Variation in Resistance Traits, Phylogenetic Backgrounds, and Virulence Genotypes among *Escherichia coli* Clinical Isolates from Adjacent Hospital Campuses Serving Distinct Patient Populations

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Escherichia coli sequence type 13 (ST131), an emergent cause of multidrug-resistant extraintestinal infections, has important phylogenetic subsets, notably the H30 and H30Rx subclones, with distinctive resistance profiles and, possibly, clinical associations. To clarify the local prevalence of these ST131 subclones and their associations with antimicrobial resistance, ecological source, and virulence traits, we extensively characterized 233 consecutive *E. coli* clinical isolates (July and August 2013) from the University of Minnesota Medical Center-Fairview Infectious Diseases and Diagnostic Laboratory, Minneapolis, MN, which serves three adjacent facilities (a children's hospital and low- and high-acuity adult facilities). ST131 accounted for 26% of the study isolates (more than any other clonal group), was distributed similarly by facility, and was closely associated with ciprofloxacin resistance and extended-spectrum β -lactamase (ESBL) production. The H30 and H30Rx subclones accounted for most ST131 isolates and for the association of ST131 with fluoroquinolone resistance and ESBL production. Unlike ST131 *per se*, these subclones were distributed differentially by hospital, being most prevalent at the high-acuity adult facility and were absent from the children's hospital. The virulence gene profiles of ST131 and its subclones were distinctive and more extensive than those of other fluoroquinolone-resistant or ESBL-producing isolates. Within ST131, *bla*_{CTX-M-15} was confined to H30Rx isolates and other *bla*_{CTX-M} variants to non-Rx H30 isolates. Pulsed-field gel electrophoresis documented a predominance of globally distributed pulsotypes and no local outbreak pattern. These findings help clarify the epidemiology, ecology, and bacterial correlates of the H30 and H30Rx ST131 subclones by documenting a high overall prevalence but significant segregation by facility, strong associations with fluoroquinolone resistance and specific ESBL variants, and distinctive virulence gene associations that may confer fitness advantages over other resistant *E. coli*.

Escherichia coli sequence type 131 (ST131) has emerged globally over the past decade as the main cause of extraintestinal *E. coli* infections (1). Treatment is challenging due to characteristic resistance to fluoroquinolones and, increasingly, third-generation cephalosporins, which is mediated by extended-spectrum β -lactamases (ESBLs), primarily CTX-M-15 (2–4). ST131 belongs to *E. coli* phylogenetic group B2 and is most often closely associated with urinary tract infections but can infect any extraintestinal site (1, 2, 5). Recent reports of carbapenemase-producing ST131 isolates add urgency to this growing problem (6, 7).

The emergence of fluoroquinolone-resistant ST131 isolates has been due almost entirely to the ST131-H30 subclone, which carries the H30 variant of the type 1 fimbrial adhesin gene *fimH* (8). Extraintestinal *E. coli* infections in young children, the elderly, and long-term care facility residents are disproportionately due to ST131, with >85% of such isolates representing the ST131-H30 subclone (9). Recent whole-genome phylogenetic analyses confirmed that most CTX-M-producing ST131 isolates represent the H30Rx subset within the H30 subclone (10, 11), supporting that the ST131 epidemic has emerged via the expansion of nested subclones with successively broader resistance capabilities (12, 13). Although the H30Rx subclone appears to comprise a large fraction of CTX-M-producing *E. coli* isolates in North American populations (14, 15), the ecological and host associations of the H30Rx subclone have only begun to be explored.

The dramatic emergence of the H30 and H30Rx subclones remains unexplained. The signature resistance phenotypes of these subclones are well defined (8, 10, 14), but whether or how viru-

lence also might have contributed to clonal expansion remains uncertain. The current prominence of these subclones as extraintestinal pathogens might suggest that they contain more virulence genes than non-ST131 *E. coli* or qualify more often as extraintestinal pathogenic *E. coli* (ExPEC). However, the available evidence supports these hypotheses only in comparison with other resistant *E. coli* (2, 16, 17).

Accordingly, we addressed three primary study questions. First, we sought to determine the prevalence of ST131 and its H30 and H30Rx subclones in comparison to that of the major *E. coli* phylogroups and classic ExPEC lineages among consecutive *E. coli* isolates from three closely located hospital campuses in Minneapolis, MN, which serve highly distinct patient populations and share a clinical microbiology laboratory. Second, we compared the ST131 subclones with other *E. coli* according to virulence gene

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carriage and resistance phenotypes and genotypes. Third, we sought to determine to what extent the local ST131, *H30*, and *H30Rx* populations are distinct from, versus part of, the global ST131 expansion and whether they represent a local clonal outbreak versus diverse strains.

MATERIALS AND METHODS

Setting and isolates. The University of Minnesota (UM) Medical Center-Fairview Infectious Diseases and Diagnostic Laboratory (UMMC IDDL) processes specimens from the medical center's East Bank and West Bank campuses and the UM Masonic Children's Hospital, which are closely colocated in central Minneapolis, MN. Each facility has an emergency department and intensive care units. The West Bank campus houses the orthopedic, behavioral health, long-term and acute rehabilitation, and obstetrical services. The East Bank campus serves primarily patients with more acute and complicated diagnoses and includes active stem cell transplant, solid organ transplant, and oncology services.

During the 7-week study period (July and August 2013), all clinical *E. coli* isolates from the IDDL were collected, deidentified, and submitted to the research laboratory. Collection date, specimen type, origin (facility, outpatient/inpatient status), ciprofloxacin phenotype, and ESBL status were recorded. The ciprofloxacin and ESBL phenotypes were determined by a Vitek 2 instrument (bioMérieux, Inc, Durham, NC), according to the manufacturer's instructions. Isolates with a ciprofloxacin MIC of ≥ 4 $\mu\text{g/ml}$ were considered resistant.

