MAJOR ARTICLE

Abrupt Emergence of a Single Dominant Multidrug-Resistant Strain of *Escherichia coli*

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Background. Fluoroquinolone-resistant *Escherichia coli* are increasingly prevalent. Their clonal origins—potentially critical for control efforts—remain undefined.

Methods. Antimicrobial resistance profiles and fine clonal structure were determined for 236 diverse-source historical (1967–2009) *E. coli* isolates representing sequence type ST131 and 853 recent (2010–2011) consecutive *E. coli* isolates from 5 clinical laboratories in Seattle, Washington, and Minneapolis, Minnesota. Clonal structure was resolved based on *fimH* sequence (fimbrial adhesin gene: *H* subclone assignments), multilocus sequence typing, *gyrA* and *parC* sequence (fluoroquinolone resistance-determining loci), and pulsed-field gel electrophoresis.

Results. Of the recent fluoroquinolone-resistant clinical isolates, 52% represented a single ST131 subclonal lineage, *H*30, which expanded abruptly after 2000. This subclone had a unique and conserved *gyrA/parC* allele combination, supporting its tight clonality. Unlike other ST131 subclones, *H*30 was significantly associated with fluoroquinolone resistance and was the most prevalent subclone among current *E. coli* clinical isolates, overall (10.4%) and within every resistance category (11%–52%).

Conclusions. Most current fluoroquinolone-resistant *E. coli* clinical isolates, and the largest share of multidrug-resistant isolates, represent a highly clonal subgroup that likely originated from a single rapidly expanded and disseminated ST131 strain. Focused attention to this strain will be required to control the fluoroquinolone and multidrug-resistant *E. coli* epidemic.

Keywords. *Escherichia coli* infections; antimicrobial resistance; extended-spectrum β-lactamase; CTX-M-15; fluoroquinolone resistance; multidrug resistance; sequence type ST131; multilocus sequence typing; molecular epidemiology; FimH.

Until recently, fluoroquinolones (FQs) were preferred agents for treating multiple types of bacterial infection, including urinary tract infections (UTIs), most of which are caused by *Escherichia coli* [1, 2]. However, FQ resistance in *E. coli* is increasingly prevalent, resulting in some patients progressing to severe illness or death despite receiving conventional empirical or prophylactic therapy [3, 4]. In part because of this, the authors of the 2010 Infectious Diseases Society of

Received 24 July 2012; accepted 29 October 2012.

Presented in part at: Symposium "Clonal Wars among Multi-resistant Gram-negative bacteria", 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Chicago, IL ("*Escherichia coli* of clonal group ST131 that produce CTX-M-β-lactamases"), 9 October 2011.

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The Journal of Infectious Diseases
Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2013.
DOI: 10.1093/infdis/jis933

America guidelines recommended nitrofurantoin over FQs for empirical treatment of uncomplicated UTIs [5].

In *E. coli*, although upregulated efflux pumps and plasmid-encoded resistance mechanisms can reduce FQ susceptibility, high-level resistance typically requires 1–2 point mutations within the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, the chromosomal genes encoding the FQ targets DNA gyrase and topoisomerase IV, respectively [6]. Because of its chromosomal basis, such FQ resistance has arisen in diverse *E. coli* clonal lineages that presumably acquired the requisite QRDR mutations independently [7–9].

Despite the high clonal diversity of FQ-resistant (FQ-R) *E. coli*, the past decade has seen the rapid emergence and global spread of a specific FQ resistance-associated clonal group, ST131, which is among ≥1000 *E. coli* sequence types (STs), as defined by multilocus sequence typing (MLST) [9–11]. As are most STs, ST131 is both genetically and phenotypically diverse. For example, although ST131 first came to attention because of its close association with extended-spectrum β-lactamases (ESBLs), especially CTX-M-15 [12], in many locales ST131 is more frequently ESBL-negative but FQ-R [9], and these resistance traits are differentially distributed among ST131's diverse pulsed-field gel electrophoresis (PFGE) types [13]. However, whether ST131's association with FQ resistance is from frequent, independent emergence of resistance in diverse ST131 strains, vs expansion of a single strain, remains unknown [8, 9, 11, 14–17].

Thus, sub-ST analysis of ST131 isolates is critical to understanding the ongoing emergence of FQ-R *E. coli*. Accordingly, we analyzed FQ resistance at the sub-ST level for over 350 archived or newly obtained ST131 isolates and over 700 non-ST131 *E. coli* isolates, by determining fine clonal diversity based on individual gene loci and PFGE analysis. We sought thereby to define the clonal history of FQ resistance in ST131 and its impact on clinical population dynamics within *E. coli* generally.

METHODS

Isolates and Patients

The clonality of FQ resistance was analyzed among historical ST131 isolates (n = 236) and recent ST131 (n = 116) and non-ST131 (n = 737) human clinical isolates. The historical ST131 isolates were a convenience sample selected from multiple collections to represent diverse years of isolation (1967–2009), FQ phenotypes, locales (201 isolates from across the United States; 35 international isolates from 10 countries on 5 continents), and sources, including humans (n = 173), food/companion animals (n = 52), and food/environment (n = 10) [13]. The 853 recent clinical isolates (both ST131 and non-ST131) were consecutive, single-patient (ostensibly), human extraintestinal isolates from October 2010 and January 2011 from 5 clinical microbiology laboratories in Seattle, Washington (Group Health Cooperative, Harborview Medical Center, Seattle Children's Hospital, University of Washington Medical Center) and Minneapolis, Minnesota (Veterans Affairs Medical Center) [18]. Local institutional review boards approved the study protocol.

