The Pandemic *H*30 Subclone of Sequence Type 131 (ST131) as the Leading Cause of Multidrug-Resistant *Escherichia coli* Infections in the United States (2011–2012)

James R. Johnson,[1,2] **Stephen Porter,**[1,2] **Paul Thuras,**[1,2] **and Mariana Castanheira**[3]

[1]Minneapolis Veterans Affairs Healthcare System; [2]Departments of Medicine and Psychiatry, University of Minnesota, Minneapolis; and [3]JMI Laboratories, North Liberty, Iowa

Background. Extraintestinal *Escherichia coli* infections are increasingly challenging due to emerging antimicrobial resistance, including resistance to extended-spectrum beta-lactams and fluoroquinolones. Sequence type 131 (ST131) is a leading contributor.

Methods. Three hundred sixty *E. coli* clinical isolates from across the United States (2011–2012), selected randomly from the SENTRY collection within 3 resistance categories (extended-spectrum cephalosporin [ECS]–reduced susceptibility [RS]; fluoroquinolone-resistant, ESC-susceptible; and fluoroquinolone-susceptible, ESC-susceptible) were typed for phylogroup, sequence type complex (STc), subsets thereof, virulence genotype, O type, and beta-lactamase genes. Molecular results were compared with susceptibility profile, specimen type, age, and sex.

Results. Phylogroup B2 accounted for most isolates, especially fluoroquinolone-resistant isolates (83%). Group B2–derived ST131 and its *H*30 subclone (divided between *H*30Rx and *H*30R1) predominated, especially among ESC-RS and fluoroquinolone-resistant isolates. In contrast, among fluoroquinolone-susceptible isolates, group B2–derived STc73 and STc95 predominated. Within each resistance category, ST131 isolates exhibited more extensive resistance and/or virulence profiles than non-ST131 isolates. ST131-*H*30 was distributed broadly by geographical region, age, and specimen type and exhibited distinctive beta-lactamase genes. Back-calculations indicated that within the source population ST131 accounted for 26.4% of isolates overall (vs 17% in 2007), including 19.8% ST131-*H*30, 13.2% ST131-*H*30R1, and 6.6% each ST131-*H*30Rx and non-*H*30 ST131.

Conclusions. ST131-*H*30, with its ESC resistance-associated *H*30Rx subset, caused most antimicrobial-resistant *E. coli* infections across the United States in 2011–2012 and, since 2007, increased in relative prevalence by >50%. Focused attention to this strain could help combat the current *E. coli* resistance epidemic.

Keywords. antimicrobial resistance; *Escherichia coli* infections; extended-spectrum beta-lactamases; fluoroquinolone resistance; ST131.

Extraintestinal infections due to *Escherichia coli* are common, costly, and clinically important [1]. Emerging antimicrobial resistance increasingly complicates their management [2, 3]. Better understanding of the growing *E. coli* resistance problem is sorely needed.

A single emergent *E. coli* clonal group, designated sequence type 131 (ST131) by multilocus sequence typing, has been identified as an important contributor to the epidemic of antimicrobial resistance in *E. coli*, especially for first-line agents such as fluoroquinolones and extended-spectrum cephalosporins (ESCs) [4]. ST131 first came to attention in the 2000s in association with the globally disseminated CTX-M-15 extended-spectrum beta-lactamase (ESBL) [4]. Its most extensively

resistant and epidemiologically successful clonal subset, designated ST131-*H*30 [5] because it contains allele 30 of the type-1 fimbriae adhesin gene *fimH* [6], appeared only in approximately 2000 and subsequently rose to prominence worldwide (Figure 1) [7, 8].

Nearly all *H*30 isolates, except for rare fluoroquinolone-susceptible variants from the ancestral *H*30S subclone, represent the *H*30R subclone (Figure 1) [5]. *H*30R strains are uniformly fluoroquinolone-resistant due to conserved replacement mutations in *gyrA* and *parC* [6]. The *H*30R subclone comprises 2 sister clades, termed *H*30R1 and *H*30Rx (Figure 1) [5, 9, 10]. Whereas *H*30R1 strains usually lack ESBL production (some do produce CTX-M-14 or CTX-M-27 [11]), *H*30Rx strains usually produce CTX-M-15 [5].

In contrast, non-*H*30 ST131 isolates are both less common and, usually, less extensively resistant. Representatives of the ancestral *H*41 clade exhibit *fimH* allele 41 and the O16 antigen, whereas representatives of the *H*22 clade usually exhibit *fimH* allele 22 and, like *H*30 strains, the O25b antigen (Figure 1) [5, 12].

Previous national surveys have identified ST131 as the leading cause of ESBL-producing, fluoroquinolone-resistant, and
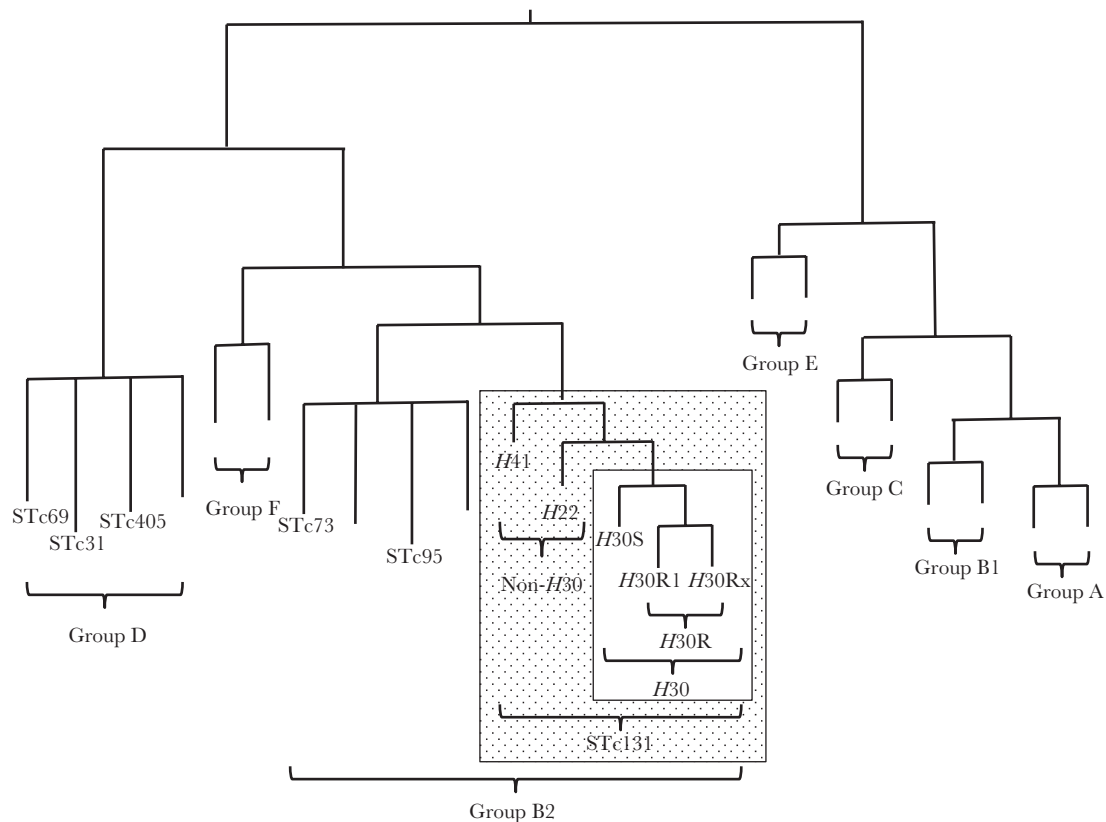
Figure 1. Phylogeny of *Escherichia coli* and sequence type complex (STc) 131. The phylogram is illustrative only; branch lengths are not to scale, and tree conformation is approximate. Phylogenetic groups are identified with brackets. Each STc included in the present study is labeled; unlabeled branches represent other (unstudied) STs within the indicated phylogenetic groups. Stippled rectangle, STc131; open rectangle within stippled rectangle, ST131-*H*30 subclone.

