

Escherichia coli Sequence Type ST131 as the Major Cause of Serious Multidrug-Resistant *E. coli* Infections in the United States

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(See the article by Lautenbach et al, on pages 280–285.)

Background. *Escherichia coli* sequence type ST131 (O25:H4), associated with the CTX-M-15 extended-spectrum β -lactamase, has emerged internationally as a multidrug-resistant pathogen but has received little attention in the United States.

Methods. From the SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance programs, 127 *E. coli* clinical isolates from hospitalized patients across the United States in 2007, stratified by extended-spectrum cephalosporin and fluoroquinolone phenotype and *bla*_{CTX-M-15} genotype, were assessed for phylogenetic group, ST131 status, susceptibility profile, virulence genotype, *gyrA* and *parC* sequence, and pulsed-field gel electrophoresis profile.

Results. The 54 identified ST131 isolates (all fluoroquinolone resistant) accounted for an estimated 17% of the source populations, including 67%–69% of isolates resistant to extended-spectrum cephalosporins or fluoroquinolones, 55% of those resistant to both fluoroquinolones and trimethoprim-sulfamethoxazole, and 52% of multidrug-resistant isolates. Their distinctive virulence profiles were more extensive compared with other antimicrobial-resistant isolates but similarly extensive compared with antimicrobial-susceptible isolates. Pulsed-field profiling suggested ongoing dissemination among locales, with concentration of *bla*_{CTX-M-15} within specific ST131 lineages. A historical ST131 isolate lacked the 2007 ST131 isolates' conserved fluoroquinolone resistance-associated single-nucleotide polymorphisms in *gyrA* and *parC*.

Conclusions. A single *E. coli* clonal group, ST131, probably caused the most significantly antimicrobial-resistant *E. coli* infections in the United States in 2007, thereby constituting an important new public health threat. Enhanced virulence and/or antimicrobial resistance compared with other *E. coli*, plus ongoing dissemination among locales, may underlie ST131's success. Urgent investigation of the sources and transmission pathways of ST131 is needed to inform mitigation efforts.

Extraintestinal infections due to *Escherichia coli* cause considerable morbidity, mortality, and increased health care costs [1]. Management is complicated by the increasing prevalence and spectrum of antimicrobial resistance [1–4]. Specifically, fluoroquinolone and trimethoprim-sulfamethoxazole resistance limit outpatient treatment options, whereas extended-spectrum cephalosporin resistance limits treatment options in the

hospital. This produces delays in appropriate therapy, higher costs, and increased use of “last resort” antimicrobials (eg, carbapenems) [1–4].

In *E. coli*, extended-spectrum cephalosporin resistance usually is from extended-spectrum β -lactamases (ESBLs). Among the various *E. coli*-associated ESBLs, the CTX-M family, notably CTX-M-1, CTX-M-14, and CTX-M-15, currently predominates [4, 5]. A recently emerged, disseminated lineage of virulent *E. coli*, designated sequence type ST131 according to multilocus sequence typing, is associated with CTX-M-15 and is usually fluoroquinolone resistant [6–9]. In addition, ESBL-negative ST131 strains are a prominent cause of fluoroquinolone-resistant (but extended-spectrum cephalosporin-susceptible) *E. coli* infections in Europe and Canada [10, 11]. Unlike most

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Table 1. Characteristics of 127 *Escherichia coli* isolates from the SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Collections (2007), According to Extended-Spectrum β -Lactamase (ESBL) and Fluoroquinolone Phenotype and *bla*_{CTX-M-15} Status.

Category and trait	No. (%) of isolates with characteristic						P ^a
	ESBL positive (n = 59)			ESBL negative (n = 68)			
	<i>bla</i> _{CTX-M-15} (subset 1) (n = 34)	Others (subset 2) (n = 25)	Fluoroquinolone resistant (subset 3) (n = 34)	Fluoroquinolone susceptible (subset 4) (n = 34)	4-Subset comparison	Subset 1 vs 2	
Phylogenetic/clonal group							
Group A	6 (18)	4 (16)	0 (0)	2 (6)		.03	
Group B1	1 (3)	4 (16)	1 (3)	4 (12)		.02	
Group B2	22 (65)	9 (36)	25 (74)	21 (62)		<.001	
Group D	5 (15)	8 (32)	8 (24)	7 (21)		.04	
ST131	22 (65)	8 (32)	24 (71)	0 (0)		<.001	
Resistance phenotype							
Trimethoprim-sulfamethoxazole resistant	22 (65)	15 (80)	18 (53)	5 (15)		<.001	
Fluoroquinolone resistant	34 (100)	21 (84)	34 (100)	0 (0)		<.001	
Piperacillin-tazobactam resistant	9 (26)	5 (20)	0 (0)	0 (0)		<.004	
Extended-spectrum cephalosporins resistant	34 (100)	17 (68)	0 (0)	0 (0)		<.001	
Aminoglycoside resistant	27 (79)	13 (52)	11 (32)	1 (3)		.047	
Trimethoprim-sulfamethoxazole and fluoroquinolone resistant	22 (65)	15 (60)	18 (53)	0 (0)		<.001	
Any of above	34 (100)	22 (88)	34 (100)	6 (18)		<.001	
Multidrug resistant	31 (91)	18 (72)	6 (18)	0 (0)		<.001	