Sequence Analysis of Individual Loci

Isolates were assigned to an ST based on MLST allele profiles, as determined by sequencing established MLST loci (http://mlst.ucc.ie/mlst/dbs/Ecoli). Within-ST clonal variation was resolved on the basis of sequence variation in the *E. coli* fimbrial adhesin gene, *fimH* (positions 64–552) [18]. The QRDR of *gyrA* (6-570) and *parC* (1-573) also was sequenced [16]. Maximum-likelihood trees were inferred for *gyrA* and *parC* using PAUP* [19]. The ST131 *gyrA* and *parC* alleles were labeled as follows: stepwise mutational derivatives of each (numbered) main allele were indicated by using lower case letters for silent (synonymous) mutations and upper case letters for replacement (nonsynonymous) mutations.

Antimicrobial Susceptibility, ESBL Status, and $bla_{CTX\text{-}M\text{-}15}$

Susceptibility to ciprofloxacin and 11 other antimicrobial agents was determined by disk diffusion, as specified by the Clinical and Laboratory Standards Institute [20]. Intermediate was considered resistant. Penicillins and cephalosporins were counted as separate antimicrobial classes. Historic isolates were previously characterized for ESBL production and presence of $bla_{CTX\text{-}M\text{-}15}$ (encoding CTX-M-15) [13].

Pulsed-field Gel Electrophoresis Analysis

The 352 historical and recent ST131 isolates underwent standardized *Xba*I pulsed-field gel electrophoresis (PFGE) analysis, with pulsotypes defined at ≥94% PFGE profile similarity to index strains for each pulsotype [13]. For dendrogram construction, a 24% subsample (n = 85) was used to allow single-page readability. The 85 ST131 isolates were selected randomly after deliberate inclusion of the earliest (plus a second, as available) representative of each *fimH-gyrA-parC* combination. The dendrogram was inferred within BioNumerics (Applied Maths) according to the unweighted pair group method based on Dice similarity coefficients.

Statistical Analysis

Fisher exact test (2-tailed) was used to test comparisons of proportions. The significance criterion was $P < .05$.

RESULTS

FQ Resistance Within ST131 Is Associated With a Specific Subclone

To explore the subclonal structure of ST131, the 352 historical and recent ST131 isolates (1967–2011) underwent *fimH* sequencing and PFGE profiling. This analysis identified 185

unique PFGE pulsotypes and 7 distinct *fimH*-based putative clonal lineages (*H*15, *H*22, *H*27, *H*30, *H*35, *H*41, *H*94).

The *fimH*-based typing corresponded broadly with PFGE profiles, as reflected in the statistically significant segregation of *fimH* alleles within the PFGE dendrogram (Figure 1). For example, the dominant *fimH*30 allele was concentrated within the upper dendrogram region (≥75% profile similarity), as compared with the lower region (<75% profile similarity; 35 of 50 [70%] vs 10 of 35 [29%]; $P < .001$). Conversely, the second-most-common *fimH*22 allele was concentrated within the lower dendrogram region (13 of 35 [37%] vs 7 of 50 [14%]; $P = .02$). However, none of the *fimH*-based lineages were confined to a single clade on the PFGE tree (Figure 1), reflecting certain level of phylogenetic incongruence between the 2 typing methods.

The *H* subclones were next analyzed for associations with FQ resistance and prevalence during the study period (1967–2011; Figure 2*A*). During the earliest period (1967–1999), only FQ-susceptible (FQ-S) subclones were encountered, predominantly *H*22 and *H*35. FQ-R isolates appeared first during 2000–2005, associated almost exclusively with the (newly detected) *H*30 subclone. Thereafter, the *H*30 subclone continued to account for >97% of FQ-R ST131 isolates and constituted an increasing proportion of ST131 isolates overall.

The FQ-R ST131 Clonal Expansion Involved Almost Exclusively a Single *gyrA/parC* Combination

Among the 352 *E. coli* ST131 study isolates, sequence analysis of *gyrA* and *parC* identified 7 *gyrA* alleles and 10 *parC* alleles (Tables 1 and 2; Figure 3). The *gyrA* alleles differed by no more than 1 single-nucleotide polymorphism (SNP), suggesting evolution by point mutation (Table 1; Figure 3*A*). All FQ-S isolates possessed either the putative ancestor allele gyrA1 or a derivative thereof containing either one silent SNP (gyrA1a) or one amino acid replacement mutation, ie, Ser-83-Leu (gyrA1A) or Asp-87-Asn (gyrA1B). In contrast, all FQ-R isolates possessed *gyrA* alleles that differed from *gyrA*1A by distinct secondary replacement mutations at Asp-87: *gyrA*1AB (Asp-87-Asn), *gyrA*1AC (Asp-87-Gly), and *gyrA*1AD (Asp-87-Tyr).

Among the 10 *parC* alleles, 6 closely related alleles likewise appeared to have evolved by point mutation (Table 2; Figure 3*B*, *gray box*). Most FQ-S isolates possessed the putative ancestor allele, *parC*1, or a variant containing 1 silent SNP (*parC*1a and *parC*1b). In contrast, most FQ-R isolates possessed *parC*1aAB (*parC*1a plus replacement mutations Ser-80-Ile and Glu-84-Val), *parC*1aABC (*parC*1aAB plus replacement mutation Pro-32-Ser), or *parC*1D (*parC*1 plus replacement mutation Ser-80-Arg). The 4 remaining *parC* alleles instead differed by multiple (≥3) silent SNPs, suggesting horizontal gene transfer. Two such alleles, *parC*4A and *parC*3A, occurred in FQ-R isolates and shared replacement mutation Ser-80-Ile.