multidrug-resistant (MDR) *E. coli* infections in the United States [13–15]. However, because these studies antedated the discovery of the fine clonal structure of ST131 (Figure 1), none tested specifically for *H*30Rx, and only 1 tested for *H*30 [15]. Additionally, 1 was limited to isolates from 2007–2009 [13], another to a convenience sample of ESBL-producing isolates from 2000–2009 [14], and a third to Veterans Affairs (VA) Medical Center isolates from 2010–2011 [15]. We undertook this study for an updated, adequately sized, nationally representative survey of *E. coli* clinical isolates that could establish the current overall prevalence in the United States of key ST131 clonal subsets, determine their contribution to relevant resistance phenotypes, and define their virulence characteristics, resistance genotypes, and host associations.

METHODS

Isolates

Each year the SENTRY Antimicrobial Surveillance Program (JMI Laboratories) monitors clinical isolates by infection site in a prevalence design mode. The core objectives (bloodstream infection [BSI], community-acquired respiratory tract infection, patients hospitalized with pneumonia, and skin and skin-structure infection [SSSI]) have been monitored annually since 1997. Other objectives vary by the year to address the needs of the sponsors or scientific requirements defined by the JMI Laboratories principal investigators.

Participating medical centers in the United States, Europe, Latin America, and Asia-Pacific countries collect consecutive bacterial isolates for the SENTRY Program (1 per patient) according to site of infection. Each center contributes either 500 or 250 total isolates, distributed by specimen type as follows (for centers supplying 500/250 isolates per year): BSI, 100/50; community-acquired respiratory tract infection, 60/30; pneumonia in hospitalized patients, 120/50; SSSI, 100/50; specified organisms of any infection site, 40/70; urinary tract infections: 50/0; and intra-abdominal infections, 30/0.

During 2011–2012, the SENTRY Antimicrobial Surveillance Programs collected systematically 4482 nonduplicate *E. coli* clinical isolates from patients hospitalized in multiple widely distributed US medical centers. Of these, 565 (13%) exhibited reduced broth microdilution susceptibility or full resistance to ESCs [16] so were classified as "ESC-reduced susceptibility" (ESC-RS). The remaining isolates, all fully ESC-susceptible, were classified based on ciprofloxacin minimum inhibitory concentration as being either fluoroquinolone-resistant (20%) or fluoroquinolone-susceptible (67%). From the 3 resulting resistance categories— that

is, ESC-RS (+/- fluoroquinolone-resistant); ESC-susceptible, fluoroquinolone-resistant (hereafter, "fluoroquinolone-resistant"); and ESC-susceptible, fluoroquinolone-susceptible (hereafter, "fluoroquinolone-susceptible")—60 isolates per category were selected randomly for both 2011 and 2012, giving 120 isolates per category, or 360 total study isolates.

Susceptibility Testing

Susceptibility to 12 antimicrobial agents was assessed by broth microdilution (JMI Laboratories) or, for ertapenem, gradient elution (J.R.J.'s laboratory), according to procedures and interpretive criteria specified by the Clinical Laboratory Standards Institute [16, 17] or, for Etest, the manufacturer's instructions (bioMérieux). Intermediate results were analyzed as resistant. Multidrug-resistant (MDR) isolates were those resistant to at least 1 representative of ≥3 antimicrobial classes—that is, penicillins (ampicillin/sulbactam, piperacillin-tazobactam), ESCs and monobactams (cefotaxime, ceftriaxone, ceftazidime, aztreonam), carbapenems (ertapenem, imipenem), aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), tetracyclines (tetracycline), and antifolate agents (trimethoprim-sulfamethoxazole) [18]. The resistance score was the number of antimicrobial classes to which resistance was detected [18].

Genotyping

Polymerase chain reaction (PCR)–based typing was done using duplicate boiled lysates as template DNA, established multiplex PCR-based assays, and relevant positive and negative controls. Phylogroups A, B1, B2, C, D, E, and F (Figure 1) were detected per Clermont et al [19]. ST131 and its clonal subsets (Figure 1), 5 other important ST complexes (ie, groups of closely related sequence types [STs]) that are associated with high prevalence and/or antimicrobial resistance (from group B2: STc73 and STc95; from group D: STc31 [O15:K52:H1], STc69 ["clonal group A," or CGA], and STc405) (Figure 1), and the ST131-associated O25b and O16 *rfb* variants were detected as described previously [12, 15, 20–25].

Extended virulence genotypes for 51 extraintestinal virulence-associated genes and variants thereof were detected using an established multiplex PCR assay [26]. The virulence gene score, which corresponds with virulence both epidemiologically and experimentally [27, 28], was the number of virulence operons detected. Isolates were classified presumptively as extraintestinal pathogenic *E. coli* (ExPEC) if positive for >2 of *papAH* and/or *papC* (P fimbriae, counted as 1), *sfa/focDE* (S and F1C fimbriae), *afa/draBC* (Dr-binding adhesins), *kpsM* II (group 2 capsule), and *iutA* (aerobactin receptor) [29].

The ESC-RS isolates were screened for broad-spectrum beta-lactamase genes using the Check-MDR CT101 kit (Check-Points) according to the manufacturer's instructions. This kit detects various CTX-M groups (1, 2, 8/25, and 9), TEM, SHV, ACC, ACT/MIR, CMYII, DHA, FOX, KPC, and NDM-1. Selected genes were sequenced to define molecular variants [30].

Statistical Methods

Using SPSS version 16 (IBM Analytics), comparisons involving dichotomous variables were tested using an "N − 1" χ^2 test for 2 × 2 tables [31] and Fisher's exact test (2-tailed) for 2 × 3 or larger tables; those involving continuous variables were tested using the Mann-Whitney U test or Kruskal-Wallis test. The significance threshold was $P < .05$. Due to the study's exploratory nature, multiple comparison adjustments were not made.

Ethics Approval

The Minneapolis VA Health Care System Institutional Review Board reviewed and approved the study.

RESULTS

Isolates

The 360 *E. coli* study isolates were derived from 64 clinical laboratories in 55 US cities, 36 states, and all nine US geographical regions. Overall, specimen types included blood (43%), urine (9%), respiratory (23%), skin/tissue/abscess (22%), abdominal (2.2%), and other (0.8%) (Table 1). Forty-nine percent of isolates were from male patients. Geographical region of origin, specimen type (Table 1), and sex distribution did not vary significantly between the 3 resistance categories. In contrast, patients with fluoroquinolone-susceptible isolates were slightly younger (median age = 57 years; range = 0–97) than those with fluoroquinolone-resistant isolates (median age = 64 years; range = 0–93), or ESC-RS isolates (median age = 60 years; range = 0–94) ($P = .009$).

Phylogenetic and Clonal Distribution

Of the 7 recognized *E. coli* phylogroups (Figure 1), group B2 dominated, both overall (72%) and within each resistance category (62%–83%) (Table 2). In contrast, each non-B2 phylogroup accounted for ≤9% of isolates overall and ≤11% within any given resistance category. Significant prevalence differences were evident across resistance categories for phylogroup B2 (most prevalent among fluoroquinolone-resistant isolates) and phylogroup F (most prevalent among ESC-RS isolates).