NOTE. Subset definitions (and prevalence in source SENTRY and MYSTIC collections): subset 1, ESBL positive and *bla*_{CTX-M-15} positive (2.1%); subset 2, ESBL positive and *bla*_{CTX-M-15} negative (4.9%); subset 3, ESBL negative and fluoroquinolone resistant (19.2%); and subset 4, ESBL negative and fluoroquinolone susceptible (73.8%)
^a P values (determined by the Fisher exact test) are shown if P < .05.

Table 2. Resistance Scores among ST131 and Non-ST131 *Escherichia coli* Isolates within 4 Resistance-Associated Subsets

Subset	Subset definition	Total		ST131 isolates		Non-ST131 isolates		<i>P</i> value, ST131 vs. non-ST131 ^a
		No.	Median score (range)	No.	Median score (range)	No.	Median score (range)	
1	ESBL positive, <i>bla</i> _{CTX-M-15}	34	6 (3–8) ^b	22	5.5 (3–7)	12	6 (4–8)	.03
2	ESBL positive, no <i>bla</i> _{CTX-M-15}	25	4 (0–8) ^b	8	5 (3–7)	17	3 (0–8)	
3	ESBL negative, fluoroquinolone resistant	34	2 (1–4) ^c	24	2 (1–4)	10	2 (1–3)	NA
4	ESBL negative, fluoroquinolone susceptible	34	0 (0–1) ^c	0	NA	34	0 (0–1)	

NOTE. ESBL, extended-spectrum β -lactamase; NA, not applicable (no ST131 isolates).

^a *P* values (determined by the Mann-Whitney *U* test) for ST131 versus non-ST131 are shown where *P* < .05.

^b For subset 1 versus subset 2, *P* < .001 (determined by the Mann-Whitney *U* test).

^c For subset 3 versus subset 4, *P* < .001 (determined by the Mann-Whitney *U* test).

historical antimicrobial-resistant *E. coli* strains, *E. coli* ST131 derives from (virulence-associated) phylogenetic group B2.

CTX-M–encoding genes are embedded in transposon-like structures, often carried in plasmids containing additional resistance genes [5]. Acquisition of these elements, which encode multiple antimicrobial resistance mechanisms, can confer resistance to β -lactams, fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole. However, high-level fluoroquinolone resistance usually also involves multiple point mutations within the “quinolone resistance–determining region” of *gyrA* (DNA gyrase) and *parC* (topoisomerase), which encode the targets of fluoroquinolones. Such mutations arise spontaneously, then are transmitted vertically.

Because of the increasing prevalence in *E. coli* of resistance to fluoroquinolones and extended-spectrum cephalosporins, evidence of CTX-M-15–positive *E. coli* in the United States [12, 13], and sporadic reports of serious *E. coli* ST131 infections in the United States [14, 15], we hypothesized that ST131 may now be contributing importantly to antimicrobial-resistant *E. coli* infections nationwide. Accordingly, using a nationally representative sample of clinical *E. coli* isolates from 2007, we assessed ST131’s current prevalence and contribution to the US antimicrobial-resistant *E. coli* population. In addition, to gain insights into the basis for ST131’s emergence, we studied ST131’s association with CTX-M-15 (vs. other ESBLs) and its virulence characteristics, fluoroquinolone resistance genetics, and clonal composition.

METHODS

Strains. During 2007, the SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Antimicrobial Surveillance Programs collected 1596 nonduplicate *E. coli* clinical isolates from patients hospitalized in 33 widely distributed US medical centers [13]. Of the 1596 isolates, 118 (7.4%) exhibited reduced broth microdilution susceptibility to extended-spectrum cephalosporins [16] and so were defined as ESBL positive. The 118 ESBL-positive isolates were tested by polymerase

chain reaction (PCR) for *bla*_{CTX-M} genes, and amplicons were sequenced and analyzed [13]. Thirty-four isolates carried the CTX-M-15–encoding gene *bla*_{CTX-M-15}. The 34 *bla*_{CTX-M-15}–positive isolates (2.1% of 1596 total isolates; 29% of 118 ESBL-positive isolates), designated subset 1, were from 17 medical centers (52% of 33 total) in 15 cities and 13 states (California, Hawaii, Indiana, Kentucky, Michigan, New Jersey, New York, Nebraska, Ohio, Texas, Utah, Washington, and Wisconsin).