The 7 *gyrA* and 10 *parC* alleles occurred in ST131 in 18 combinations (Figure 3*C*). Among FQ-S isolates the *gyrA*1/*parC*1 ancestral allele combination (Figure 3*C*) occurred in slightly more than half of isolates and in most *H* subclones (Figure 2*B*). In contrast, among FQ-R isolates the *gyrA*1AB/*parC*1aAB combination predominated overwhelmingly (98% of FQ-R isolates; Figure 3*C*), associated almost exclusively with the *H*30 subclone (Figure 2*B*).

The *H*30 ST131 Subclone Is Associated With FQ Resistance Globally, Regardless of Source, and With CTX-M-15

Among the 236 historical ST131 isolates, the *H*30 ST131 subclone was closely associated with FQ resistance regardless of locale and source, and with ESBL production and $bla_{\text{CTX-M-15}}$. Specifically, among US isolates the *H*30 subclone accounted for 122 of 126 (97%) FQ-R isolates, vs 1 of 75 (1%) FQ-S isolates ($P < .001$), and among international isolates for 26 of 27 (96%) FQ-R isolates, vs 0 of 8 (0%) FQ-S isolates ($P < .001$). Similarly, among human-source isolates it accounted for 136 of 140 (97%) FQ-R isolates, vs 0 of 32 (0%) FQ-S isolates ($P < .001$), and among non-human-source isolates for 13 of 13 (100%) FQ-R isolates, vs 1 of 51 (2%) FQ-S isolates ($P < .001$). The 13 non-human-source FQ-R *H*30 subclone isolates, all with the *gyrA*1AB/*parC*1aAB combination, represented diverse animal hosts, including dogs, cats, a primate, and a dolphin (data not shown). Regarding cephalosporin resistance, the *H*30 subclone accounted for 92 of 108 (85%) ESBL-positive isolates, vs 57 of 128 (45%) ESBL-negative isolates ($P < .001$), and among ESBL-positive isolates for 63 of 68 (93%) $bla_{\text{CTX-M-15}}$-positive isolates, vs 29 of 40 (73%) $bla_{\text{CTX-M-15}}$-negative isolates ($P < .01$).

ST131's Principal FQ-R *gyrA/parC* Combination Is Confined to ST131

The isolates *gyrA* and *parC* were sequenced also from selected non-ST131 recent clinical isolates, including all 78 non-ST131 FQ-R isolates, plus 63 FQ-S isolates from the same STs. To avoid false inferences of evolutionary relatedness due to repeated occurrence of the same resistance-associated replacement mutations in different lineages, phylogenetic trees were inferred for *gyrA* and *parC* based only on silent SNPs (green: Figure 4), after which the replacement mutations were added to the corresponding tree branches (red: Figure 4).

In contrast to ST131's mutation-evolved *gyrA* and *parC* alleles, the 4 *parC* alleles suspected of being horizontally transferred into ST131 (Figure 3*B*) clearly derived from non-ST131 regions of the *parC* tree (Figure 4). Moreover, horizontal transfer of *gyrA* and *parC* occurred extensively throughout the species, with most alleles appearing in multiple STs (as indicated by the diverse STs listed for certain alleles) and/or in diverse combinations (as indicated by the cross-links connecting certain *gyrA* alleles to multiple *parC* alleles, and vice versa; Figure 4). In contrast, ST131's signature *gyrA*1AB and *parC*1aAB alleles, and their combination (heavy cross-link), occurred only within ST131 (Figure 4).