Overall, ST131 was by far the most prevalent identified clonal group (44% overall); it accounted for 61% of all phylogroup B2 isolates and was more prevalent than all non-B2 isolates combined (28%) (Table 2). ST131 was represented mainly by its *H*30 subclone (38% overall prevalence), with its *H*30R1 and *H*30Rx subsets (23% and 15%, respectively), but also by the O25b- and O16-associated non-*H*30 ST131 lineages (3% each; 6% combined). It was broadly distributed by specimen type, differing from non-ST131 isolates only for the proportion from blood versus urine within the fluoroquinolone-resistant category (Table 1).

The relative prevalence of the ST131 clonal subsets varied greatly by resistance category. That is, *H*30Rx was associated mainly with ESC-RS isolates (33% prevalence), and *H*30 and *H*30R1 were associated mainly with fluoroquinolone-resistant

Table 1. Distribution of Isolates by Specimen Type Within Each Resistance Category, Overall and Stratified by ST131-H30 Status

Specimen Type[a] No. (%) n = 358	ESC-RS No. of Isolates (Column %)				FQ-R No. of Isolates (Column %)				FQ-S[c] No. of Isolates (Column %)	
	Total n = 118	Non-H30[b] n = 66	H30[b] n = 52	P Value[d], Non-H30 vs H30	Total n = 120	Non-H30[b] n = 35	H30[b] n = 85	P Value[d], Non-H30 vs H30	Total n = 120	P Value[d], ESC-RS vs FQ-R vs FQ-S
Blood: 155 (45%)	46 (39)	28 (42)	18 (35)	.45	56 (47)	25 (71)	31 (37)	**.001**	53 (44)	.46
Genitourinary: 33 (9%)	9 (8)	7 (11)	2 (4)	.30	12 (10)	0 (0)	12 (14)	**.02**	12 (10)	.83
Respiratory: 82 (23%)	32 (27)	14 (21)	18 (35)	.14	30 (25)	7 (20)	23 (27)	.49	20 (17)	.12
Skin/tissue/abscess: 77 (22%)	28 (24)	15 (23)	13 (25)	.83	18 (15)	2 (6)	16 (19)	.09	31 (26)	.09
Abdominal: 8 (2.2%)	2 (3)	2 (3)	1 (2)	1.0	2 (2)	1 (3)	1 (1)	.50	3 (3)	.91
Other: 3 (0.8%)	0 (0)	0 (0)	0 (0)	n.a.	2 (2)	0 (0)	2 (2)	1.0	1 (1)	.78

Abbreviations: ESC-RS, extended-spectrum cephalosporin-reduced susceptibility; FQ-R, fluoroquinolone-resistant; FQ-S, fluoroquinolone-susceptible.

[a] Specimen type was unknown for two isolates; % values shown are for the 358 isolates with known specimen type.

[b] ST131-H30 (= H30), H30 subclone of *Escherichia coli* sequence type 131. Non-H30, all *E. coli* other than those from the ST131-H30 subclone.

[c] No FQ-S isolates were H30 subclone members.

[d] P values (by Fisher's exact test, two-tailed) are for comparisons between H30 and non-H30 isolates within a given resistance category (ESC-RS or FQ-R), or across the 3 resistance categories. P values are shown in boldface where P < .05.

isolates (71% and 58% prevalence, respectively). In contrast, non-H30 ST131 isolates were the sole ST131 representatives among the fluoroquinolone-susceptible isolates (8% prevalence).

Regarding non-ST131 clonal groups (Figure 1), group B2-derived STc95 and STc73 each accounted for 6% of total isolates, whereas group D-derived STc31 (the O15 clonal group), STc69, and STc405 each accounted for only 2%–3% of total isolates (Table 2). Their distribution also varied greatly by resistance category, with STc95, STc73, and STc69 concentrated significantly among fluoroquinolone-susceptible isolates; STc73 and STc405 prominent among ESC-RS isolates; and STc31 concentrated among fluoroquinolone-resistant isolates.

Prevalence in Source Population

Based on the above findings, the absolute contribution of each ST or clonal subset to the SENTRY source population was estimated by multiplying the particular subset's prevalence within a given resistance category by that resistance category's fractional contribution to the SENTRY source population, then summing the resulting values across the 3 resistance categories [15]. The final overall prevalence estimates were as follows: ST131, 26.4%; ST131-H30, 19.8%; ST131-H30R1, 13.2%; ST131-H30Rx, 6.6%; and non-H30 ST131, 6.6%. ST131-H30 was more prevalent overall than any other phylogenetic group or ST; the next most prevalent STs were ST95 (11.4%) and ST73 (10.6%).

Co-resistance

The prevalence of resistance to the studied antimicrobial agents ranged overall from 1% (imipenem) to 70% (ampicillin-sulbactam) and differed significantly by resistance category (not shown). The ESC-RS isolates exhibited the highest resistance prevalence for all agents, including non-beta-lactams other than ciprofloxacin (100% resistance among the fluoroquinolone-resistant isolates, by definition) (not shown). Accordingly, resistance scores were highest for ESC-RS isolates (median = 4; range = 0–6), intermediate for fluoroquinolone-resistant isolates (median = 2; range = 1–4), and lowest for fluoroquinolone-susceptible isolates (median = 0; range = 0–2) ($P < .001$ for each pairwise comparison, Mann-Whitney test).

Virulence Genes

Of the studied virulence genes, all but 4 were detected in ≥1 isolate each, with overall prevalence values ranging from 1% (*papG* allele 1; variant P adhesin) to 99% (*fimH*) (Table 3). Most virulence genes varied significantly in prevalence by resistance category. That is, fluoroquinolone-susceptible isolates had the highest virulence gene prevalence for the greatest number of genes (n = 22), followed by fluoroquinolone-resistant isolates (n = 7), then ESC-RS isolates (n = 2). Accordingly, virulence scores were significantly higher among fluoroquinolone-susceptible isolates (median = 11; range = 1–20) than among fluoroquinolone-resistant isolates (median = 9; range = 1–15; $P < .001$) or ESC-RS isolates (median = 10; range = 1–17; $P < .001$).

Table 2. Prevalence of Phylogroups and Clonal Groups in Relation to Resistance Category Among 360 *Escherichia coli* Clinical Isolates

Category	Specific trait	Total n = 360	ESC-RS n = 120	FQ-R n = 120	FQ-S n = 120	P Value[b]
		Prevalence[a] of trait, No. (Column %)				
Phylogroup	Group A	22 (6)	10 (8)	3 (3)	9 (8)	.13
	Group B1	22 (6)	8 (7)	3 (3)	11 (9)	.50
	Group B2	258 (72)	74 (62)	**100 (83)**	84 (70)	**.001**
	Group C	6 (2)	5 (4)	0 (0)	1 (1)	.051
	Group D	34 (9)	10 (8)	11 (9)	13 (11)	.85
	Group E	5 (1.4)	3 (3)	1 (1)	1 (1)	.63
	Group F	13 (4)	**10 (8)**	2 (2)	1 (1)	**.006**
Clonal group(s)	STc131 (group B2)	159 (44)	60 (50)	**90 (75)**	9 (8)	**< .001**
	STc131-*H*30	138 (38)	53 (44)	**85 (71)**	0 (0)	**< .001**
	*H*30R1	84 (23)	14 (12)	**70 (58)**	0 (0)	**< .001**
	*H*30Rx	54 (15)	**39 (33)**	15 (13)	0 (0)	**< .001**
	Non-*H*30 STc131	21 (6)	7 (6)	5 (4)	9 (8)	.55
	O25b non-H30	10 (3)	4 (3)	2 (2)	4 (3)	.64
	O16 non-H30	11 (3)	3 (3)	3 (3)	5 (4)	.69
	STc95 (group B2)	21 (6)	0 (0)	1 (1)	**20 (17)**	**< .001**
	STc73 (group B2)	23 (6)	5 (4)	0 (0)	**18 (15)**	**< .001**
	All other group B2 clonal groups	55 (15)	9 (8)	9 (8)	**37 (31)**	**< .001**
	STc31/O15 (group D)	6 (2)	0 (0)	5 (4)	1 (1)	.051
	STc69/CGA (group D)	12 (3)	0 (0)	3 (3)	**9 (8)**	**.003**
	STc405 (group D)	7 (2)	5 (4)	1 (1)	1 (1)	.23
	All other group D clonal groups	9 (3)	5 (5)	2 (2)	2 (2)	.69

Abbreviations: ESC-RS, extended-spectrum cephalosporin-reduced susceptibility (whether or not fluoroquinolone-resistant); FQ-R, fluoroquinolone-resistant and ESC-susceptible; FQ-S, fluoroquinolone-susceptible and ESC-susceptible.