Three control groups (ie, subsets 2–4) were selected. For subset 2, all non-CTX-M-15, ESBL-positive isolates that were available for molecular analysis (*n* = 25) were used. For subsets 3 and 4 (non-ESBL; fluoroquinolone resistant and fluoroquinolone susceptible, respectively), representatives were chosen randomly from among the available isolates from a particular city to equal the number of subset 1 isolates from that city. This gave 59 ESBL-positive isolates (ie, 34 CTX-M-15 [subset 1] plus 25 non-CTX-M-15 [subset 2]) and 68 ESBL-negative isolates (ie, 34 fluoroquinolone susceptible [subset 3] plus 34 fluoroquinolone resistant [subset 4]). The prevalence of the 4 subsets within the combined SENTRY and MYSTIC populations was 2.1% (subset 1), 4.9% (subset 2), 19.2% (subset 3), and 73.8% (subset 4).

Susceptibility testing. Coresistance was assessed by broth microdilution and/or disk diffusion according to procedures and interpretive criteria specified by the Clinical Laboratory Standards Institute [13, 16, 17]. Intermediate susceptibility was analyzed as resistance. Multidrug-resistant (MDR) isolates were those resistant to at least 1 representative of ≥ 3 antimicrobial classes, including β -lactam/ β -lactamase inhibitors (piperacillin-tazobactam), extended-spectrum cephalosporins (ceftriaxone, ceftazidime, cefepime), aminoglycosides (gentamicin, tobramycin), fluoroquinolones (ciprofloxacin), and trimethoprim-sulfamethoxazole. The resistance score was the number of agents to which resistance was evident.

Molecular methods. *E. coli* phylogenetic group (A, B1, B2, and D) was determined by PCR [18]. Group B2 isolates were assessed for ST131 status by O25b *rfb* detection [7], random amplified polymorphic DNA profiling [10], and detection of

Table 3. Estimated Contribution of ST131 to Antimicrobial-Resistant *Escherichia coli* Populations, 2007

Resistance phenotype	Overall prevalence of phenotype in population, %	Estimated fraction due to ST131
Extended-spectrum cephalosporins ^a	5.4	0.69
Fluoroquinolones ^a	25.4	0.67
Trimethoprim-sulfamethoxazole	25.4	0.31
Aminoglycosides ^a	12.6	0.55
Piperacillin-tazobactam	3.8	0.24
Fluoroquinolones ^a plus trimethoprim-sulfamethoxazole	14.5	0.55
Any of above	38.9	0.43
Multidrug resistance (≥ 3 of above drug classes)	7.1	0.52

^a Extended-spectrum cephalosporins, resistant to ≥ 1 of cefepime, ceftriaxone, and ceftazidime; fluoroquinolones, resistant to ciprofloxacin; and aminoglycosides, resistant to gentamicin, tobramycin, or both.

ST131-specific single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* [10]. Selected putative ST131 isolates underwent confirmatory multilocus sequence typing on the basis of partial sequence for *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* (<http://mlst.ucc.ie>) [10], which uniformly confirmed their ST131 status.

Presence of 62 extraintestinal virulence genes was assessed by multiplex PCR [10, 19, 20]. The virulence score was the number of virulence genes detected, adjusted for multiple detection of certain operons (ie, *pap* [P fimbriae], *sfa/foc* [S and F1C fimbriae], and *kps* [group 2 capsule]). For selected isolates, SNPs within the quinolone resistance-determining region of *gyrA* and *parC* were identified by sequence analysis [21], in comparison with strains MG1655 [22] and H17 (a fluoroquinolone-susceptible, virulence gene-deficient ST131 urosepsis isolate from 1985) [20].

*Xba*I pulsed-field gel electrophoresis (PFGE) analysis was used for pulsotype assignment (on the basis of a 94% Dice similarity coefficient threshold) and dendrogram construction [23]. Profiles also were compared with a private reference library containing 150 pulsotypes, from 275 ST131 isolates (J.R.J., unpublished data).

Statistical analyses. Comparisons of proportions and scores were tested using the Fisher exact test and the Mann-Whitney *U* test, respectively. (Alternate methods that account for by-locale clustering—that is, linear mixed-model regression and logistic generalized estimating equations, gave essentially identical results.) Principal coordinates analysis, a multidimensional scaling method analogous to principle components analysis, was used to collapse the molecular dataset for simplified between-group comparisons [24]. Groups were compared on each of the first 3 coordinates, which capture most of the variance within the dataset, using a 2-tailed *t* test.

RESULTS

Source, phylogenetic distribution, and ST131 prevalence.

The 127 systematically selected *E. coli* clinical isolates from the SENTRY and MYSTIC collections (United States, 2007) were from bloodstream (73%); wounds, abscesses, tissue, or bone (15%); respiratory samples (9%); and miscellaneous sources (2%). Phylogenetic group B2 predominated within each resistance subset, accounting for >50% of isolates in subsets 1, 3, and 4 and a plurality of those in subset 2 (Table 1). ST131 accounted for 54 (70%) of the 77 total group B2 isolates but was strikingly distributed by subset, accounting for nearly all group B2 isolates within subsets 1–3 but none within subset 4 ($P < .001$) (Table 1). Accordingly, ST131 was significantly associated with *bla*_{CTX-M-15} among ESBL-positive isolates (subsets 1 and 2) and with fluoroquinolone resistance among ESBL-negative isolates (subsets 3 and 4). ST131 and non-ST131 isolates were distributed similarly by specimen type. On the basis of the prevalence of ST131 within each subset (Table 1) and each subset's prevalence within the source SENTRY and MYSTIC collections, the estimated overall prevalence of ST131 within the source collections was 16.6%.