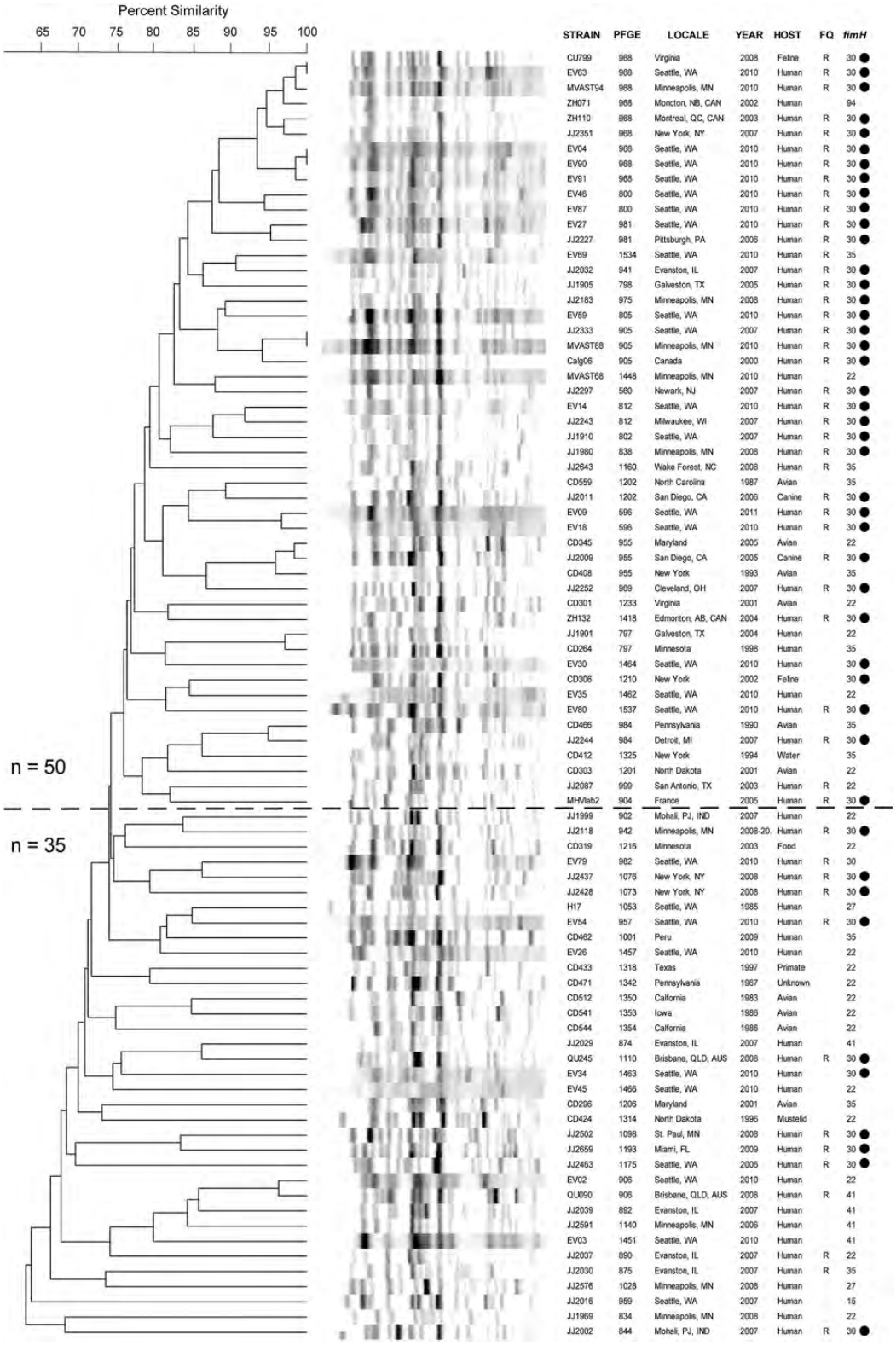


Figure 1. *Xba*I pulsed-field gel electrophoresis-based dendrogram for 85 sequence type ST131 *Escherichia coli* isolates (1967–2011). FQ, fluoroquinolone phenotype (R, resistant; S, susceptible); *fimH*, allele of *fimH* (type 1 fimbrial adhesin); PFGE, pulsotype; Year, year of isolation or submission to reference laboratory. Bullets (to right) mark isolates with the *fimH*30 allele. Horizontal line separates isolates with ≥75% overall profile similarity (top, n = 50) from less-similar isolates (bottom, n = 35).

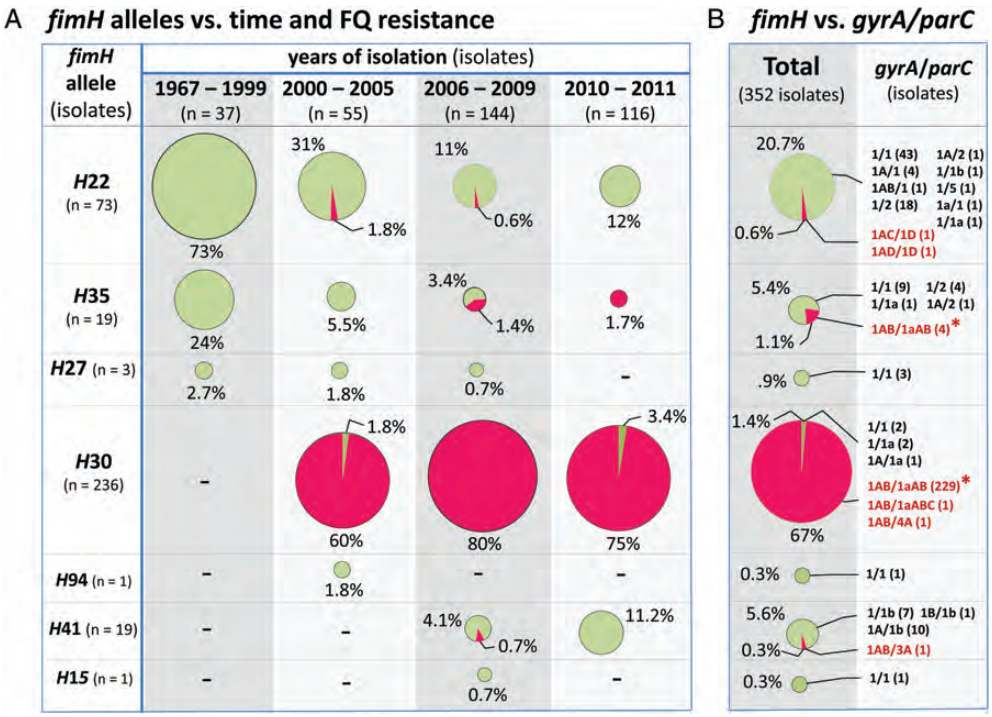


Figure 2. Distribution of fluoroquinolone-susceptible (FQ-S) and resistant (FQ-R) isolates among the 7 *fimH*-based (*H*) ST131 subclones. Area of circle is proportional to the relative abundance of the particular *H* subclone within the particular time period. Percentage values are shown relative to the total no. of isolates within the time period. (*A*) Clonal distribution by time period. (*B*) Overall clonal distribution. The *gyrA* and *parC* allele combinations observed among FQ-S (in green) and FQ-R (in red) isolates are labeled according to the nomenclature shown in Figure 3. Asterisks identify the ST131 isolates' principal *gyrA/parC* allele combination (ie, *gyrA*1AB/*parC*1aAB).

The *H*30 ST131 Subclone Represents the Largest Clonal Expansion in *E. coli* and Is Associated With Extensively Antimicrobial-resistant Infections

When analyzed across the 853 recent clinical *E. coli* isolates, the *H*30 ST131 subclone was strongly associated with FQ resistance, accounting for 86 of 166 (52%) FQ-R isolates but only 4 of 687 (0.6%) FQ-S isolates ($P < .001$). In contrast, the combined non-*H*30 ST131 subclones exhibited no such association, accounting for 2 of 166 (1.2%) FQ-R isolates and 29 of 687 (4.2%) FQ-S isolates ($P > .10$).