Molecular methods. Isolates were characterized molecularly using previously described methods (16). Major *E. coli* phylogenetic group, i.e., A, B1, B2, C (formerly typed as A), D, E (formerly typed as D), and F (also formerly typed as D) was determined by the revised multiplex PCR-based approach of Clermont et al. (18, 19). ST131 genotype was determined by PCR-based detection of ST131-specific single-nucleotide polymorphisms (SNPs) in *gyrB* and *mdh* (20). ST131 isolates were further tested for membership in the *H30* and *H30Rx* ST131 subclones by subclone-specific SNP-based PCR (10). Additional lineage-specific PCR assays were used to detect other prominent ExPEC clonal lineages, including ST95 and ST73 (group B2) and ST69, clonal complex 31 (CC31), and ST405 (group D) (20–23). Additionally, a dual-locus sequence typing system based on *fumC* and *fimH* (CH typing) was used to presumptively define the ST complex of origin for 10 randomly selected group B2 isolates that were not from ST131, ST73, or ST95 (24).

The presence of 50 ExPEC-associated virulence genes (VGs) was assessed by multiplex PCR. A virulence score was the total number of virulence genes detected, adjusted for the multiple detection of *pap* (P fimbriae), *sfa/foc* (S and F1C fimbriae, respectively), and *kps* II (group 2 capsule) operons. Isolates were classified as ExPEC if positive for ≥ 2 of the following: *papAH* and/or *papC* (P fimbriae), *sfa/focDE*, *afa/draBC* (Dr family adhesins), *iutA* (aerobactin receptor), and *kpsM* II (25).

CTX-M-type β -lactamase genes were identified with two sets of previously described primers, one for the generic detection of all *bla*_{CTX-M} variants, and the second specific for *bla*_{CTX-M-15} (26, 27).

Pulsed-field gel electrophoresis analysis. For a more discriminating analysis of the genetic similarities of the ST131 isolates, pulsed-field gel electrophoresis (PFGE) analysis was done as described previously (28), based on a standardized protocol (29) (Fig. 1). BioNumerics software version 6.6 (Applied Maths) was used for digital capture and analysis of profiles. Based on pairwise Dice similarity coefficients, isolates exhibiting $>94\%$ profile similarity (≈ 3 -band difference) to the index isolate for an established pulsotype, implying close genetic similarity, were assigned to that pulsotype. A PFGE profile dendrogram was constructed according to the unweighted pair group method.

Statistical methods. Comparisons of proportions and continuous variables were tested using Fisher's exact test and the Mann-Whitney *U* test, respectively (all 2-tailed). The significance criterion was a *P* value of <0.05 .

RESULTS

Study population. During the 7-week study period (July and August 2013), the UMMC IDDL recovered 233 *E. coli* isolates. Of these, 211 (91%) were from unique patients, and 22 (9%) were from 11 patients (two per patient). The East Bank facility contributed the most isolates (150 [64%]), the West Bank facility fewer (65 [29%]), and the children's hospital the fewest (18 [7%]). The commonest specimen type was urine (80%). Most isolates (69%) were collected from outpatients in one of the three emergency departments.

Overall, 65 (28%) of the 233 isolates were ciprofloxacin resistant, and 16 (7%) had an ESBL-positive phenotype. Fifteen (94%) of the 16 ESBL-positive isolates contained *bla*_{CTX-M}, and 10 (63%) contained *bla*_{CTX-M-15}. Ciprofloxacin resistance and ESBL production were closely correlated, with all 16 (100%) ESBL-positive isolates being ciprofloxacin resistant versus only 49/217 (23%) ESBL-negative isolates ($P < 0.001$).

Phylogenetic group, clonal group, and pulsotype distribution. The 233 study isolates were predominantly (70%) from phylogenetic group B2, and the remainder were from groups A, B1, and D (8 to 9% each) and C, E, and F (1 to 2% each) (Table 1). According to clonal group-specific PCR, ST131 accounted for 61 (26%) of the 233 study isolates, ST131-*H30* for 42 (18%), and ST131-*H30Rx* for 11 (5%). The *H* type of the non-*H30* ST131 isolates was not determined, although previous epidemiologic studies suggest that O16 ST131 isolates are likely *H41*, and non-O16 ST131 isolates are likely *H22* (30).

ST95 accounted for only 24 (10%), ST73 for 20 (9%), ST69 for 4 (2%), and ST405 for 3 (1%) isolates, whereas CC31 was not detected. Supplemental CH typing of 10 randomly selected group B2 isolates from the 57 isolates that did not type as ST131, ST73, or ST95 identified six minor STs, including ST12 ($n = 3$) ST14 ($n = 2$), ST127 ($n = 2$), ST141 ($n = 1$), and 2 undefined STs ($n = 1$ each). Based on this distribution and the overall prevalence of non-ST131, -ST73, and -ST95 group B2 isolates (24%), and each of these minor STs was estimated to account for 3 to 7% of the total population. Thus, ST131 and its *H30* subset, with 26% and 18% total prevalence rates, respectively, were by far the most prevalent clonal subsets within the population, outstripping all other group B2 STs and even the non-B2 major phylogenetic groups.

XbaI PFGE analysis resolved 38 different pulsotypes, which accounted for one to 12 isolates each (Fig. 1). The three most prevalent pulsotypes, i.e., types 968 (20%), 800 (7%), and 812 (10%), represented the top pulsotypes in a previous global PFGE survey of ST131 isolates (28). There were only two instances of paired isolates with indistinguishable profiles, in each of which pairs of matching isolates were collected from different sites from the same patient at the same facility.

Resistance versus phylogeny. In contrast to the expectation that group B2 isolates should be more susceptible than others (31), ciprofloxacin resistance showed no significant phylogenetic group associations, and ESBL production was significantly associated with group C only (Table 2). In contrast, at the clonal group level, both ciprofloxacin resistance and ESBL production were strongly associated with ST131, *H30*, and *H30Rx* (Table 2). Specifically, all *H30* and *H30Rx* isolates, and 44 (72%) of ST131 isolates overall, were ciprofloxacin resistant. Similarly, ST405 (group D) was significantly associated with ESBL production. In contrast,

FIG 1 Pulsed-field gel electrophoresis profile dendrogram of ST131 study isolates. The scale is the percent profile similarity according to Dice similarity coefficients. Columns to the right of the dendrogram give associated data on the isolates. cath, catheter; ESBL, extended-spectrum β -lactamase; FQ-R, fluoroquinolone resistant; midstr, midstream; neph, nephrostomy; PFGE, pulsed-field gel electrophoresis pulsotype (as defined within a large private PFGE library); und, undefined; Ur, urethral. Hyphens indicate absence of a particular characteristic.

ST73 and ST95 (group B2) were significantly associated with ciprofloxacin susceptibility.