Antimicrobial resistance. Overall, resistance to antimicrobial agents other than those that defined the 4 subsets was common and varied significantly by subset (Table 1). Among ESBL-positive isolates (subsets 1 and 2), subset 1 isolates (*bla*_{CTX-M-15} positive) were significantly more likely than subset 2 isolates (*bla*_{CTX-M-15} negative) to be fluoroquinolone resistant, extended-spectrum cephalosporin resistant, or aminoglycoside resistant; similar trends were obtained for most other resistance phenotypes (Table 1). Likewise, among ESBL-negative isolates (subsets 3 and 4), subset 3 isolates (fluoroquinolone resistant) were significantly more likely than subset 4 isolates (fluoroquinolone susceptible) to be trimethoprim-sulfamethoxazole

Table 4. Molecular Characteristics of 54 *Escherichia coli* ST131 Isolates and 73 Geographically Matched Controls, According to Antimicrobial Resistance Subset

Specific trait ^a	No. (%) of isolates with trait					<i>P</i> ^b			
	ST131 (<i>n</i> = 54)	Non-ST131				ST131 vs. subset 1 (non-ST131)	ST131 vs. subset 2 (non-ST131)	ST131 vs. subset 3 (non-ST131)	ST131 vs. subset 4 (non-ST131)
		Subset 1 (<i>n</i> = 12)	Subset 2 (<i>n</i> = 17)	Subset 3 (<i>n</i> = 10)	Subset 4 (<i>n</i> = 34)				
Phylogenetic group									
Group A	0 (0)	6 (50)	4 (24)	0 (0)	2 (6)	<.001	.002		
Group B1	0 (0)	1 (8)	4 (24)	1 (10)	4 (12)		.002	.02	.02
Group B2	54 (100)	0 (0)	1 (6)	1 (10)	21 (62)	<.001	<.001	<.001	<.001
Group D	0 (0)	5 (42)	8 (47)	8 (80)	7 (21)	<.001	<.001	<.001	<.001
O antigen									
O25b <i>rfb</i>	54 (100)	0 (0)	0 (0)	0 (0)	0 (0)	<.001	<.001	<.001	<.001
Adhesins									
F10 <i>papA</i>	52 (96)	1 (8)	4 (24)	0 (0)	8 (24)	<.001	<.001	<.001	<.001
<i>papACEFG</i>	5 (9)	0 (0)	2 (12)	6 (60)	18 (53)			.001	<.001
<i>sfa/foc</i>	0 (0)	0 (0)	0 (0)	0 (0)	7 (21)				.001
<i>focG</i>	0 (0)	0 (0)	0 (0)	0 (0)	4 (12)				.002
<i>iha</i>	54 (100)	0 (0)	6 (35)	5 (50)	10 (29)	<.001	<.001	.001	<.001
<i>fimH</i>	53 (98)	8 (67)	14 (82)	9 (90)	33 (97)	.003	.04		
<i>tsh</i>	15 (28)	0 (0)	1 (6)	3 (30)	2 (6)				.01
Toxins									
<i>hlyD</i>	1 (2)	1 (8)	1 (6)	1 (10)	8 (24)				.002
<i>sat</i>	51 (94)	1 (8)	0 (0)	5 (50)	11 (32)	<.001	<.001	.001	<.001
<i>pic</i>	0 (0)	0 (0)	1 (6)	0 (0)	5 (15)				.007
<i>vat</i>	0 (0)	0 (0)	1 (6)	0 (0)	18 (53)				<.001
<i>astA</i>	0 (0)	2 (17)	2 (12)	0 (0)	1 (3)	.03			
Siderophores									
<i>iroN</i>	0 (0)	0 (0)	1 (6)	1 (10)	9 (26)				<.001
<i>fyuA</i>	54 (100)	9 (75)	10 (56)	9 (90)	27 (79)	.005	<.001		.001
<i>ireA</i>	1 (2)	0 (0)	1 (6)	0 (0)	10 (29)				<.001
<i>iutA</i>	52 (96)	8 (67)	9 (53)	7 (70)	12 (35)	.008	<.001	.02	<.001
Capsule									
<i>kpsM</i> II	41 (76)	2 (25)	5 (29)	10 (100)	27 (79)	.001	.001	.01	
K1	0 (0)	0 (0)	2 (12)	0 (0)	16 (47)				<.001
K2	10 (19)	0 (0)	0 (0)	3 (30)	3 (9)	.01		.02	
K5	17 (31)	2 (17)	0 (0)	0 (0)	5 (15)	.03	.007	.01	
Miscellaneous									
<i>usp</i>	54 (100)	0 (0)	3 (18)	3 (30)	26 (76)	<.001	<.001	<.001	<.001
<i>traT</i>	49 (91)	7 (58)	13 (76)	7 (70)	23 (7)	.01			.01
<i>ompT</i>	50 (93)	3 (25)	1 (6)	1 (10)	21 (62)	<.001	<.001	<.001	<.001
<i>iss</i>	0 (0)	0 (0)	0 (0)	2 (20)	2 (6)			.02	
H7 <i>fliC</i>	0 (0)	0 (0)	1 (6)	0 (0)	15 (44)				<.001
<i>malX</i>	54 (100)	6 (50)	4 (24)	3 (30)	25 (74)	<.001	<.001	<.001	<.001