Overall, based on *fimH* typing (combined with MSLT), 185 total *H* subclones were identified among the 853 recent clinical *E. coli* isolates. The *H*30 ST131 subclone was by far the most prevalent (10.4% overall), followed only distantly by *H* subclones from historically dominant lineages such as ST95, ST69, ST127, and ST73 (Figure 5). The *H*30 subclone also dominated for resistance to each studied antimicrobial, for example, FQs (52% *H*30) and ceftriaxone (21% *H*30), and for multidrug resistance (Figure 5).

Table 1. Single-Nucleotide Polymorphisms in *gyrA* Among 352 Recent and Historical *Escherichia coli* Isolates of Sequence Type ST131

	Nucleotide at Indicated Position[a] (Associated Amino Acid Shift, if Any)[b]			
gyrA allele	bp 248	bp 259	bp 260	bp 516
1	c	g	a	t
1a				c
1A	t (S83L)			
1AB	t (S83L)	a (D87N)		
1AC	t (S83L)		g (D87G)	
1B		a (D87N)		
1AD	t (S83L)	t (D87Y)		

[a] Only polymorphic sites are shown. Bases are shown for ancestral allele (allele 1) and for other alleles, only if different from allele 1. Blank cells, identity with allele 1.

[b] Amino acid code: D, aspartate; G, glycine; L, leucine; N, asparagine; S, serine; Y, tyrosine.

DISCUSSION

Here we show that, despite the repeated independent emergence of FQ resistance within *E. coli*, most current FQ-R *E. coli* clinical isolates appear to have originated about a decade ago from a single strain that emerged within ST131 as the *fimH*-based *H*30 subclone. This subclone expanded rapidly to become what is now the dominant and most extensively

Table 2. Single-Nucleotide Polymorphisms in *parC* Among 352 Recent and Historical *Escherichia coli* Isolates of Sequence Type ST131

parC allele	Nucleotide at Indicated Position[a] (Associated Amino Acid Shift, if Any)[b]																		
	bp 54	bp 87	bp 94	bp 105	bp 129	bp 238	bp 239	bp 240	bp 251	bp 261	bp 321	bp 348	bp 372	bp 387	bp 391	bp 399	bp 408	bp 411	bp 432	bp 471
1	c	t	c	t	c	a	g	t	a	c	c	g	g	c	c	g	g	g	g	g
1a											g									
1b											t									
1aAB							t (S80I)		t (E84V)		g									
1aABC			t (P32S)				t (S80I)		t (E84V)		g									
1D						c (S80R)														
2					g						t								c	a
3A							t (S80I)				t		t	t	t	a	t	a		
4A	t	c					t (S80I)	c											a	a
5	t	c		c						t		a								

[a] Only polymorphic sites are shown. Bases are shown for ancestral allele (allele 1) and, for other alleles, only if different from allele 1. Blank cells, identity with allele 1.

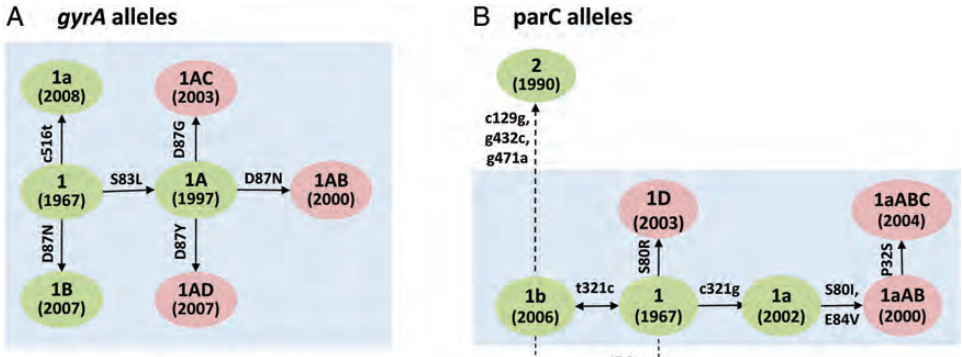
[b] Amino acid codes: E, glutamate; I, isoleucine; P, proline; R, arginine; S, serine; V, valine.

multidrug-resistant lineage of extraintestinal pathogenic *E. coli* worldwide. Horizontal transfer and recombination involving *gyrA* and *parC* were newly identified as a widespread mechanism for acquisition of chromosomal FQ resistance in *E. coli*, including ST131. However, these genetic mechanisms have not affected the dominating *H*30 subclone of ST131.

Although MLST has become the preferred method for clonal typing of bacterial pathogens, MLST-based clonal lineages (ie, STs) of *E. coli* commonly comprise highly heterogeneous strains. For example, ST73, a major ST among extraintestinal pathogenic *E. coli*, also includes nonpathogenic strains Nissle1917 and ABU83972, which are used as probiotics [18, 21]. Recently, we showed that STs can be divided into ecologically distinct subclones by sequencing an internal region of *fimH* [18]. This gene, encoding the type 1 fimbrial adhesin, is subject to more rapid evolution and horizontal gene transfer than the traditional MLST loci, which are expected to evolve neutrally [22]. Our results suggest that FQ resistance, a hallmark phenotype of (clonally diverse) ST131, is associated almost exclusively with a single *fimH*-based subclone, *H*30, carrying a distinct *gyrA* and *parC* allele combination. The close relatedness of *H*30 isolates strongly suggests that, remarkably, the H30 subclone—and, thus, most current FQ-R *E. coli*—arose from a single strain as recently as 1 decade ago. Notably, the single-strain origin of *H*30 subclone was more apparent from sequence analysis of *gyrA* and *parC* (which are widely separated on the chromosome) than from PFGE analysis, consistent with the limited phylogenetic validity of PFGE [7].