These findings suggested that the paucity of overall phylogenetic group associations with ciprofloxacin resistance and ESBL

production might be due to a “diluting” effect from the presence of ST131 within group B2. Supporting this hypothesis, repetition of the analysis without ST131 yielded a quite different picture, with group B2 being significantly associated with ciprofloxacin

TABLE 1 Characteristics of 233 *E. coli* clinical isolates according to source hospital

Trait ^a	Prevalence of trait (no. of isolates [column %]) at:				<i>P</i> value ^b
	Total (<i>n</i> = 233)	Children's (<i>n</i> = 18)	West Bank (<i>n</i> = 65)	East Bank (<i>n</i> = 150)	
Cip ^r	65 (28)	1 (6)	14 (22)	50 (33)	0.02
ESBL	16 (7)	0 (0)	0 (0)	16 (11)	<0.001
Group A	20 (9)	1 (6)	2 (3)	17 (11)	
Group B1	22 (9)	1 (6)	8 (12)	13 (9)	
Group B2	162 (70)	13 (72)	48 (74)	101 (67)	
ST131	61 (26)	4 (22)	16 (25)	41 (27)	
H30	42 (18)	0 (0)	11 (17)	31 (21)	0.049
H30Rx	11 (5)	0 (0)	1 (2)	10 (7)	
ST73	20 (9)	1 (6)	7 (11)	12 (8)	
ST95	24 (10)	2 (11)	10 (15)	12 (8)	
Group C	4 (2)	0 (0)	0 (0)	4 (3)	
Group D	19 (8)	3 (17)	5 (8)	11 (7)	
ST69	4 (2)	2 (11) ^c	1 (2)	1 (1)	0.03 ^c
ST405	3 (1)	0 (0)	0 (0)	3 (2)	
Group E	2 (1)	0 (0)	1 (2)	1 (1)	
Group F	4 (2)	0 (0)	1 (2)	3 (2)	

^a Cip^r, ciprofloxacin resistant; ESBL, extended-spectrum β -lactamase production.

^b *P* values (for 3-group comparison across the 3 facilities, unless specified otherwise) are shown for comparisons that yielded a *P* value of ≤ 0.05 . For all other comparisons, *P* > 0.05.

^c For children's hospital versus other sites, *P* = 0.03.

susceptibility and ESBL negativity (*P* = 0.004 and 0.002, respectively) (Table 3). Additionally, phylogenetic group C now exhibited a significant association with ciprofloxacin resistance (*P* = 0.006) and an even stronger association with ESBL production (*P* = 0.008) (Table 3); the same was true for ST405 (for ciprofloxacin-resistant isolates, 67% [ST405] versus 11% [others], *P* = 0.04; for ESBL production, 67% [ST405] versus 3% [others], *P* = 0.004). In contrast, ST73 and ST95 lost their significant associations with ciprofloxacin susceptibility (not shown).

Hospital distribution. Hospital campus of origin was significantly associated with ciprofloxacin resistance, ESBL phenotype, and ST131 subclone. Specifically, 64 (98%) of 65 ciprofloxacin-resistant isolates were from the East and West Bank adult facilities (versus 0% for the children's facility, *P* = 0.02; Table 1). Similarly, all 16 ESBL-expressing isolates (100%) originated from the East Bank facility (versus none from the West Bank and children's facilities; *P* < 0.001) (Table 1).

The major phylogenetic groups and ST131 were distributed

TABLE 2 Ciprofloxacin and extended-spectrum β -lactamase phenotypes by phylogenetic and clonal group

Phylogenetic/clonal group	Prevalence of phylogenetic/clonal group (no. of isolates [column %]) for: ^a				Prevalence of phylogenetic/clonal group (no. of isolates [column %]) for:		<i>P</i> value ^b
	Total (<i>n</i> = 233)	Cip ^s (<i>n</i> = 168)	Cip ^r (<i>n</i> = 65)	<i>P</i> value ^b	ESBL negative (<i>n</i> = 217)	ESBL positive (<i>n</i> = 16)	
Group A	20 (9)	17 (10)	3 (5)		18 (8)	2 (13)	
Group B1	22 (9)	18 (11)	4 (6)		22 (10)	0 (0)	
Group B2	162 (70)	112 (67)	50 (77)		153 (71)	9 (56)	
ST131	61 (26)	17 (10)	44 (68)	<0.001	52 (24)	9 (56)	0.008
H30	42 (18)	0 (0)	42 (65)	<0.001	34 (16)	8 (50)	0.003
H30Rx	11 (5)	0 (0)	11 (17)	<0.001	5 (2)	6 (38)	<0.001
ST73	20 (9)	20 (12)	0 (0)	0.001	20 (9)	0 (0)	
ST95	24 (10)	24 (14)	0 (0)	<0.001	24 (11)	0 (0)	
Group C	4 (2)	1 (1)	3 (5)		2 (1)	2 (13)	0.02
Group D	19 (8)	15 (9)	4 (6)		17 (8)	2 (13)	
ST69	4 (2)	4 (2)	0 (0)		4 (2)	0 (0)	
ST405	3 (1)	1 (1)	2 (3)		1 (0)	2 (13)	0.01
Group E	2 (1)	2 (1)	0 (0)		2 (1)	0 (0)	
Group F	4 (2)	3 (2)	1 (2)		3 (1)	1 (6)	

^a Cip^s, ciprofloxacin susceptible; Cip^r, ciprofloxacin resistant.

^b *P* values are shown where *P* < 0.05; for all other comparisons, *P* $\geq$ 0.05.