[a]Boldface indicates the resistance category with the highest prevalence value.

[b]*P* values (by Fisher's exact test) are for three-group comparisons: ESC-RS, FQ-R, FQ-S.

Comparison of ST131 and Non-ST131 Isolates

To gain insights into ST131's predominance among ESC-RS and fluoroquinolone-resistant isolates, ST131 and non-ST131 isolates were compared within each resistance category. According to the prevalence of resistance to individual agents (Table 4) and resistance scores (Table 5), ST131 isolates were more extensively resistant than non-ST131 isolates within the ESC-RS and fluoroquinolone-susceptible categories, but not the fluoroquinolone-resistant category. They also had distinctive virulence gene profiles within each resistance category and a higher ExPEC fraction within the ESC-RS and fluoroquinolone-susceptible categories (Table 4). Additionally, they tended to derive from somewhat older patients within the ESC-RS category (median age = 61 years vs 56 years) and fluoroquinolone-resistant category (median age = 64 vs 63 years) but from somewhat younger patients within the fluoroquinolone-susceptible category (median age = 53 vs 56.5 years).

Comparisons Among ST131 Clonal Subsets

The non-*H*30, *H*30R1, and *H*30Rx ST131 clonal subsets also differed significantly from one another for multiple resistance phenotypes and virulence and resistance genes (Table 6) plus host characteristics. Non-*H*30 ST131 isolates had the highest prevalence for ertapenem resistance and 4 virulence genes, *H*30R1 isolates had the highest prevalence for K5 capsule, and *H*30Rx isolates had the highest prevalence for 5 resistance phenotypes,

10 virulence genes, and ExPEC status (Table 6). Accordingly, resistance scores were higher among *H*30R isolates (median = 3; range = 1–5) and *H*30Rx isolates (median = 3; range = 1–6) than among non-*H*30 isolates (median = 2; range = 0–6) (*P* < .001), whereas virulence scores were higher among non-*H*30 isolates (median =10; range = 7–13) and *H*30Rx isolates (median = 10; range = 6–14.75) than among *H*30R isolates (median = 9; range = 6–12) (*P* < .001). Additionally, the source patients for non-*H*30 isolates were more commonly female (71%, vs 41–43%; *P* = .04) and slightly younger (median age = 55 years, vs 58–59 years; *P* = .75) than those for *H*30R1 and *H*30Rx isolates.

Additionally, among non-*H*30 ST131 isolates, the O25b subset (typically *H*22; Figure 1), as compared with the O16 subset (typically *H*41; Figure 1), exhibited a higher prevalence of *pap* genes and *hlyD* (for each, 40% vs 0%; *P* = .04) and of *kpsM* II and ExPEC status (for each, 100% vs 64%; *P* = .04). In contrast, the O16 isolates were more commonly from blood samples (64% vs 20%; *P* = .049) and exhibited a higher prevalence of the K15 (36% vs 0%; *P* = .04) and K2/K100 (64% vs 30%; *P* = .049) capsule variants. Host age and sex did not vary with O25b versus O16 status.

Beta-Lactamase Genes

Of the 120 ESC-RS isolates, 107 (89%) contained ≥1 of the studied beta-lactamase genes, in decreasing prevalence as follows: CTX-M group 1 (49%), CTX-M group 9 (22%), CMYII

Table 3. Prevalence of Virulence Traits in Relation to Resistance Category Among 360 *Escherichia coli* Clinical Isolates

| Category | Specific Trait[a,b,c,d] | Prevalence of Trait[e], No. (Column %) | | | | P Value[f] |
		Total n = 360	ESC-RS n = 120	FQ-R n = 120	FQ-S n = 120	
Adhesins	*papA/C/EF/G*	80 (22)	26 (22)	8 (7)	**46 (38)**	< .001
	papG allele II	66 (18)	22 (18)	8 (7)	**36 (30)**	< .001
	papG allele III	21 (6)	7 (6)	0 (0)	**14 (12)**	< .001
	sfa/focDE	40 (11)	7 (6)	0 (0)	**33 (28)**	< .001
	sfaS	7 (2)	1 (1)	0 (0)	**6 (5)**	.02
	focG	24 (7)	3 (3)	0 (0)	**21 (18)**	< .001
	afa/draBC	25 (7)	**13 (11)**	3 (3)	9 (8)	.03
	iha	204 (57)	67 (56)	**101 (84)**	36 (30)	< .001
	hra	76 (21)	35 (29)	4 (3)	**37 (31)**	< .001
Toxins	*hlyD*	57 (16)	20 (17)	2 (2)	**35 (29)**	.005
	hlyF	20 (6)	5 (4)	3 (3)	**12 (10)**	.04
	cnf1	35 (10)	14 (12)	0 (0)	**21 (18)**	< .001
	cdtB	10 (3)	1 (1)	0 (0)	**9 (8)**	.001
	sat	199 (55)	65 (54)	**101 (84)**	33 (28)	< .001
	pic	17 (5)	4 (3)	0 (0)	**13 (11)**	< .001
	vat	99 (28)	15 (13)	9 (8)	**75 (63)**	< .001
Siderophores	*iroN*	59 (16)	11 (9)	3 (3)	**45 (38)**	< .001
	ireA	30 (8)	5 (4)	1 (1)	**24 (20)**	< .001
	iutA	242 (67)	87 (73)	**107 (89)**	48 (40)	< .001
Protectins	K1 capsule	54 (15)	7 (6)	8 (7)	**39 (33)**	< .001
	K5 capsule	66 (18)	18 (15)	**36 (30)**	12 (10)	< .001
	K2/K100 capsule	48 (13)	**31 (26)**	9 (8)	8 (7)	< .001
	iss	20 (6)	3 (4)	3 (3)	**13 (11)**	.02
Miscellaneous	*usp*	260 (72)	73 (61)	**102 (85)**	85 (71)	< .001
	ibeA	36 (10)	3 (3)	3 (3)	**30 (25)**	< .001
	ompT	280 (78)	82 (68)	**103 (86)**	95 (79)	.005
	H7 *fliC*	20 (6)	1 (1)	0 (0)	**19 (16)**	< .001
	malX	265 (74)	88 (73)	**104 (87)**	73 (61)	< .001
	clbB/N	66 (18)	12 (10)	0 (0)	**54 (45)**	< .001

Abbreviations: ESC-RS, extended-spectrum cephalosporin-reduced susceptibility (whether or not fluoroquinolone-resistant); FQ-R, fluoroquinolone-resistant and ESC-susceptible; FQ-S, fluoroquinolone-susceptible and ESC-susceptible.

[a]Traits shown are those that yielded P < .05 for the 3-group comparison.