The finding of a predominantly single-strain origin of FQ resistance within ST131 and *E. coli* generally is quite surprising. Indeed, FQ resistance can potentially emerge in any *E. coli* strain by appropriate, analogous point mutations in the QRDR of the ubiquitous housekeeping genes *gyrA* and *parC* [6, 23]. Our study confirms this and newly documents that FQ resistance-conferring *gyrA* and *parC* alleles also exhibit extensive horizontal mobility, thereby disseminating FQ resistance rapidly among different *E. coli* STs. Even within ST131, FQ resistance-conferring *parC* alleles have entered multiple times via horizontal transfer, as illustrated in Figure 4. Nevertheless, a single allelic combination—*gyrA*1AB/*parC*1aAB – has achieved predominance within ST131 and, consequently, the species overall.

The observed tight linkage between a single *gyrA*/*parC* combination and the *H*30 ST131 subclone conceivably could be due to this lineage's superior FQ resistance, commensal fitness, and/or pathogenicity, compared with other *E. coli*. Greater FQ resistance, if present, could represent an effect of the distinctive Glu-84-Val *parC* replacement mutation, which occurs in ST131's hallmark *parC*1aAB allele along with the widespread Ser-80-Leu mutation. Further, although plasmid-borne FQ resistance and up-regulated efflux pumps are uncommon in



C gyrA/parC combinations and FQ resistance

parC \ gyrA		1	1A	1AB	1AC	1AD	1B	1a
		-	S83L	S83L, D87N	S83L, D87G	S83L, D87Y	D87N	-
1	-	59	4	1	-	-	-	1
1a	-	4	1	-	-	-	-	-
1b	-	8	10	-	-	-	1	-
2	-	22	2	-	-	-	-	-
5	-	1	-	-	-	-	-	-
1aAB	**S80I, E84E**	-	-	**233**	-	-	-	-
1aABC	S80I, E84E, P32S	-	-	1	-	-	-	-
3A	S80I	-	-	1	-	-	-	-
4A	S80I	-	-	1	-	-	-	-
1D	S80R	-	-	-	1	1	-	-

Figure 3. ST131-associated *gyrA* and *parC* alleles: gene phylogeny and combinations. Green, alleles (or combinations) associated with fluoroquinolone-susceptible isolates. Red, alleles (or combinations) associated with fluoroquinolone-resistant isolates. Single letter amino acid code: G (Gly), D (Asp), E (Glu), I (Ile), L (Lys), N (Asn), P (Pro), R (Arg), S (Ser), V (Val), and Y (Tyr). (*A* and *B*) Phylogeny of the ST131-associated *gyrA* and *parC* alleles. Labels inside circles: allele designations. In parentheses: earliest known year of isolation for the allele. Along the branches: lower-case numbers are nucleotide positions with silent mutations, upper-case numbers are amino acid positions with amino acid replacement mutations. Arrows: putative evolutionary order of mutations (double arrow between allele 1 and 1′ indicates uncertainty of the order). Gray boxes: phylogenetic clades within which nearest alleles differ by no more than one silent nucleotide change. (*C*) *gyrA* and *parC* allele combinations. Numbers inside cells: number of ST131 isolates with the corresponding allele combination. The predominant allele combination among FQ-R isolates is shown in boldface.

ST131 [15, 16], other traits of the *H*30 subclone conceivably could augment its resistance, for example, by blocking FQ entry or increasing intracellular FQ inactivation [6]. Comparative assessment of FQ minimum inhibitory concentrations among FQ-R ST131 vs non-ST131 *E. coli* isolates is needed.

Regarding commensalism, resistance-conferring mutations in housekeeping genes may decrease overall bacterial fitness when antimicrobial selective pressure is absent, unless compensatory mutations have coevolved with the resistance [24], possibly resulting in a competitive advantage in the commensal niche over other *E. coli*. In this regard, the *H*30 subclone's broad host range may also promote its dissemination through the population, both within and among locales [25].

Finally, the *H*30 subclone might be more fit in the pathogenic niche, and this, in combination with FQ resistance, could underlie its rapid rise to clinical dominance over other *E. coli*. The current dominance of the *H*30 subclone clearly surpasses such common lineages as ST69 ("clonal group A"; associated with trimethoprim-sulfamethoxazole resistance [7]) and classic extraintestinal pathogenic lineages ST95, ST73, and ST127. We are currently exploring the successful features of the *H*30 subclone through comparative genomics and molecular epidemiological surveys.

Recognition of the *H*30 ST131 subclone has direct clinical implications. First, rapid diagnostics that detect this single-strain lineage, analogous to those currently used for rapid detection of methicillin-resistant *Staphylococcus aureus*, could allow better targeted selection of empirical antimicrobial therapy for patients in whom FQ-R *E. coli* are of concern [5]. Second, identification of relevant reservoirs and transmission pathways of the *H*30 subclone, and development of effective interventions against them, could limit its spread [3]. Third, development of an effective vaccine directed toward *H*30 subclone-associated antigens (eg, surface-exposed virulence factors, and/or the O25 somatic antigen or H4 flagellar antigen) could help protect at-risk hosts [11, 26]. Thus, focused attention to the highly successful *H*30 ST131 subclone can potentially lead to substantially improved prevention and management of FQ-R *E. coli* infections.