TABLE 3 Ciprofloxacin and extended-spectrum β -lactamase phenotypes by phylogenetic group after excluding the 61 ST131 isolates

Phylogenetic group	Total no. of isolates (% of 172)	Prevalence of phylogenetic/clonal group (no. of isolates [column %]) for: ^a			Prevalence of phylogenetic/clonal group (no. of isolates [column %]) for: ^a		
		Cip ^s (n = 151)	Cip ^r (n = 21)	P value ^a	ESBL negative (n = 165)	ESBL positive (n = 7)	P value ^a
A	20 (12)	17 (11)	3 (14)		18 (11)	2 (29)	
B1	22 (13)	18 (12)	4 (19)		22 (13)	0 (0)	
B2 (non-ST131)	101 (59)	95 (63)	6 (29)	0.004	101 (61)	0 (0)	0.002
C	4 (2)	1 (1)	3 (14)	0.006	2 (1)	2 (29)	0.008
D	19 (11)	15 (10)	4 (19)		17 (10)	2 (29)	
E	2 (1)	2 (1)	0 (0)		2 (1)	0 (0)	
F	4 (2)	3 (2)	1 (5)		3 (2)	1 (14)	

^a P values are shown where $P < 0.05$; for all other comparisons, $P \geq 0.05$.

similarly across the 3 hospital campuses (Table 1). In contrast, the ST131-*H30* subclone exhibited a marked gradient across facilities, being most abundant at the East Bank facility (21% of all isolates), intermediate at the West Bank facility (11%), and absent from the children's facility ($P = 0.049$) (Table 1). The *H30Rx* group followed this trend, accounting for 7% of the East Bank isolates, 2% of the West Bank isolates, and no children's hospital isolates.

Clonal associations of *bla*_{CTX-M} and *bla*_{CTX-M-15}. *bla*_{CTX-M} was significantly more prevalent among ST131 (13% versus 4%), ST131-*H30* (19% versus 4%), and ST131-*H30Rx* (55% versus 4%) isolates than others ($P < 0.05$ for each comparison) (Table 4). Likewise, *bla*_{CTX-M-15} was significantly more frequent among ST131 (10% versus 2%), ST131-*H30* (14% versus 2%), and ST131-*H30Rx* (55% versus 2%) isolates than others ($P < 0.05$ for each comparison) (Table 4). Within ST131, *bla*_{CTX-M-15} ($n = 6$) was confined to the *H30Rx* subclone, whereas other *bla*_{CTX-M} variants ($n = 2$) were confined to the non-Rx subset within *H30* (Table 4).

Virulence genes. Of the 50 tested VGs, 31 (62%) were significantly associated (negatively or positively) with ST131 and/or its clonal subsets (Table 5). Although most associations were negative, nine VGs were significantly overrepresented in ST131, including *iha* (adhesin siderophore receptor), *sat* and *usp* (toxins), *fyuA* and *iutA* (siderophore receptors), *traT* and *ompT* (outer membrane proteins), *kpsM* II (group 2 capsule), and *malX* (pathogenicity island marker). These same nine genes were also significantly overrepresented among *H30* isolates and seven also among *H30Rx* isolates, despite the smaller group size. Molecular ExPEC status was significantly associated with both ST131 and its *H30Rx* subclone, with the *H30* subclone (100% ExPEC) exhibiting a similar trend.

Virulence scores in relation to ST131 and its subclones. Aggregate virulence scores did not differ significantly between the ST131, *H30*, or *H30Rx* isolates and other isolates, either overall or

specifically among ciprofloxacin-susceptible or ESBL-negative isolates (Table 6). In contrast, among ciprofloxacin-resistant and ESBL-positive isolates, the ST131, *H30*, and *H30Rx* isolates had significantly higher aggregate virulence scores than those of other isolates by 1 to 4 points (ciprofloxacin-resistant isolates) or 6 points (ESBL-positive isolates), according to the group medians (Table 6).

DISCUSSION

In this molecular-epidemiological survey, we assessed the prevalence of multiple *E. coli* lineages, including the pandemic ST131 clonal group and its recently described *H30* and *H30Rx* subclones, among 233 consecutive clinical *E. coli* isolates from three UM Medical Center hospital campuses over a 2-month period in 2013. We also studied the associations of these clonal groups with source, resistance phenotype, and virulence genotype.

Our main findings are 3-fold. First, ST131 was the dominant lineage both within group B2 and overall, and it was similarly prevalent (22% to 26%) across specimen types and hospitals. In contrast, the dominant *H30* subclone of ST131 was negatively associated with the children's hospital (0% prevalence) and was most prevalent at the (high-acuity) East Bank facility. Second, ST131 and its subclones were the main carriers of fluoroquinolone resistance and ESBLs in the study population. Third, although aggregate virulence scores were comparable for ST131 and other *E. coli* isolates overall, within the fluoroquinolone-resistant and ESBL-producing subsets, ST131 isolates had a significant virulence score advantage over non-ST131 isolates. These findings indicate that ST131 *E. coli* comprises a significant population of virulent and resistant clinical isolates in our Midwestern locale and that its subclones have distinct ecological and, likely, epidemiological and clinical associations.

According to our PFGE analysis, the ST131 isolates comprise 38 different pulsotypes, the most prevalent of which were pulso-

TABLE 4 Associations of *bla*_{CTX-M} and *bla*_{CTX-M-15} with ST131 and its clonal subsets

Gene	Total no. of isolates (n = 233)	No. with trait (column %)			No. with trait (column %)			No. with trait (column %)		
		ST131 (n = 61)	Others (n = 172)	P value ^a	<i>H30</i> (n = 42)	Others (n = 191)	P value ^a	<i>H30Rx</i> (n = 11)	Others (n = 222)	P value ^a
<i>bla</i> _{CTX-M}	15	8 (13)	7 (4)	0.03	8 (19)	7 (4)	0.001	6 (55)	9 (4)	<0.001
<i>bla</i> _{CTX-M-15}	10	6 (10)	4 (2)	0.02	6 (14)	4 (2)	0.003	6 (55)	4 (2)	<0.001

^a P value by Fisher's exact test.