[b]Trait definitions: *papA/C/EF/G*, P fimbriae major subunit, assembly, minor subunits, and adhesin (which gave similar results); *papG* alleles II and III, variant P adhesins; *sfa/focDE*, S and F1C fimbriae; *sfaS*, S fimbriae adhesin; *focG*, F1C fimbriae adhesin; *afa/draBC*, Dr-binding adhesins; *iha*, adhesin-siderophore receptor; *hra*, heat-resistant agglutinin; *hlyD*, alpha hemolysin; *hlyF*, variant hemolysin; *cnf1*, cytotoxic necrotizing factor 1; *cdtB* cytolethal distending toxin; *sat*, secreted autotransporter toxin; *pic*, protein associated with intestinal colonization; *vat*, vacuolating toxin; *iroN*, salmochelin receptor; *ireA*, catecholate siderophore receptor; *iutA*, aerobactin receptor; K2, K5, K2/K100, group 2 capsule variants; *iss*, increased serum survival; *usp*, uropathogenic-specific protein; *ibeA*, invasion of brain endothelium A; *ompT*, outer membrane protease; H7 *fliC*, flagellar variant; *malX*, pathogenicity island marker; *clb/N*, colibactin (polyketide) synthesis.

[c]Traits detected in ≥1 isolate but not significantly associated with resistance category (definition, % of 360 with trait): *papG* allele 1 (variant P adhesin, 1%), *gafD* (G fimbriae, 1%), *fimH* (type-1 fimbriae adhesin, 99%), *tsh* (temperature-sensitive hemagglutinin, 2%), *astA* (enteroaggregative *E. coli* toxin, 3%), *fyuA* (yersiniabactin receptor, 88%), *kpsM* II (group 2 capsules, 69%), K15 (variant group 2 capsule, 1%), *kpsMT* III (group 3 capsules, 3%), *rfc* (O4 lipopolysaccharide, 2%), *traT* (serum-resistance associated, 74%), *cvaC* (microcin V, 4%), ExPEC (extraintestinal pathogenic *E. coli*, 64%).

[d]Traits sought but not detected in any isolate: *afaE8, clpG,* F17, *bmaE* (variant mannose-resistant adhesins).

[e]Boldface indicates the resistance category with the highest prevalence value.

[f]P values (by Fisher's exact test) are for 3-group comparisons: ESC-RS, FQ-R, FQ-S.

(14%), KPC (2.5%), and SHV and TEM (1.7% each). CTX-M group 1 was significantly associated with ST131-*H30* (66% [*H30*] vs 36% [others]; P = .002), whereas CMYII was significantly associated with non-*H30* isolates (22% vs 4%; P = .004). Within *H30*, CTX-M group 1 was associated with *H30*Rx (90% vs 0%; P < .001) and CTX-M group 9 with *H30*R1 (86% vs 3%; P < .001). Selective sequence analysis showed that CTX-M group 1 was represented by *bla*$_{CTX-M-15}$ (24/24, 100%), CTX-M group 9 mainly by *bla*$_{CTX-M-14}$ (7/12, 58%) and *bla*$_{CTX-M-27}$ (4/12, 33%), and CMY II by *cmy2* (9/9, 100%).

Overall Contribution of ST131 to Resistance

Back-calculations similar to those described for overall prevalence estimates were used to estimate ST131's net contribution to resistance within the SENTRY source population (Table 7) [15]. ST131 was estimated to account for 65%–71% of gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, and multidrug resistance; 52%–55% of ESC and aztreonam resistance; 41%–44% of ampicillin-sulbactam, piperacillin-tazobactam, and tetracycline resistance; and 28%–33% of carbapenem resistance.

Table 4. Prevalence of Resistance Phenotypes and Virulence Traits in Relation to Sequence Type 131 (ST131) Status Within Each Resistance Category Among 360 *Escherichia coli* Clinical Isolates

Category	Specific Trait[a]	ESC-RS: Trait prevalence, No. (Column %)[b]			FQ-R: Trait Prevalence, No. (Column %)[b]			FQ-S: Trait Prevalence, No. (Column %)[b]		
		Non-ST131 n = 60	ST131 n = 60	P Value[c]	Non-ST131 n = 30	ST131 n = 90	P Value[c]	Non-ST131 n = 111	ST131 n = 9	P Value[c]
Resistance	Amp-sulbactam	**59 (98)**	53 (88)	.03	22 (73)	74 (82)	.29	35 (32)	**8 (89)**	.001
	Cefotaxime	52 (87)	**58 (97)**	.048	1 (3)	1 (1)	.44	0 (0)	0 (0)	NA
	Ceftriaxone	47 (78)	**57 (95)**	.007	1 (3)	0 (0)	.08	0 (0)	0 (0)	NA
	Gentamicin	16 (27)	23 (38)	.17	7 (23)	22 (24)	.90	2 (2)	**4 (44)**	< .001
	Ciprofloxacin	35 (58)	**57 (95)**	< .001	30 (100)	90 (100)	NA	0 (0)	0 (0)	NA
	MDR (≥ 3 classes)	38 (63)	**55 (92)**	< .001	12 (40)	35 (39)	.54	0 (0)	0 (0)	NA
Adhesins	*papAH/C/EG/G*	9 (15)	17 (28)	.06	**7 (23)**	1 (1)	< .001	45 (41)	1 (11)	.08
	papG allele II	5 (8)	**17 (28)**	.004	**7 (23)**	1 (1)	< .001	35 (32)	1 (11)	.28
	papG allele III	**7 (12)**	0 (0)	.006	0 (0)	0 (0)	NA	14 (13)	0 (0)	.31
	sfa/focDE	**7 (12)**	0 (0)	.006	0 (0)	0 (0)	NA	32 (29)	1 (11)	.23
	afa/draBC	1 (2)	**12 (20)**	.001	2 (6)	1 (1)	.15	5 (5)	**4 (44)**	.002
	iha	10 (17)	**57 (95)**	< .001	15 (50)	**86 (96)**	< .001	29 (26)	**7 (78)**	.003
Toxins	*hlyF*	5 (8)	0 (0)	.06	3 (10)	0 (0)	**.01**	12 (11)	0 (0)	.60
	sat	9 (15)	**56 (93)**	< .001	16 (53)	**85 (94)**	< .001	27 (24)	**6 (67)**	.01
	vat	**13 (21)**	2 (3)	.004	**9 (30)**	0 (0)	< .001	75 (68)	0 (0)	< .001
Siderophores	*iroN*	**11 (18)**	0 (0)	.001	**3 (10)**	0 (0)	.01	44 (40)	1 (11)	.15
	fyuA	41 (68)	**60 (100)**	< .001	21 (70)	**90 (100)**	< .001	95 (86)	9 (100)	.60
	iutA	30 (50)	**57 (95)**	< .001	21 (70)	**86 (96)**	< .001	41 (37)	**7 (78)**	.03
Protectins	*kpsM* II	25 (42)	**57 (95)**	< .001	22 (73)	57 (63)	.38	79 (72)	9 (100)	.11
	kpsMT III	**7 (12)**	0 (0)	.01	1 (3)	0 (0)	.25	4 (4)	0 (0)	1.0
	K1	**7 (12)**	0 (0)	.01	**8 (27)**	0 (0)	< .001	39 (35)	0 (0)	.03
	K5	2 (3)	**16 (27)**	.001	0 (0)	**36 (40)**	< .001	10 (9)	2 (22)	.22
	K2/K100	4 (7)	**27 (45)**	< .001	4 (13)	5 (6)	.23	3 (3)	**5 (56)**	< .001
	iss	4 (7)	0 (0)	.12	**3 (10)**	0 (0)	.01	13 (12)	0 (0)	.60
	traT	44 (73)	52 (87)	.11	9 (30)	**76 (84)**	< .001	78 (70)	9 (100)	.06
Miscellaneous	*usp*	13 (22)	**60 (100)**	< .001	12 (40)	**90 (100)**	< .001	76 (69)	9 (100)	.06
	ibeA	3 (5)	0 (0)	.24	**3 (10)**	0 (0)	.01	27 (24)	3 (33)	.69
	ompT	24 (40)	**58 (97)**	< .001	16 (53)	**87 (97)**	< .001	86 (77)	9 (100)	.20
	malX	28 (53)	**60 (100)**	< .001	14 (47)	**90 (100)**	< .001	64 (58)	**9 (100)**	.01
	clbB/N	**12 (20)**	0 (0)	< .001	0 (0)	0 (0)	NA	**66 (33)**	0 (0)	.004
	ExPEC	23 (38)	**56 (93)**	< .001	18 (60)	57 (63)	.83	68 (61)	**9 (100)**	.03