NOTE. Non-ST131 isolates were stratified into 4 subsets, defined as follows: subset 1, extended-spectrum β -lactamase (ESBL) positive and *bla*_{CTX-M-15} positive; subset 2, ESBL positive and *bla*_{CTX-M-15} negative; subset 3, ESBL negative and fluoroquinolone resistant; and subset 4, ESBL negative and fluoroquinolone susceptible. (Subset 4 included only non-ST131 isolates.) ST131 isolates (from subsets 1–3) were analyzed collectively, without regard for subset.

^a Traits shown are those that yielded *P* < .05 for at least 1 comparison. Definitions are as follows: O25 *rfb*, O antigen-specific lipopolysaccharide; F10 *papA*, P fimbriae subunit variant; *papACEFG*, genes of P fimbriae operon; *sfa/foc*, S or F1C fimbriae; *focG*, F1C fimbriae adhesin; *iha*, adhesin-siderophore; *fimH*, type 1 fimbriae; *tsh*, temperature-sensitive hemagglutinin; *hlyD*, α -hemolysin; *sat*, secreted autotransporter toxin; *pic*, serine protease; *vat*, vacuolating toxin; *iroN*, salmochelin (siderophore) receptor; *fyuA*, yersiniabactin (siderophore) receptor; *ireA*, siderophore receptor; *iutA*, aerobactin (siderophore) receptor; *kpsM* II, group 2 capsule; K1, K2, and K5, group 2 capsule variants; *usp*, uropathogenic-specific protein; *traT*, serum resistance-associated; *ompT*, outer membrane protease T; *iss*, increased serum survival; H7 *fliC*, flagellin variant; and *malX*, pathogenicity island marker.

^b *P* values (by Fisher's exact test) are shown where *P* < .05.

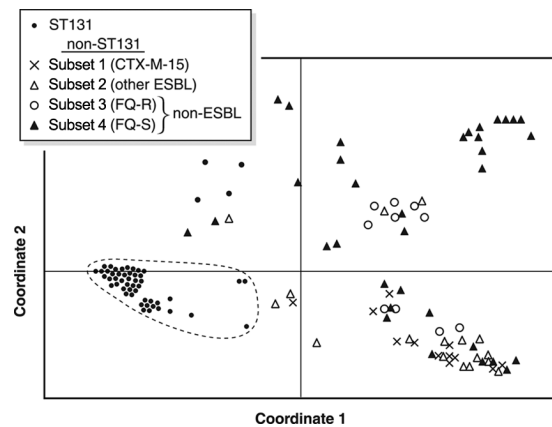


Figure 1. Principle coordinates analysis plot for 127 *Escherichia coli* isolates from the SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programs (2007). The principle coordinates analysis was based on 60 virulence genes. Coordinates 1 and 2 capture 34% and 32% of variance in the dataset, respectively (total, 66%). Fifty (93%) of the 54 ST131 were placed within the region of the plane encircled by the dashed line, which contained no non-ST131 isolates. ESBL, extended-spectrum β -lactamase; FQ-R, fluoroquinolone resistant; FQ-S, fluoroquinolone susceptible. Subsets 1–4 were defined according to ESBL, *bla*_{CTX-M-15}, and FQ resistance status. The ST131 isolates ($n = 54$) included representatives from subsets 1–3.

resistant, aminoglycoside resistant, dually trimethoprim-sulfamethoxazole and fluoroquinolone resistant, resistant to any drug class, or MDR (Table 1). Aggregate resistance scores were highest in subset 1 (median score, 6) and decreased progressively through subsets 2, 3, and 4 (median scores, 4, 2, and 0, respectively) (Table 2).

Within subsets 1–3 the ST131 and non-ST131 isolates did not differ significantly for the prevalence of any phenotype excepting aminoglycoside resistance and “any resistance,” which within subset 2 were significantly more prevalent among ST131 isolates (ie, for aminoglycoside resistance, 7 [88%] of 8 ST131 isolates vs 6 [35%] of 17 non-ST131 [$P = .03$]; for any resistance, 8 [100%] of 8 ST131 isolates vs 14 [83%] of 17 non-ST131 isolates [$P = .01$]). Accordingly, aggregate resistance scores were similar (subsets 1 and 3) or significantly greater (subset 2) among ST131 isolates than non-ST131 isolates (Table 2).