Study limitations included the exclusive focus on *gyrA*/*parC*-mediated FQ resistance, the use of convenience sample historical isolates (albeit broadly distributed by year, locale,

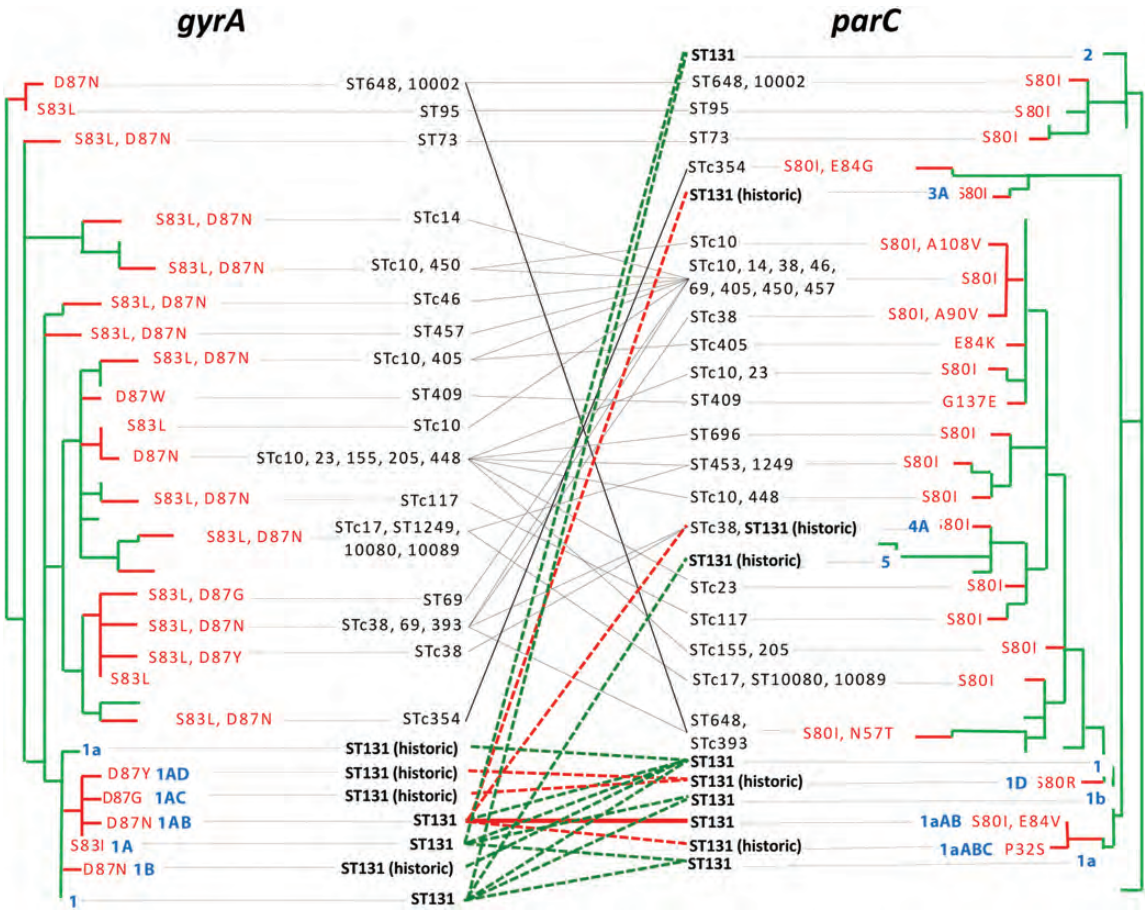


Figure 4. *gyrA* and *parC* phylogenetic trees for ST131 isolates and diverse fluoroquinolone (FQ)-resistant non-ST131 *Escherichia coli* isolates. Trees were built based on silent variation only (green branches); FQ-resistance-determining amino acid replacement changes were then added manually (red branches and labels). STc: ST complexes (groups of closely related STs) within which the indicated alleles are found. Diagonal lines indicate *gyrA*/*parC* combinations (dashed green, in FQ-susceptible ST131 isolates; dashed red, in FQ-R ST131 isolates; solid black, in FQ-R non-ST131 isolates). Blue/boldface labels: the 17 alleles found in ST131, named according to the nomenclature used in Figure 3. "ST131 (historic)" marks ST131-associated alleles found only in historic ST131 isolates.

and source/host), and the current isolates' geographical restriction to 2 US cities (albeit from 5 separate institutions serving distinct patient populations). Strengths include the large and diverse study population for comparison of ST131 and non-ST131 *E. coli*, the use of multiple advanced molecular modalities to define sub-ST131 clonal structure, the clonal phylogenetic analysis of mutations in *gyrA* and *parC*, and the clonal assessment of resistance.

In summary, we provide evidence suggesting that the major source of the current FQ resistance epidemic in *E. coli* is the rapid clonal expansion of a single strain within ST131, which over the past decade has become the most successful lineage of extraintestinal *E. coli* overall, particularly of multidrug-resistant *E. coli*. Although confirmation of these findings in other locales and host populations is needed, this discovery provides a novel perspective on the *E. coli* antimicrobial resistance epidemic, with profound implications and exciting opportunities.

Notes

Acknowledgments. Ruth Anway (Minneapolis VA Medical Center) performed medical record abstraction. Dave Prentiss prepared the dendrogram image. Alena Gileva and Elena Linardopoulou provided excellent technical assistance. Sandip Paul provided computational assistance. Drs. Steve Moseley and Oliwia Zurek provided critical reading and discussion of the manuscript. Walter E. Stamm, MD (deceased) provided formative inspiration, guidance, and support.