Abbreviations: Amp, ampicillin; ESC-RS, extended-spectrum cephalosporin-reduced susceptibility (whether or not fluoroquinolone-resistant); ExPEC, extraintestinal pathogenic *Escherichia coli*; FQ-R, fluoroquinolone-resistant and ESC-susceptible; FQ-S, fluoroquinolone-susceptible and ESC-susceptible; MDR, multidrug-resistant; NA, not applicable (prevalence 0% in both comparison groups)

[a] Traits shown are those that yielded *P* < .05 for at least 1 comparison (ST131 vs non-ST131). Trait definitions are as shown in Table 3.

[b] Boldface indicates comparison group with higher resistance prevalence.

[c] *P* values (by "N – 1" χ^2 test) are show in boldface where *P* < .05.

Table 5. Resistance Score and Virulence Score in Relation to Sequence Type 131 (ST131) Status Within Each Resistance Category Among 360 *Escherichia coli* Clinical Isolates

Variable	Resistance Category	Non-ST131		ST131		P Value[b]
		No. of Isolates	Median Score (Range)	No. of Isolates	Median score[a] (Range)	
Resistance score	ESC-RS	60	3 (0–6)	60	**4 (2–6)**	.02
	FQ-R	30	2 (1–4)	90	2 (1–4)	.85
	FQ-S	111	0 (0–2)	9	**1 (0–2)**	.003
Virulence score	ESC-RS	60	6 (1–17)	60	**10 (6–14.75)**	< .001
	FQ-R	30	8 (1–15)	90	**9 (6–12)**	< .001
	FQ-S	111	11 (1–20)	6	11 (9–13)	.95

Abbreviations: ESC-RS, extended-spectrum cephalosporin-reduced susceptibility (whether or not fluoroquinolone-resistant); FQ-R, fluoroquinolone-resistant and ESC-susceptible; FQ-S, fluoroquinolone-susceptible and ESC-susceptible.

[a] Boldface indicates results for the comparison group with a significantly higher score.

[b] *P* values (by Mann-Whitney test, 2-tailed) are in boldface where *P* < .05.

Table 6. Prevalence of Resistance Phenotypes and Virulence Traits in Relation to Clonal Subset Among 159 *Escherichia coli* Sequence Type 131 (ST131) Clinical Isolates

Category	Specific Trait[a]	Prevalence[b] of Trait, No. (Column %)				P Value[c]
		Total ST131 n = 159	non-*H*30 n = 21	*H*30R1 n = 84	*H*30Rx n = 53	
Resistance	Piperacillin-tazobactam	26 (16)	1 (5)	11 (13)	**14 (26)**	.05
	Cefotaxime	59 (37)	3 (33)	12 (14)	**40 (74)**	< .001
	Ceftazidime	39 (25)	6 (29)	5 (6)	**28 (52)**	< .001
	Ceftriaxone	57 (36)	7 (33)	12 (14)	**38 (70)**	< .001
	Aztreonam	50 (31)	3 (33)	9 (11)	**34 (63)**	< .001
	Ertapenem	4 (3)	**2 (10)**	0 (0)	2 (4)	.02
	Ciprofloxacin	147 (93)	9 (43)	**84 (100)**	54 (100)	< .001
	MDR (≥3 classes)	90 (57)	9 (43)	43 (51)	**38 (70)**	.03
Adhesins	*papA/HC/EF/G*	19 (12)	4 (19)	1 (1)	**14 (26)**	< .001
	papG allele II	19 (12)	4 (19)	1 (1)	**14 (26)**	< .001
	afa/draBC	17 (11)	**5 (24)**	0 (0)	12 (22)	< .001
	hra	18 (11)	1 (5)	1 (1)	**16 (30)**	< .001
	hlyD	13 (8)	4 (19)	1 (1)	**8 (15)**	< .001
	cnf1	8 (5)	1 (5)	1 (1)	**6 (11)**	.03
	sat	147 (93)	16 (76)	79 (94)	**52 (96)**	.02
	iutA	150 (94)	17 (81)	80 (95)	**53 (98)**	.03
	kpsM II	17 (77)	17 (81)	53 (63)	**53 (98)**	< .001
	K2/K100	37 (23)	**10 (48)**	1 (1.2)	**26 (48)**	< .001
	K5	54 (34)	2 (10)	**44 (52)**	8 (15)	< .001
	K15	4 (3)	**4 (19)**	0 (0)	0 (0)	< .001
	ibeA	3 (2)	**3 (14)**	0 (0)	0 (0)	.002
	malX	159 (100)	21 (100)	84 (100)	53 (100)	.001
	ExPEC	122 (77)	17 (81)	52 (62)	**53 (98)**	< .001

Abbreviations: ExPEC, extraintestinal pathogenic *Escherichia coli*; MDR, multidrug resistant.

[a]Traits shown are those that yielded *P* < .05 for the 3-group comparison (non-*H*30, *H*30R1, *H*30Rx). Trait definitions are as shown in Table 3.

[b]Boldface indicates the resistance category with the highest prevalence value.

[c]*P* values (by Fisher's exact test) are for the 3-group comparison.

Table 7. Estimated Overall Contribution of Sequence Type 131 (ST131) to Antimicrobial Resistance Within the SENTRY Collection

Resistance Phenotype	Overall Prevalence, %[a]	ST131 fraction, %[b]
Ampicillin-sulbactam	51.9	43.2
Piperacillin-tazobactam	8.4	42.0
Cefotaxime	12.0	52.7
Ceftazidime	7.8	52.7
Ceftriaxone	11.2	54.0
Aztreonam	9.6	54.9
Ertapenem	1.5	28.6
Imipenem	3.2	33.3
Gentamicin	12.3	67.7
Tetracycline	31.4	43.4
Ciprofloxacin	29.7	70.7
Trimethoprim-sulfamethoxazole	22.5	64.9
Multidrug resistance (≥ 3 classes)	17.7	65.9

[a]Overall resistance prevalence was imputed for each agent by multiplying the prevalence of resistance to that agent within a given resistance category by the fractional contribution of that resistance category to the total population, then summing these values across the 3 resistance categories.

[b]ST131's fractional contribution to overall resistance prevalence was imputed for each agent by calculating, for each resistance category, the net contribution of ST131 to resistance within that resistance category, then adjusting for the fractional contribution of that resistance category to the total population, and summing these values across the three resistance categories.