On the basis of the proportional contribution of ST131 to specific resistance phenotypes within each subset and each subset’s contribution to the source population, the net contribution of ST131 to various resistant subpopulations with the source SENTRY and MYSTIC collections was calculated (Table 3). According to these estimates, ST131 accounted for >40% of all isolates exhibiting any resistance; >50% of aminoglycoside-resistant, MDR, and fluoroquinolone plus trimethoprim-sulfamethoxazole-resistant isolates; and 67%–69% of extended-spectrum cephalosporin-resistant and fluoroquinolone-resistant isolates.

Virulence traits. To identify possible explanations for ST131’s remarkably high prevalence, especially among antimicrobial-resistant strains, phylogenetic background and virulence profiles were examined. The ST131 isolates exhibited a significantly greater prevalence of group B2 (100%) than did the non-ST131 isolates within any subset, including group B2–dominated subset 4 ($P < .001$ for each comparison) (Table 4). Likewise, according to the first 3 coordinates of a principle coordinates analysis on the basis of all 62 studied virulence genes, the ST131 isolates exhibited highly distinctive virulence profiles that significantly differentiated them from non-ST131 isolates, whether these were considered collectively ($P < .001$: coordinate 1 only) or by individual subset ($P < .01$ for 2 or 3 coordinates per comparison). In a plot of the coordinate 1–coordinate 2 plane, which captured 66% of total variance, the ST131 isolates clustered tightly in the lower left quadrant, clearly separated from the non-ST131 isolates (Figure 1).

The basis for these aggregate virulence profile differences was explored through univariate comparisons that involved individual virulence genes. Thirteen virulence traits were significantly more prevalent among the 54 total ST131 isolates than among the non-ST131 isolates within at least 1 of the 4 subsets, whereas 11 virulence traits were significantly more prevalent among the non-ST131 isolates (subset 4 only) (Table 4). Accordingly, aggregate virulence scores among the combined ST131 isolates were significantly higher than among the non-ST131 subset 1–3 isolates and were only slightly (and nonsignificantly) lower than among the subset 4 isolates (Table 5).

PFGE analysis. To clarify the relationship of ST131 to other *E. coli* and to identify additional explanations for ST131’s prominence, PFGE was used to assess (1) genomic diversity among ST131 and non-ST131 isolates, (2) the clonal distribution of *bla*_{CTX-M-15}, and (3) the geographic distribution of ST131-associated pulsotypes. Consistent with their common genetic background, ST131 isolates exhibited more homogeneous PFGE profiles than did non-ST131 isolates (Figure 2). For example, in the PFGE-based dendrogram the ST131 cluster extended to 76% similarity versus 45% overall. Likewise, compared with non-ST131 isolates, ST131 isolates were significantly more likely to exhibit the same pulsotype (59% vs 20%; $P < .001$) or frankly indistinguishable profiles (15% vs 3%; $P = .04$).

Among the ST131 isolates, *bla*_{CTX-M-15} positivity was concentrated within specific subclusters, suggesting vertical transmission (Figure 2). However, several highly similar isolates were *bla*_{CTX-M-15} discordant, suggesting horizontal gene transfer or gene loss. ST131 was encountered in 14 of the 17 study locales. Within each of the 6 ST131-associated pulsotypes that included ≥ 3 isolates, multiple locales were represented; one 5-isolate pulsotype actually spanned 5 distinct locales.