TABLE 5 Virulence gene distribution by ST131 and its H30 and H30Rx subclones

Category	Gene ^a	Prevalence of trait (no. of isolates [column %])				P value vs all others for ^b :		
		Total (n = 233)	ST131 (n = 61)	H30 (n = 42)	H30Rx (n = 11)	ST131	H30	H30Rx
Adhesins	<i>papAH</i>	75 (32)	6 (10)	2 (5)	1 (9)	<0.001	<0.001	
	<i>papC</i>	79 (34)	6 (10)	2 (5)	1 (9)	<0.001	<0.001	
	<i>papEF</i>	75 (32)	6 (10)	2 (5)	1 (9)	<0.001	<0.001	
	<i>papG</i>	62 (27)	6 (10)	1 (2)	1 (9)	<0.001	<0.001	
	Allele <i>GII</i>	46 (20)	5 (8)	2 (5)	1 (9)	0.008	0.005	
	Allele <i>GIII</i>	29 (12)	1 (2)	0 (0)	0 (0)	0.001	0.003	
	<i>sfa/focDE</i>	40 (17)	0 (0)	0 (0)	0 (0)	<0.001	<0.001	
	<i>sfaS</i>	18 (8)	0 (0)	0 (0)	0 (0)	0.005	0.05	
	<i>focG</i>	13 (6)	0 (0)	0 (0)	0 (0)	0.02		
	<i>afa/draBC</i>	21 (9)	11 (18)	4 (10)	4 (36)	0.008		0.01
	<i>iha</i>	84 (36)	54 (89)	39 (93)	11 (100)	<0.001	<0.001	<0.001
Toxins	<i>hlyD</i>	56 (24)	4 (7)	1 (2)	1 (9)	<0.001	<0.001	
	<i>cnf1</i>	42 (18)	1 (20)	1 (2)	1 (9)	<0.001	0.002	
	<i>sat</i>	86 (37)	55 (90)	40 (95)	11 (100)	<0.001	<0.001	<0.001
	<i>vat</i>	97 (42)	1 (2)	1 (2)	0 (0)	<0.001	<0.001	0.003
	<i>usp</i>	155 (67)	61 (100)	42 (100)	11 (100)	<0.001	<0.001	
	<i>pic</i>	21 (9)	0 (0)	0 (0)	0 (0)	0.002		
Siderophores	<i>iroN</i>	58 (25)	3 (5)	0 (0)	0 (0)	<0.001	<0.001	
	<i>fyuA</i>	189 (81)	61 (100)	42 (100)	11 (100)	<0.001	<0.001	
	<i>ireA</i>	30 (13)	1 (2)	1 (2)	0 (0)	0.001	0.022	
	<i>iutA</i>	114 (49)	57 (93)	40 (95)	11 (100)	<0.001	<0.001	<0.001
Protectins	<i>kpsM II</i>	154 (66)	46 (75)	27 (64)	11 (100)			0.02
	K1	53 (23)	2 (3)	1 (2)	0 (0)	<0.001	<0.001	
	K2/K100	22 (9)	14 (23)	5 (12)	5 (46)	<0.001		0.002
	K5	35 (15)	22 (36)	15 (36)	3 (27)	<0.001	<0.001	
	<i>traT</i>	160 (69)	58 (91)	39 (93)	11 (100)	<0.001	<0.001	0.02
	<i>iss</i>	21 (9)	3 (5)	0 (0)	0 (0)		0.02	
Other	<i>clbB</i>	74 (32)	1 (2)	1 (2)	0 (0)	<0.001	<0.001	0.02
	<i>clbN</i>	74 (32)	1 (2)	1 (2)	0 (0)	<0.001	<0.001	0.02
	<i>ibeA</i>	34 (15)	10 (16)	0 (0)	0 (0)		0.001	
	<i>ompT</i>	180 (77)	61 (100)	42 (100)	11 (100)	<0.001	<0.001	0.07
	<i>malX</i>	165 (71)	61 (100)	42 (100)	11 (100)	<0.001	<0.001	0.04
	H7 <i>fliC</i>	30 (13)	1 (2)	1 (2)	0 (0)	0.001	0.02	
ExPEC ^c	NA ^d	143 (61)	44 (72)	27 (64)	11 (100)	0.05		0.008

^a Of the 50 virulence genes tested, the 34 shown yielded $P < 0.05$ in at least one comparison: *afa/draBC*, Dr family adhesins; *iha*, adhesion siderophore; alleles *GII* and *GIII*, P-fimbriae adhesin variants; *clbB* and *clbN*, colibactin synthesis; *cnf1*, cytotoxic necrotizing factor; *focG*, SF1C fimbriae; *fyuA*, yersiniabactin receptor; *hlyD*, hyaluronidase; *hlyA*, heat-resistant agglutinin; *ibeA*, invasion-associated gene; *ireA*, siderophore receptor; *iroN*, salmochelin receptor; *iss*, increased serum survival gene; *iutA*, aerobactin receptor; K1, K2/K100, and K5, group 2 capsule variants; *kpsM II*, group 2 capsule; H7, *fliC* (flagella) variant; *malX*, pathogenicity island marker; *ompT*, outer membrane protein T (protease); *papAH*, *papC*, *papEF*, and *papG*, P-fimbrial structural subunit, assembly, tip pilins, and adhesion, respectively; *pic*, serine protease autotransporters; *sat*, secreted autotransporter toxin; *sfa/focDE*, S or F1C fimbriae; *sfaS*, S fimbriae; *traT*, serum-resistance associated; *usp*, uropathogenic-specific protein; and *vat*, vacuolating toxin. Genes associated positively with at least one ST131 clonal subset are shown in bold. Genes detected in ≥ 1 isolate each but not yielding $P < 0.05$, included (total prevalence): *afaE8*, variant Dr family adhesion (0.4%); *astA*, enterotoxigenic *E. coli* toxin (4%); *bmaE*, M fimbriae (0.4%); *cdtB*, cytolethal distending toxin (3%); *cvaC*, microcin V (7%); *hlyF*, variant hemolysin (8%); *kpsM III*, group 3 capsule (2%); *rfe*, O antigen polymerase (4%); and *tsh*, serine protease autotransporters (5%). Genes sought but not detected included allele *GI*, P fimbriae adhesin variants; *clpG*, major subunit of CS31A fimbriae; F17, F17 fimbriae; *gafD*, G fimbriae; and K15, group 2 capsule variant.

^b P values (by Fisher's exact test) are shown where $P < 0.05$.

^c ExPEC, extraintestinal pathogenic *E. coli*, defined operationally as the presence of >2 of (*papA* and/or *papC*, counted as one), *sfa/focDE*, *afa/draBC*, *iutA*, and *kpsM II*.

^d NA, not applicable.

types 968 (20%), 800 (7%), and 812 (10%). These pulsotypes were described previously as common *E. coli* ST131 U.S. strains, suggesting that our local ST131 population reflects part of the national (and global) ST131 ecology rather than being a localized regional phenomenon (28). Further, the absence of PFGE profile commonality across patients effectively excludes a local point source outbreak, favoring instead either the importation of di-

verse strains into the institutions from the community or clonally diverse within-institution reservoirs.