DISCUSSION

Here we assessed 360 diverse-source clinical *E. coli* isolates, as collected systematically from non-VA hospitals across the United States in 2011–2012, for phylogroup, clonal background, virulence genes, and beta-lactamase genes and compared these results with existing data for resistance phenotype, specimen type, and host age. This analysis yielded 3 main findings, with potentially important implications. First, ST131, and specifically its dominant *H*30R subclone, not only was the most prevalent strain overall but accounted for ≥50% of ESC-RS, fluoroquinolone-resistant, and MDR isolates, confirming its status as the main driver in the United States of the current epidemic of resistant *E. coli*. Second, the various ST131 clonal subsets exhibited distinctive associations with resistance phenotypes, virulence genotypes, and host subsets, confirming the importance of considering them individually rather than lumping them together as simply ST131. Third, ST131 isolates differed, or failed to differ, from relevant comparator non-ST131 isolates in ways that may explain their high prevalence among resistant isolates and low prevalence among susceptible isolates and that offer potential targets for interventions.

To our knowledge, this is the first study to screen for ST131 and its main clonal subsets—including *H*30R1, *H*30Rx, and non-*H*30 strains (divided as O16 vs O25b)—within a nationally representative reference set of systematically collected *E. coli* clinical isolates. Previous studies, including several to which the authors contributed, screened for ST131 subclones, but within more geographically, epidemiologically, and/or microbiologically restricted populations [14, 15, 18, 21, 32–34]—or examined broadly representative isolates, but without in-depth attention to key ST131 clonal subsets, or to recent (ie, post-2010) isolates [15, 35]. The present findings extend the results of these previous studies to the US population generally. In so doing, they confirm that ST131-*H*30R is the leading antimicrobial-resistant *E. coli* strain in the United States today, that its *H*30R1 subset is the leading cause of ESC-susceptible, fluoroquinolone-resistant *E. coli* infections, and that its *H*30Rx subset is the leading cause of ESC-RS *E. coli* infections, especially those involving co-resistance to fluoroquinolones, which has important implications for empirical therapy [3]. Likewise, they show that non-*H*30 ST131 strains, although minor contributors to each resistance category, are more extensively resistant than other fluoroquinolone-susceptible isolates and have distinctive host associations (eg, younger females).

The 26.4% absolute prevalence estimate for ST131 can be compared with similar estimates from previous national surveys, including of 2007 SENTRY isolates (17% ST131) [13] and 2011 VA isolates (28% ST131) [15]. These comparisons suggest that ST131's prevalence in the United States increased relatively by >50% from 2007 to 2011–2012 and that the prevalence difference between 2007 SENTRY isolates and 2011 VA isolates reflected this temporal emergence rather than SENTRY vs VA host population differences. The fractional contribution of ST131 (mainly its ST131-*H*30R subclone) to different resistance phenotypes (eg, Table 7) was similar here and among the (contemporaneous) 2011 VA isolates [15] but generally higher in both these studies than among the 2007 SENTRY isolates [13], consistent with clonal expansion of *H*30R between 2007 and 2011–2012.

The present findings support the potential clinical utility of rapid diagnostic tests that can identify ST131 and its key clonal subsets. Such tests are under development that can return results in <1 hour, thereby potentially allowing their use for empirical antibiotic selection, even at the point of care [36]. Guidance from such tests conceivably could protect against both "bug–drug" mismatch (from unanticipated resistance) and excessively broad empirical therapy, thereby providing the double benefit of improved clinical outcomes and enhanced antimicrobial stewardship [37].

The basis for the ST131-*H*30R subclone's impressive epidemiological success, which contrasts with the limited success of non-*H*30 ST131 strains, remains speculative. We found that,

among ESC-RS isolates, ST131 strains (mainly *H*30Rx) have multiple advantages over non-ST131 strains, including more extensive resistance, more extensive virulence gene profiles, a greater ExPEC fraction, and a large disparity for group B2 background (100% vs 23%). Likewise, among fluoroquinolone-resistant isolates, although ST131 strains (mainly *H*30R1) have similarly extensive resistance profiles and a similar ExPEC fraction as non-ST131 strains, they have more extensive virulence profiles and a large disparity for group B2 background (100% vs 33%). In contrast, among fluoroquinolone-susceptible isolates, ST131 strains (all non-*H*30; equally O16 vs O25b) have only minor advantages over non-ST131 strains for group B2 background (100% vs 67%), extent of resistance, and ExPEC fraction and have comparably extensive virulence profiles. Thus, whereas, among ESC-RS and fluoroquinolone-resistant strains, ST131 strains (mainly *H*30Rx and *H*30R1) have multiple factors favoring their success over non-ST131 strains, among fluoroquinolone-susceptible strains, competition with other group B2 strains may have limited the non-*H*30 ST131 strains' epidemiologic success.

Conserved accessory traits across the ST131 clonal subsets conceivably could provide useful targets for preventive or therapeutic interventions. Here, as in previous studies (reviewed in [4]), high-prevalence ST131-associated virulence genes included specific adhesins (*fimH*, *iha*), toxins (*sat*), siderophores (*fyuA*, *iutA*), group 2 capsule variants (*kpsM* II and others), and miscellaneous other traits (*ompT*, *traT*, *malX*). The UTI-preventing efficacy in mice of a vaccine based on the siderophores aerobactin and yersiniabactin, as documented recently using a non-ST131 group B2 challenge strain [38], suggests that such an approach might be effective also against ST131 strains, which typically produce both factors. Likewise, the nearly universal presence of the O25b *rfb* variant among ESC-RS and/or fluoroquinolone-resistant ST131 strains suggests that a recently described O25b-specific bacteriophage [39], or O25b protein conjugate vaccine [40], could protect broadly against such concerning ST131 variants. Such interventions should retain their effectiveness against even those O25b-positive ST131 variants that acquire new resistance mechanisms, including against carbapenems [41] and polymyxins [42].

The study has limitations. Clinical and epidemiologic data were largely lacking, precluding assessments of predisposing host factors, clinical presentation, management, and outcomes. The virulence and resistance genotyping was not comprehensive and did not address gene expression, copy number, or molecular variants. Some subgroups were small, limiting statistical power. Finally, the use of multiple comparisons risked finding putative differences by chance alone.

The study also has important strengths. These include the large, systematically assembled, broadly representative, post-2010 study population; the high-resolution clonal subtyping within ST131; attention to key non-ST131 clonal groups; and

analysis of extended resistance profiles and virulence and resistance genotypes in relation to clonal background.

In summary, by analyzing a broadly representative panel of *E. coli* clinical isolates from non-VA hospitals across the United States (2011–2012), we documented that the ST131-*H*30R lineage, with its *H*30R1 and *H*30Rx subclones, was the leading cause of *E. coli* infections overall and was especially prominent among ESC-RS and fluoroquinolone-resistant isolates, having expanded by >50% since 2007. Focused attention to these ST131 clonal subsets, including with surveillance, diagnostic tests, treatment algorithms, and preventive measures, should help in confronting the ever-growing challenge of MDR *E. coli* infections.

References

1. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect. **2003**;5:449–56.

2. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. **2011**;52:e103–e20.

3. Talan DA, Takhar SS, Krishnadasan A, et al. Fluoroquinolone-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* infections in patients with pyelonephritis, United States. Emerg Inf Dis. **2016**;22:1594–603.

4. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev. **2014**;27:543–74.

5. Price LB, Johnson JR, Aziz M, et al. The epidemic of extended-spectrum-β-lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30-Rx. MBio. **2013**;4:e00377–13.

6. Johnson JR, Tchesnokova V, Johnston B, et al. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. J Infect Dis. **2013**;207:919–28.

7. Peirano G, van der Bij AK, Freeman JL, et al. Characteristics of *Escherichia coli* sequence type 131 isolates that produce extended-spectrum β-lactamases: global distribution of the H30-Rx sublineage. Antimicrob Agents Chemother. **2014**;58:3762–7.