gyrA and parC. Sequence analysis of the quinolone resis-

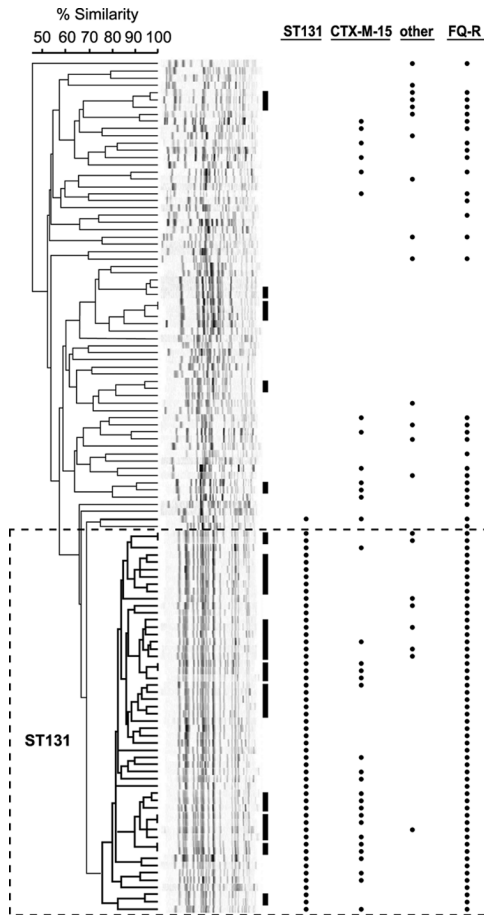


Figure 2. Dendrogram of pulsed-field gel electrophoresis (PFGE) profiles for 127 *Escherichia coli* isolates from the SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programs (2007). Fifty-three (98%) of the 54 ST131 isolates occurred within the cluster enclosed by the dashed rectangle, with no interposed non-ST131 isolates. Branches leading to ST131 isolates are bolded. Heavy vertical bars to the right of the PFGE profiles identify groups of isolates with the same pulsotype. Bullets identify isolates that are ST131, exhibit CTX-M-15 or another extended-spectrum β -lactamase (ESBL), and/or are fluoroquinolone resistant (FQ-R). Dendrogram was inferred according to the unweighted pair group method with averaging on the basis of Dice similarity coefficients.

tance-determining region of *gyrA* and *parC* for 15 ST131 isolates (14 fluoroquinolone resistant [present study] and 1 fluoroquinolone susceptible [historical]) and 14 non-ST131 isolates (7 fluoroquinolone resistant [present study] and 8 fluoroquinolone susceptible [7 present study, 1 reference]) identified SNPs at 30 positions. Only 5 SNPs were nonsynonymous (ie, altered the inferred peptide sequence).

All 14 fluoroquinolone-resistant ST131 isolates (excepting 1 with an obviously recombined *parC*) exhibited identical sequence across both genes, including 4 conserved nonsynonymous SNPs, 2 each in *gyrA* (c248t and g259a) and *parC* (g239t and a251t). The first 3 of these nonsynonymous SNPs were

also present in all 7 fluoroquinolone-resistant non-ST131 isolates (which exhibited diverse phylogenetic backgrounds), whereas the fourth SNP was ST131 specific. A fifth non-synonymous SNP, *gyrA* g166a, occurred in a single fluoroquinolone-resistant non-ST131 isolate. In contrast, multiple synonymous (ie, silent) SNPs differentiated ST131 isolates from non-ST131 isolates and distinguished among the various non-ST131 isolates. The historical fluoroquinolone-susceptible ST131 isolate exhibited identical *gyrA* and *parC* sequence to the present fluoroquinolone-resistant ST131 isolates except for the 4 conserved nonsynonymous SNPs and a single synonymous SNP (c321g) in *parC*.

DISCUSSION

Our analysis of 127 systematically selected *E. coli* isolates from the nationally representative SENTRY and MYSTIC surveillance programs (United States, 2007) found that a single *E. coli* clonal group, ST131, was highly prevalent, particularly among antimicrobial-resistant isolates. By extrapolation, we estimated that within the source collections ST131 accounted for (1) ~17% of isolates overall, (2) 44% of antimicrobial-resistant isolates, (3) >50% of MDR isolates and those dually resistant to fluoroquinolones and trimethoprim-sulfamethoxazole, and (4) 67%–69% of those resistant to fluoroquinolones or extended-spectrum cephalosporins. These remarkably high prevalence values are unprecedented within *E. coli*, which is a highly diverse species that in clinical collections is usually represented by multiple minority clonal groups [25, 26]. This suggests that ST131 has emerged as an important, new, disseminated MDR extraintestinal pathogen.

The clinical and public health implications of the observed high prevalence of ST131, a pathogen with the ability to cause serious or fatal extraintestinal infections [14, 15], are potentially profound. Because fluoroquinolones are increasingly relied on for empirical therapy of urinary tract infections [2, 27], fluoroquinolone resistance in *E. coli* poses a substantial threat among ambulatory patients, increasing the risk of treatment failure despite use of what traditionally has been a predictably effective regimen. Dual fluoroquinolone and trimethoprim-sulfamethoxazole resistance is even more problematical, eliminating both current mainstays for empirical oral urinary tract infection therapy [27]. Both MDR status and resistance to extended-spectrum cephalosporins also threaten hospitalized patients, for whom extended-spectrum cephalosporins and other antimicrobials, such as aminoglycosides and β -lactam/ β -lactamase inhibitors, constitute standard empirical therapy.

The estimated overall contribution of ST131 to these important phenotypes (ie, 67%–69% of fluoroquinolone or extended-spectrum cephalosporin resistance, 55% of combined fluoroquinolone and trimethoprim-sulfamethoxazole resistance, and 52% of MDR isolates) suggests that as of 2007 ST131 had

Table 5. Virulence Scores of 54 *Escherichia coli* ST131 Isolates and 73 Geographically Matched Controls, by Antimicrobial Resistance Subset

Group	Subset definitions	No. of isolates	Virulence score, median (range)	<i>P</i> ^a for ST131 vs non-ST131
ST131 (subsets 1–3)	...	54	10 (6–13)	NA
Non-ST131 (subset 1)	ESBL positive, <i>bla</i> _{CTX-M-15}	12	3 (1–7)	<.001
Non-ST131 (subset 2)	ESBL positive, no <i>bla</i> _{CTX-M-15}	17	5 (1–14)	<.001
Non-ST131 (subset 3)	ESBL negative, fluoroquinolone resistant	10	7.5 (3–12)	<.001
Non-ST131 (subset 4)	ESBL negative, fluoroquinolone susceptible	34	12 (1–19)	

NOTE. ESBL, extended-spectrum β -lactamase; NA, not applicable (no non-ST131 comparator).