Our study uniquely compared multiple adjacent hospitals within the same health care system, which share a clinical microbiology laboratory, to assess the prevalence of ST131 and its H30 and H30Rx subclones. The three study sites constituted a spectrum from a children's hospital, to a lower-acuity primarily out-

TABLE 6 Distribution of aggregate virulence gene scores in relation to ST131, *H30*, and *H30Rx* status and ciprofloxacin and extended-spectrum β -lactamase (ESBL) phenotype

Population	Subgroup	No. of isolates in:		Virulence gene score (median [range]) in:		<i>P</i> value ^a
		Subgroup	Others	Subgroup	Others	
Total	ST131	61	172	10 (5, 17)	10 (0, 20)	
	<i>H30</i>	42	191	10 (5, 15)	11 (0, 20)	
	<i>H30Rx</i>	11	222	10 (10, 14)	10 (0, 20)	
Ciprofloxacin susceptible	ST131	17	151	12 (8, 17)	12 (0, 20)	ND ^c
	<i>H30</i>	0	168	NA ^b	12 (0, 20)	
	<i>H30Rx</i>	0	168	NA	12 (0, 20)	
Ciprofloxacin resistant	ST131	44	21	10 (5, 15)	6 (1, 10)	<0.001
	<i>H30</i>	42	23	10 (5, 15)	8 (1, 10)	<0.001
	<i>H30Rx</i>	11	54	10 (10, 14)	9 (1, 15)	<0.001
ESBL negative	ST131	52	165	10 (5, 17)	10 (0, 20)	
	<i>H30</i>	34	183	10 (5, 15)	11 (0, 20)	
	<i>H30Rx</i>	5	212	10 (10, 10)	10 (0, 20)	
ESBL positive	ST131	9	7	11 (10, 14)	5 (3, 8)	<0.001
	<i>H30</i>	8	8	11 (10, 14)	5 (3, 10)	0.001
	<i>H30Rx</i>	6	10	12 (10, 14)	6 (3, 10)	0.001

^a *P* values (by the Mann-Whitney test) are shown where *P* < 0.05.^b NA, not applicable (no ciprofloxacin-susceptible *H30* or *H30Rx* isolates).^c ND, not done (Mann-Whitney test not performed with empty groups).

patient adult facility (West Bank), and to a higher-acuity adult tertiary care center (East Bank). The overall prevalence of ST131 (26%) corresponded closely with values noted in recent surveys of the U.S. veteran population (27.7%) and Olmstead County, MN, laboratories (27%) (9, 16), suggesting concordance with what is known about the nationwide prevalence of this *E. coli* lineage. Moreover, ST131 *per se* was fairly evenly distributed across the three study facilities, as were the major *E. coli* phylogenetic groups (except group A) and selected non-ST131 clonal groups.

In contrast, we found marked disparities in the across-facility distribution of the ST131 subclones. That is, although overall the *H30* and *H30Rx* subclones accounted for 18% and 4.7% of all *E. coli* isolates and for 69% and 18% of ST131 isolates, respectively, they were absent from the children's hospital, at intermediate prevalence at the West Bank facility, and highly prevalent at the East Bank facility.

This relationship parallels the observed distribution of resistance phenotypes. Ciprofloxacin resistance and ESBL carriage were associated with the non-children's facilities and the East Bank facility, respectively, and with ST131-*H30* and ST131-*H30Rx*. Although these associations with facility were statistically significant for *H30* only, the trends for *H30Rx* were numerically similar. Thus, future studies of ST131 epidemiology should distinguish among these ST131 subclones, which have distinctive resistance and epidemiological correlates. An analysis of ST131 as a unitary entity, which is the traditional approach, may fail to detect important associations and lead to erroneous conclusions. Additionally, ecological context appears to influence significantly the antimicrobial resistance prevalence and clonal mix of the *E. coli* population, even within a given locale.

The negative association between ST131-*H30* and the relatively small number of children's hospital isolates corresponds

with the findings of Banerjee et al. (9) from their Olmstead County cohort. There, although ST131 was present in all age cohorts, non-*H30* ST131 isolates predominated in the 11-to-20-year-old cohort, within which nearly all ST131 isolates were non-*H30*. Isolates from children <10 years included slightly more *H30* than non-*H30* isolates. This association of non-*H30* ST131 *E. coli* with older children likely reflects multiple factors, including distinctive patterns of antibiotic exposure and comorbidities. An additional patient characteristic association was recently reported by Burgess et al. (32), whose data suggest that the *H30* subclone is overrepresented among long-term care facility residents, and that a subset of these isolates have the *H30Rx* ESBL phenotype. While our West Bank facility includes a long-term rehabilitation facility, there are multiple other care services at this site. Our study does not allow for age-specific analysis; larger studies in both children and long-term care facility residents are needed to examine these issues.

Also notable were the associations of ST131-*H30* and ST131-*H30Rx* with the East Bank facility. The East Bank facility patients tend to be severely ill, extensively antibiotic exposed, and multiply compromised (our unpublished data). This would favor extensively resistant pathogens while not requiring as much intrinsic virulence as for infections in intact hosts. Although ST131-*H30Rx* has been proposed as an aggressive sepsis-causing pathogen (8, 10), recent data suggest that it behaves more as a multidrug-resistant opportunist (33). More refined epidemiological analyses are needed to clarify the host correlates and associated clinical outcomes of the different ST131 subclones.

Regarding our third main finding, virulence gene content may contribute to the success of the ST131-*H30* and ST131-*H30Rx* subclones. Here, although aggregate virulence scores of the ST131, *H30*, and *H30Rx* isolates did not differ significantly from those of other *E. coli* generally, they were significantly higher than those of

non-ST131 ciprofloxacin-resistant and ESBL-producing *E. coli* isolates, similar to previous findings by our group and others (2, 16, 17). Additionally, many individual virulence genes were significantly associated with ST131 and its subclones. Nine such genes were overrepresented among the ST131 isolates, including specific adhesins, toxins, and siderophore receptors. These lineage-specific patterns of virulence gene carriage coincide closely with previous findings from other locales (9, 10, 14, 16), suggesting that the present ST131 study isolates are part of the global ST131 expansion and not a distinctive local population. Further, while the ST131 subclones may have nonsuperior virulence overall, they may be more virulent than other resistant strains, promoting their rise to predominance within the resistant subpopulation (1, 7).