8. Stoesser N, Sheppard AE, Pankhurst L, et al; Modernizing Medical Microbiology Informatics Group (MMMIG). Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. MBio. **2016**;7:e02162.

9. Johnson JR, Davis G, Clabots C, et al. Household clustering of *Escherichia coli* sequence type 131 clinical and fecal isolates according to whole genome sequence analysis. Open Forum Infect Dis. **2016**;3:ofw129.

10. Petty NK, Ben Zakour NL, Stanton-Cook M, et al. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A. **2014**;111:5694–9.

11. Matsumura Y, Johnson JR, Yamamoto M, et al; Kyoto–Shiga Clinical Microbiology Study Group; Kyoto-Shiga Clinical Microbiology Study Group. CTX-M-27- and CTX-M-14-producing, ciprofloxacin-resistant *Escherichia coli* of the H30 subclonal group within ST131 drive a Japanese regional ESBL epidemic. J Antimicrob Chemother. **2015**;70:1639–49.

12. Johnson JR, Clermont O, Johnston B, et al. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within *Escherichia coli* sequence type 131. J Clin Microbiol. **2014**;52:1358–65.

13. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States (2007). Clin Infect Dis. **2010**;51:286–94.

14. Johnson JR, Urban C, Weissman SJ, et al. Molecular epidemiology of *Escherichia coli* sequence type ST131 (O25:H4) and *bla*CTX-M-15 among extended-spectrum cephalosporinase-producing *E. coli* in the United States (2000–2009). Antimicrob Agents Chemother. **2012**;56:2364–70.

15. Colpan A, Johnston B, Porter S, et al; VICTORY (Veterans Influence of Clonal Types on Resistance: Year 2011) Investigators. *Escherichia coli* sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among US veterans. Clin Infect Dis. **2013**;57:1256–65.

16. Clinical and Laboratory Standards Institute. M100-S23: Performance standards for antimicrobial susceptibility testing. 23rd informational supplement document. Wayne, PA: CLSI; **2013**.

17. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard–ninth edition (M07-A9). Wayne, PA: CLSI, **2012**.

18. Johnson JR, Johnston B, Thuras P, Launer B, Sokurenko EV, Miller L. *Escherichia coli* sequence type 131 *H*30 is the main driver of emerging extended-spectrum-β-lactamase-producing *E. coli* at a tertiary care center. mSphere. **2016**;1:e00314–16.

19. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. **2013**;5:58–65.

20. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002–2004. Antimicrob Agents Chemother. **2009**;53:2733–9.

21. Banerjee R, Strahilevitz J, Johnson JR, et al. Predictors and molecular epidemiology of community-onset extended-spectrum β-lactamase-producing *Escherichia coli* infection in a Midwestern community. Infect Control Hosp Epidemiol. **2013**;34:947–53.

22. Clermont O, Christenson JK, Daubié AS, et al. Development of an allele-specific PCR for *Escherichia coli* B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. J Microbiol Methods. **2014**;101:24–7.

23. Johnson JR, Owens K, Sabate M, Prats G. Rapid and specific detection of the O15:K52:H1 clonal group of *Escherichia coli* by gene-specific PCR. J Clin Microbiol. **2004**;42:3841–3.

24. Johnson JR, Owens K, Manges AR, Riley LW. Rapid and specific detection of *Escherichia coli* clonal group A by gene-specific PCR. J Clin Microbiol. **2004**;42:2618–22.

25. Matsumura Y, Yamamoto M, Nagao M, et al; Kyoto-Shiga Clinical Microbiology Study Group. Emergence and spread of B2-ST131-O25b, B2-ST131-O16 and D-ST405 clonal groups among extended-spectrum-β-lactamase-producing *Escherichia coli* in Japan. J Antimicrob Chemother. **2012**;67:2612–20.

26. Johnson JR, Porter S, Johnston B, et al. Host characteristics and bacterial traits predict experimental virulence for *Escherichia coli* bloodstream isolates from patients with urosepsis. Open Forum Infect Dis. **2015**;2:ofv083.

27. Johnson JR, Owens K, Gajewski A, Kuskowski MA. Bacterial characteristics in relation to clinical source of *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women. J Clin Microbiol. **2005**;43:6064–72.

28. Johnson JR, Porter SB, Zhanel G, et al. Virulence of *Escherichia coli* clinical isolates in a murine sepsis model in relation to sequence type ST131 status, fluoroquinolone resistance, and virulence genotype. Infect Immun. **2012**;80:1554–62.

29. Johnson JR, Murray AC, Gajewski A, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother. **2003**;47:2161–8.

30. Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN. Prevalence of beta-lactamase-encoding genes among Enterobacteriaceae bacteremia isolates collected in 26 U.S. hospitals: report from the SENTRY Antimicrobial Surveillance Program (2010). Antimicrob Agents Chemother. **2013**;57:3012–20.

31. Campbell I. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. Stat Med. **2007**;26:3661–75.

32. Banerjee R, Robicsek A, Kuskowski M, et al. Molecular epidemiology of *Escherichia coli* sequence type ST131 and its H30 and H30-Rx subclones among extended-spectrum beta-lactamase-positive and -negative *E. coli* clinical isolates from the Chicago region (2007–2010). Antimicrob Agents Chemother. **2013**;57:6385–8.

33. Drawz SM, Porter S, Kuskowski MA, et al. Variation in resistance traits, phylogenetic backgrounds, and virulence genotypes among *Escherichia coli* clinical isolates from adjacent hospital campuses serving distinct patient populations. Antimicrob Agents Chemother. **2015**;59:5331–9.

34. Salipante SJ, Roach DJ, Kitzman JO, et al. Large-scale genomic sequencing of extraintestinal pathogenic *Escherichia coli* strains. Genome Res. **2015**;25:119–28.

35. Day MJ, Doumith M, Abernethy J, et al. Population structure of *Escherichia coli* causing bacteraemia in the UK and Ireland between 2001 and 2010. J Antimicrob Chemother. **2016**;71:2139–42.

36. Tchesnokova VL, Avagyan H, Rechkina E, et al. Bacterial clonal diagnostics as a tool for evidence-based empiric antibiotic selection. PLoS One. **2017**;12:e0174132.

37. Johnson JR, Thuras P, Johnston BD, et al. The pandemic H30 subclone of *Escherichia coli* sequence type 131 is associated with persistent infections and adverse outcomes independent from its multidrug resistance and associations with compromised hosts. Clin Infect Dis. **2016**;62:1529–36.

38. Mike LA, Smith SN, Sumner CA, et al. Siderophore vaccine conjugates protect against uropathogenic *Escherichia coli* urinary tract infection. Proc Natl Acad Sci U S A. **2016**;113:13468–73.

39. Dufour N, Clermont O, La Combe B, et al. Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b:H4 *Escherichia coli* clonal complex. J Antimicrob Chemother. **2016**;71:3072–80.

40. van den Dobbelsteen GP, Faé KC, Serroyen J, et al. Immunogenicity and safety of a tetravalent *E. coli* O-antigen bioconjugate vaccine in animal models. Vaccine. **2016**;34:4152–60.

41. Peirano G, Bradford PA, Kazmierczak KM, et al. Global incidence of carbapenemase-producing *Escherichia coli* ST131. Emerg Infect Dis. **2014**;20:1928–31.

42. Ewers C, Göttig S, Bülte M, et al. Genome sequence of avian *Escherichia coli* strain IHIT25637, an extraintestinal pathogenic *E. coli* strain of ST131 encoding colistin resistance determinant MCR-1. Genome Announc. **2016**;4:e00863–16.