Additional providers of historic ST131 isolates and associated data were the RESIST ("Resistance in *E. coli* ST131 as Investigated Using Sequence Typing") Study Group members, who included: Jo Ellen Abraham (Abbott Northwestern Hospital, Minneapolis, MN), Javier Adachi (The University of Texas M. D. Anderson Cancer Center, Houston, TX), Aristides Assimacopoulos (University of South Dakota, Sioux Falls, SD), Robert L. Bergsbaken (Health Partners and Regions Medical Center, St. Paul, MN), Mariana Castanheira (JMI Laboratories, North Liberty, IA), Timothy Cleary (University of Miami, Miami, FL), Michael Cooperstock (University of Missouri School of Medicine, Columbia, MO), Yohei Doi (University of Pittsburgh, Pittsburgh, PA), Paul Edelstein (Hospital of the University of Pennsylvania, Philadelphia, PA), Nancy Hanson (Creighton University, Omaha, NE), John Holter (Veterans Affairs Medical Center,

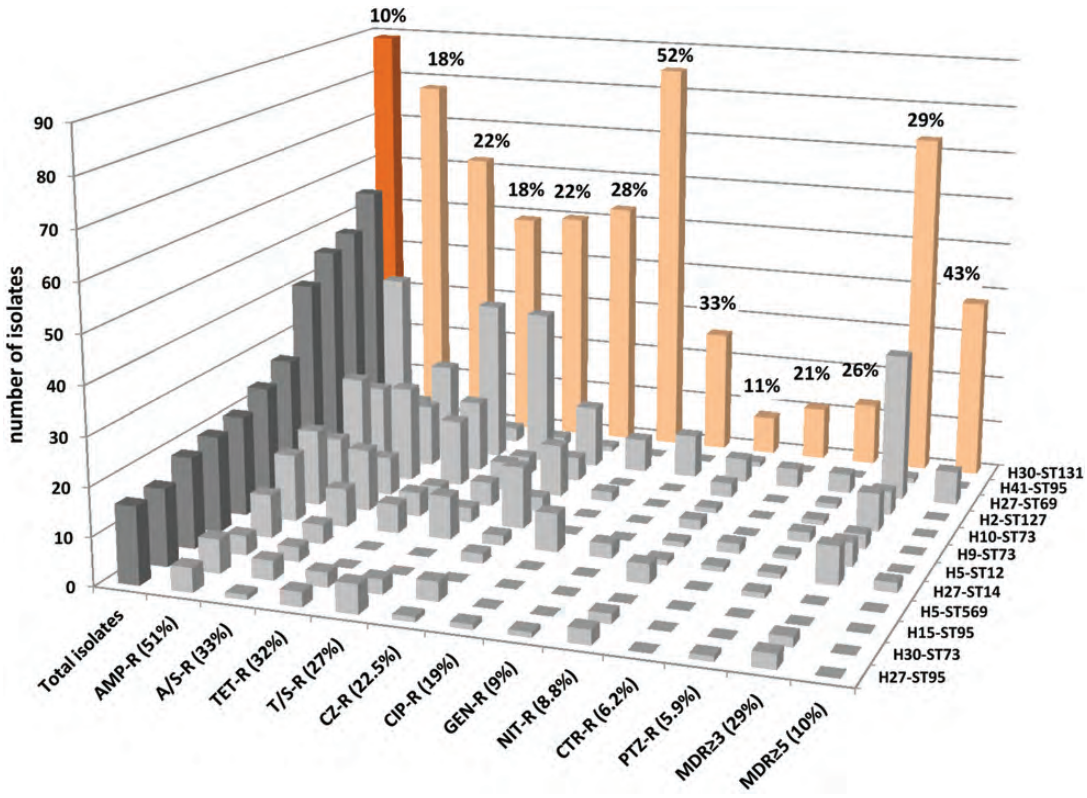


Figure 5. Prevalence of the *H*30 ST131 subclone among 853 recent clinical *Escherichia coli* isolates. Orange, *H*30 ST131 subclone. Gray, 11 most prevalent non-ST131 *H* subclones. Each *H* subclone is labeled along the Z-axis (right side of chart) with its *fimH* allele and ST number. "Total isolates" columns (at left) are darker than the rest. Antimicrobials are listed along the X-axis (front of chart) in descending order of resistance prevalence (shown in parentheses). Y-axis (vertical) gives number of total or resistant isolates. Percentage numbers above the pink *H*30 columns indicate the *H*30 subclone's relative prevalence within each category (total, or specific resistance phenotypes). Abbreviations: AMP, ampicillin; A/S, ampicillin/sulbactam; CIP, ciprofloxacin; CZ, cefazolin; GM, gentamicin; NIT, nitrofurantoin; PTZ, piperacillin/tazobactam; TET, tetracycline; T/S, trimethoprim/sulfamethoxazole. No imipenem resistance was detected. MDR, multidrug resistant (ie, to ≥3 or ≥5 classes, with penicillins and cephalosporins counted separately).

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Financial support. This material is based upon work supported by Office of Research and Development, Medical Research Service, Department of Veterans Affairs, Merit Review grant 1 I01 CX000192 01 (J. R. J.), and NIH ARRA award 1RC4AI092828 (E. V. S.).

Potential conflicts of interest. The following authors report these conflicts of interest: F. C. F.: Consultancy, research grants, and educational presentations with Cepheid. D. C. H.: Research grant from Rib-X Pharmaceuticals, consultancies with Fab Pharma and Pfizer, speakers bureau with Pfizer. J. R. J.: Research grants and contracts from Merck, Rochester Medical, and Syntiron; patent pending for detection of the H30 ST131 subclone. L. B. P.: Patent pending for detection of the H30 ST131 subclone. M.-H. N.-C.: Expert testimony for Region Wallon; research grant from Direction de la Recherche Clinique AP-HP; lectures for Novartis; travel reimbursement for 2012 ICAAC speaker. E. V. S.: Research grant from Group Health; patent pending for detection of the H30 ST131 subclone. D. T.: Research grant from ARC Linkage; consultancy from Pfizer and Bayer; speaker for Bayer Animal Health. C. U.: Consultancy with Pfizer, lecture/speaker for Forest Pharmaceuticals, Cubist, and Pfizer. G. Z.: Research grants from Achaogen, Merck, Pfizer, Cubist, Affinium, and Sunovion. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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