Our study has several limitations. First, although consecutive sampling reflected the number of ST131 isolates originating from each facility, the relatively few isolates from the children's hospital limited the statistical power for certain comparisons. Second, given the relatively small sample size, conclusions must be interpreted cautiously. Third, we lacked patient-specific data and relied instead on characteristics of the specimens (facility, inpatient versus outpatient source, and specimen type). Third, for phylogenetic group assignments and inferences regarding virulence potential, we relied on PCR-based detection of SNPs and virulence genes, which have well-recognized limitations at the individual strain level (18, 34).

The study strengths include our ability to examine *E. coli* diversity within three adjacent hospitals in the same health care system that share a clinical microbiology laboratory and yet serve very different patient populations. Our findings underscore the importance of understanding intraregional differences when drawing conclusions about how microbiologic niches may differ between regions. Another strength was the extensive molecular characterization of the *E. coli* isolates, including phylotyping using the updated Clermont system (19) and the detection of multiple individual clonal groups.

In summary, we documented a high overall and individual hospital prevalence of ST131 among consecutive clinical *E. coli* isolates from three adjacent Midwestern hospital campuses. The H30 and H30Rx subclones were strongly associated with ciprofloxacin and CTX-M-mediated cephalosporin resistance and varied significantly in prevalence by hospital, being notably absent from the children's facility. Although these subclones are part of a global clonal expansion, any virulence advantage (as inferred from virulence genotypes) was found only in comparison with other resistant isolates. Our findings demonstrate the importance of delineating the ST131-H30 and ST131-H30Rx subclones in epidemiologic surveys and the effect of host characteristics (as inferred here from hospital of origin) in determining the composition of the local clinical *E. coli* population.

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for tests to detect specific *E. coli* clones. The other authors declare no financial conflicts of interest.

REFERENCES

- Nicolas-Chanoine MH, Bertrand X, Madec JY. 2014. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev 27:543–574. <http://dx.doi.org/10.1128/CMR.00125-13>.
- Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. 2010. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis 51:286–294. <http://dx.doi.org/10.1086/653932>.
- Rogers BA, Sidjabat HE, Paterson DL. 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 66:1–14. <http://dx.doi.org/10.1093/jac/dkq415>.
- Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Canica MM, Park YJ, Lavigne JP, Pitout J, Johnson JR. 2008. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 61:273–281.
- Williamson DA, Freeman JT, Porter S, Roberts S, Wiles S, Paterson DL, Johnson JR. 2013. Clinical and molecular correlates of virulence in *Escherichia coli* causing bloodstream infection following transrectal ultrasound-guided (TRUS) prostate biopsy. J Antimicrob Chemother 68:2898–2906. <http://dx.doi.org/10.1093/jac/dkt276>.
- Accogli M, Giani T, Monaco M, Giuffrè M, García-Fernández A, Conte V, D'Ancona F, Pantosti A, Rossolini GM, Cerquetti M. 2014. Emergence of *Escherichia coli* ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy. J Antimicrob Chemother 69:2293–2296. <http://dx.doi.org/10.1093/jac/dku132>.
- O'Hara JA, Hu F, Ahn C, Nelson J, Rivera JJ, Pascalle AW, Doi Y. 2014. Molecular epidemiology of KPC-producing *Escherichia coli*: occurrence of ST131-*fimH30* subclone harboring pKpQIL-like IncFIIK plasmid. Antimicrob Agents Chemother 58:4234–4237. <http://dx.doi.org/10.1128/AAC.02182-13>.
- Johnson JR, Tchesnokova V, Johnston B, Clabots C, Roberts PL, Billig M, Riddell K, Rogers P, Qin X, Butler-Wu S, Price LB, Aziz M, Nicolas-Chanoine MH, Debroy C, Robicsek A, Hansen G, Urban C, Platell J, Trott DJ, Zhanel G, Weissman SJ, Cookson BT, Fang FC, Limaye AP, Scholes D, Chattopadhyay S, Hooper DC, Sokurenko EV. 2013. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. J Infect Dis 207:919–928. <http://dx.doi.org/10.1093/infdis/jis933>.
- Banerjee R, Johnston B, Lohse C, Chattopadhyay S, Tchesnokova V, Sokurenko EV, Johnson JR. 2013. The clonal distribution and diversity of extraintestinal *Escherichia coli* isolates vary according to patient characteristics. Antimicrob Agents Chemother 57:5912–5917. <http://dx.doi.org/10.1128/AAC.01065-13>.
- Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, Nordstrom L, Billig M, Chattopadhyay S, Stegger M, Andersen PS, Pearson T, Riddell K, Rogers P, Scholes D, Kahl B, Keim P, Sokurenko EV. 2013. The epidemic of extended-spectrum- β -lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30Rx. mBio 4:e00377–00313.
- Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan MD, Gomes Moriel D, Peters KM, Davies M, Rogers BA, Dougan G, Rodriguez-Baño J, Pascual A, Pitout JD, Upton M, Paterson DL, Walsh TR, Schembri MA, Beatson SA. 2014. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A 111:5694–5699. <http://dx.doi.org/10.1073/pnas.1322678111>.
- Banerjee R, Johnson JR. 2014. A new clone sweeps clean: the enigmatic experience of *Escherichia coli* sequence type 131. Antimicrob Agents Chemother 58:4997–5004. <http://dx.doi.org/10.1128/AAC.02824-14>.
- Olesen B, Frimodt-Møller J, Leihof RF, Struve C, Johnston B, Hansen DS, Scheut F, Krogfelt KA, Kuskowski MA, Clabots C, Johnson JR. 2014. Temporal trends in antimicrobial resistance and virulence-associated traits within the *Escherichia coli* sequence type 131 clonal group and its H30 and H30Rx subclones, 1968 to 2012. Antimicrob Agents Chemother 58:6886–6895. <http://dx.doi.org/10.1128/AAC.03679-14>.
- Banerjee R, Robicsek A, Kuskowski MA, Porter S, Johnston BD, Sokurenko E, Tchesnokova V, Price LB, Johnson JR. 2013. Molecular epidemiology of *Escherichia coli* sequence type 131 and Its H30 and H30Rx subclones among extended-spectrum- β -lactamase-positive and -nega-