^a *P* values (determined by the Mann-Whitney *U* test) for ST131 versus indicated group are shown where *P* < .05.

become the major cause of significantly antimicrobial-resistant *E. coli* infections in the United States. By extension, the emergence of ST131 is likely the main explanation for recent increases in antimicrobial resistance prevalence in *E. coli*, with the resulting adverse consequences for clinical outcomes and costs.

Our findings suggest possible explanations for ST131's remarkable success as an emerging pathogen, including phylogenetic background, virulence characteristics, and antimicrobial resistance capability. ST131 is from phylogenetic group B2, which is associated epidemiologically and experimentally with extraintestinal virulence [28, 29]. Although much of its enhanced virulence potential is due to defined virulence traits, undefined group B2-associated qualities also likely contribute. Because the ST131 isolates were significantly more likely to be from group B2 than were the non-ST131 isolates, they may have a fitness advantage because of their group B2 genomic backbone, independent of accessory traits.

In addition, the ST131 isolates significantly exceeded the non-ST131 isolates for extent of resistance and/or virulence profiles. According to individual traits and aggregate scores, compared with resistant non-ST131 isolates (from subsets 1–3), the ST131 isolates were as or more antimicrobial resistant and significantly more virulent appearing. Likewise, compared with susceptible non-ST131 isolates (from subset 4) the ST131 isolates were approximately as virulent appearing but significantly more antimicrobial resistant. Thus, ST131 appears to combine resistance and virulence, which in *E. coli* traditionally have been somewhat mutually exclusive [30]. This resistance-plus-virulence combination may give ST131 a competitive advantage over other *E. coli*, promoting its clonal expansion and dominance within niches traditionally occupied by less virulent and/or more susceptible clones.

Our findings provide other insights into ST131's current predominance among antimicrobial-resistant *E. coli*. Sequence analysis of *gyrA* and *parC*, which encode the drug targets of fluoroquinolones [21], suggested that current fluoroquinolone-resistant ST131 isolates derive from a recent common fluoroquinolone-resistant ST131 ancestor, which in turn descended from an earlier (perhaps in the 1980s) fluoroquinolone-sus-

ceptible progenitor by accumulating 4 nonsynonymous SNPs within *gyrA* and *parC*. Likewise, during this same interval the ST131 ancestor apparently acquired the characteristic suite of ST131-associated virulence genes, which are fairly uniformly present among current (fluoroquinolone-resistant) ST131 isolates but are largely absent from the historical fluoroquinolone-susceptible ST131 reference strain. In contrast, *bla*_{CTX-M-15} seemingly entered ST131 more recently, likely by horizontal transfer of a mobile genetic element into ≥ 1 already fluoroquinolone-resistant ST131 lineages.

Layered on this postulated evolutionary scenario is the apparent widespread distribution of ST131 pulsotypes among locales [6, 8], likely reflecting ongoing dissemination of ST131, another probable contributor to ST131's emergence. Although ST131's historical acquisition of fluoroquinolone resistance, *bla*_{CTX-M-15} (and other resistance elements), and virulence genes represents a *fait accompli*, ongoing spread of ST131 suggests the possibility of identifying transmission pathways and reservoirs to interrupt further dissemination. Investigation is urgently needed into the sources of ST131 (including, possibly, certain human groups, food animals, and/or companion animals) and the factors contributing to ST131's widespread dissemination (including, possibly, host-to-host or foodborne transmission vs environmental contamination). On the basis of the findings of such studies, appropriate public health interventions (to block transmission) and interventions toward more parsimonious antimicrobial use (to decrease selection pressure) should be aggressively pursued to curtail further emergence of this threatening pathogen.

In summary, we found that among the SENTRY and MYSTIC programs' US *E. coli* isolates (2007), ST131 accounted for an estimated 17% of the isolates overall but for 52% of isolates resistant to ≥ 3 antimicrobial classes, 55% of those dually resistant to fluoroquinolones and trimethoprim-sulfamethoxazole, and 67%–69% of those resistant to extended-spectrum cephalosporins or fluoroquinolones. Although *bla*_{CTX-M-15} was concentrated within specific lineages, implying recent acquisition, 4 distinctive fluoroquinolone resistance-associated *gyrA* and *parC* SNPs were ubiquitous within ST131, indicating that

they antedate ST131's recent expansion. ST131's distinctive combination of resistance and virulence, plus widespread dissemination among locales, may underlie its epidemiologic success. These findings establish ST131 as a major drug-resistant pathogen in the United States and, consequently, an important new public health threat in need of urgent investigation and remediation efforts.

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