- tive *E. coli* clinical isolates from the Chicago Region, 2007 to 2010. Antimicrob Agents Chemother 57:6385–6388. <http://dx.doi.org/10.1128/AAC.01604-13>.
15. Peirano G, Pitout JD. 2014. Fluoroquinolone-resistant *Escherichia coli* sequence type 131 isolates causing bloodstream infections in a Canadian region with a centralized laboratory system: rapid emergence of the H30Rx sublineage. Antimicrob Agents Chemother 58:2699–2703. <http://dx.doi.org/10.1128/AAC.00119-14>.
16. Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, Kuskowski MA, Tchesnokova V, Sokurenko EV, Johnson JR, VICTORY (Veterans Influence of Clonal Types on Resistance: Year 2011) Investigators. 2013. *Escherichia coli* sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among U.S. veterans. Clin Infect Dis 57:1256–1265. <http://dx.doi.org/10.1093/cid/cit503>.
17. Olesen B, Hansen DS, Nilsson F, Frimodt-Møller J, Leihof RF, Struve C, Scheutz F, Johnston B, Krogfelt KA, Johnson JR. 2013. Prevalence and characteristics of the epidemic multiresistant *Escherichia coli* ST131 clonal group among extended-spectrum beta-lactamase-producing *E. coli* isolates in Copenhagen, Denmark. J Clin Microbiol 51:1779–1785. <http://dx.doi.org/10.1128/JCM.00346-13>.
18. Clermont O, Bonacorsi S, Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66:4555–4558. <http://dx.doi.org/10.1128/AEM.66.10.4555-4558.2000>.
19. Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58–65. <http://dx.doi.org/10.1111/1758-2229.12019>.
20. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. 2009. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob Agents Chemother 53:2733–2739. <http://dx.doi.org/10.1128/AAC.00297-09>.
21. Bidet P, Metais A, Mahjoub-Messai F, Durand L, Dehem M, Aujard Y, Bingen E, Nassif X, Bonacorsi S. 2007. Detection and identification by PCR of a highly virulent phylogenetic subgroup among extraintestinal pathogenic *Escherichia coli* B2 strains. Appl Environ Microbiol 73:2373–2377. <http://dx.doi.org/10.1128/AEM.02341-06>.
22. Clermont O, Christenson JK, Daubié AS, Gordon DM, Denamur E. 2014. Development of an allele-specific PCR for *Escherichia coli* B2 subtyping, a rapid and easy to perform substitute of multilocus sequence typing. J Microbiol Methods 101:24–27. <http://dx.doi.org/10.1016/j.mimet.2014.03.008>.
23. Matsumura Y, Yamamoto M, Nagao M, Hotta G, Matsushima A, Ito Y, Takakura S, Ichihara S, Kyoto-Shiga Clinical Microbiology Study Group. 2012. 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 67:2612–2620. <http://dx.doi.org/10.1093/jac/dks278>.
24. Weissman SJ, Johnson JR, Tchesnokova V, Billig M, Dykhuizen D, Riddell K, Rogers P, Qin X, Butler-Wu S, Cookson BT, Fang FC, Scholes D, Chattopadhyay S, Sokurenko E. 2012. High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. Appl Environ Microbiol 78:1353–1360. <http://dx.doi.org/10.1128/AEM.06663-11>.
25. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, Smith KE. 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother 47:2161–2168. <http://dx.doi.org/10.1128/AAC.47.7.2161-2168.2003>.
26. Johnson JR, Urban C, Weissman SJ, Jorgensen JH, Lewis JS, Hansen G, Edelstein PH, Robicsek A, Cleary T, Adachi J, Paterson D, Quinn J, Hanson ND, Johnston BD, Clabots C, Kuskowski MA, AMERECUS Investigators. 2012. Molecular epidemiological analysis of *Escherichia coli* sequence type ST131 (O25:H4) and bla_{CTX-M-15} among extended-spectrum-β-lactamase-producing *E. coli* from the United States, 2000 to 2009. Antimicrob Agents Chemother 56:2364–2370. <http://dx.doi.org/10.1128/AAC.05824-11>.
27. Leflon-Guibout V, Jurand C, Bonacorsi S, Espinasse F, Guelfi MC, Duportail F, Heym B, Bingen E, Nicolas-Chanoine MH. 2004. Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob Agents Chemother 48:3736–3742. <http://dx.doi.org/10.1128/AAC.48.10.3736-3742.2004>.
28. Johnson JR, Nicolas-Chanoine MH, DebRoy C, Castanheira M, Robicsek A, Hansen G, Weissman S, Urban C, Platell J, Trott D, Zhanel G, Clabots C, Johnston BD, Kuskowski MA, Investigators M. 2012. Comparison of *Escherichia coli* ST131 pulsotypes, by epidemiologic traits, 1967–2009. Emerg Infect Dis 18:598–607. <http://dx.doi.org/10.3201/eid1804.111627>.
29. Ribot EM, Fair MA, Gautam R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 3:59–67. <http://dx.doi.org/10.1089/fpd.2006.3.59>.
30. Johnson JR, Clermont O, Johnston B, Clabots C, Tchesnokova V, Sokurenko E, Junka AF, Maczynska B, Denamur E. 2014. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within *Escherichia coli* sequence type 131. J Clin Microbiol 52:1358–1365. <http://dx.doi.org/10.1128/JCM.03502-13>.
31. Johnson JR, Kuskowski MA, Gajewski A, Sahm DF, Karlowsky JA. 2004. Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of *Escherichia coli* from across the United States, 2000–2001. J Infect Dis 190:1739–1744. <http://dx.doi.org/10.1086/425018>.
32. Burgess MJ, Johnson JR, Porter SB, Johnston B, Clabots C, Lahr BD, Uhl JR, Banerjee R. 2015. Long-term care facilities are reservoirs for antimicrobial-resistant sequence type 131 *Escherichia coli*. Open Forum Infect Dis 2:ofv011. <http://dx.doi.org/10.1093/ofid/ofv011>.
33. Drekonja DM, Johnston B, Porter SB, Clabots C, Amway R, Kuskowski MA, Johnson JR. 2014. Epidemiology of *Escherichia coli* sequence type 131 in a Veterans Affairs medical center, abstr 342, IDWeek, 8 to 12 October 2014, Philadelphia, PA.
34. Johnson JR, Porter SB, Zhanel G, Kuskowski MA, Denamur E. 2012. 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 80:1554–1562. <http://dx.doi.org/10.1128/IAI.06388-11>.