Clinical Infectious Diseases

BRIEF REPORT

Rapid and Extensive Expansion in the United States of a New Multidrug-resistant *Escherichia coli* Clonal Group, Sequence Type 1193

Veronika L. Tchesnokova,[1] Elena Rechkina,[2] Lydia Larson,[1] Kendra Ferrier,[1] Jamie Lee Weaver,[1] David W. Schroeder,[1] Rosemary She,[3] Susan M. Butler-Wu,[3] Maria E. Aguero-Rosenfeld,[4] Danielle Zerr,[5] Ferric C. Fang,[6] James Ralston,[7,8] Kim Riddell,[7] Delia Scholes,[7,8] Scott Weissman,[5] Kaveri Parker,[2] Brad Spellberg,[3] James R. Johnson,[9] and Evgeni V. Sokurenko[1]

[1]Department of Microbiology, University of Washington School of Medicine, and [2]ID Genomics, Inc, Seattle; [3]Keck School of Medicine, University of Southern California, Los Angeles; [4]Langone Hospital, New York University; and [5]Seattle Children's Hospital, [6]Department of Microbiology and Harborview Medical Center, University of Washington School of Medicine, [7]Kaiser Permanente Washington, and [8]Kaiser Permanente Washington Research Institute, Seattle; and [9]Veterans Affairs Medical Center and University of Minnesota, Minneapolis

We describe the rapid and ongoing emergence across multiple US cities of a new multidrug-resistant *Escherichia coli* clone—sequence type (ST) 1193—resistant to fluoroquinolones (100%), trimethoprim-sulfamethoxazole (55%), and tetracycline (53%). ST1193 is associated with younger adults (age <40 years) and currently comprises a quarter of fluoroquinolone-resistant clinical *E. coli* urine isolates.

Keywords. urinary tract infections; *Escherichia coli*; fluoroquinolone resistance.

Antibiotic resistance is rising globally at an alarming rate. Urinary tract infections, caused primarily by *Escherichia coli*, are a major reason for antibiotic use, and fluoroquinolones (FQs) are among the most commonly used agents. One decade ago, an FQ-resistant (FQ-R) multilocus sequence type (ST) of *E. coli*, ST131, was found to have emerged globally in a pandemic fashion. Subsequent studies showed that the ST131 pandemic is driven by the *H*30 subclone, which emerged in the late 1990s, likely on the US East Coast [1]. No other *E. coli* clonal group has yet been identified that matches the rapid expansion and dominance of *H*30, which limits our ability to pinpoint the mechanisms whereby successful drug-resistant *E. coli* strains can emerge and disseminate. However, starting in 2012, reports from individual hospitals in the United States, Australia, China, South Korea, and Norway have documented the frequent occurrence among FQ-R *E. coli* isolates of the clonal group ST1193 [2–6]. Here, we analyze whether, besides *H*30, any other clonal expansion is evident among FQ-R *E. coli* isolates collected in the course of a multicenter surveillance study.

METHODS

As part of a multicenter surveillance study, 6349 consecutive clinical *E. coli* isolates from urine (96.0%), blood (3.5%), or wounds (0.5%) were obtained in 2016–2017. The study involved 9 clinical microbiology laboratories (sites) in 4 geographically dispersed cities, including Seattle, Washington (Kaiser Permanente Washington [KPWA], Seattle Children's Hospital, and Harborview Medical Center); Los Angeles, California (Keck and Los Angeles County + University of Southern California medical centers); Minneapolis, Minnesota (Veterans Affairs and Hennepin medical centers); and New York, New York (New York University Langone Medical Center and Langone Brooklyn Hospital). Susceptibility to 12 antibiotics, representing 8 drug classes, and production of extended-spectrum β-lactamases (ESBLs) were tested by disk diffusion according to Clinical and Laboratory Standards Institute guidelines [7], with intermediate isolates designated as resistant. FQ-R isolates were analyzed further for clonal identity by using a single-nucleotide polymorphism (SNP)–based clonotyping test (7-SNP test) [8] and *fumC/fimH* sequence typing, which identified the isolate's ST-*fimH* clonal group [9]. Mutations in the quinolone resistance–determining regions (QRDRs) of *gyrA* and *parC* were identified by sequencing. Presence of the *fimH*64 allele was identified in quantitative polymerase chain reaction (PCR) using a designed *fimH*64 SNP-specific primer, *H*64-F2, 5′-GAACGGATAAGCCGTGACT-3′ (forward), in combination with previously described primer 488/483-R, 5′-TCTGCGGTTGTGCCGGATAGG-3′ (reverse) [8], which yields a 316-bp PCR product. EnteroBase was used to identify the differences between the ST14 and ST1193 multilocus sequence type (MLST) alleles (https://enterobase.warwick.ac.uk/species/ecoli).

At each study site, the prevalence of *H*30 and ST1193 was compared statistically using the likelihood ratio test. To compare current and historic prevalence values for *H*30 and ST1193, we used urine *E. coli* isolates from a previously described study done in 2011 at 4 of the present study sites (KPWA, Harborview, Seattle Children's Hospital, and Veterans Affairs) according to the same protocol as used here [10]. Temporal changes in clonal group prevalence among FQ-R *E. coli* isolates were estimated using multiple logistic regression, with by-site adjustment for either 4 sites (2011 to 2016–2017 analysis) or 8 sites (2016–2017 analysis). Association of FQ-R *E. coli* isolates with age was estimated

Received 3 May 2018; editorial decision 21 June 2018; accepted 27 June 2018; published online June 29, 2018.

Correspondence: E. V. Sokurenko, Department of Microbiology, University of Washington School of Medicine, Box 357735, Seattle, WA 98195-7735 (evs@uw.edu).

Clinical Infectious Diseases® 2019;68(2):334–7

DOI: 10.1093/cid/ciy525

using data from the above-described 2011 study and a 2015 study performed at KPWA [10, 11]. A 2-sample Kolmogorov-Smirnov (K-S) test was used to evaluate differences in age distribution for patients with *H*30 vs ST1193; the binary age cutoff was chosen based on the age with the largest K-S statistic [12]. Associations of clonal background with age group were assessed using a χ^2 test; multiple logistic regression was used to account for possible confounding due to different studies. Resistance prevalence among *H*30 and ST1193 isolates from 9 sites (2016–2017) was compared using a χ^2 or 2-tailed Fisher exact test, as appropriate. Statistical analysis was performed using Stata/IC 14.0 software (StataCorp, College Station, Texas).

RESULTS

Of the 6349 total *E. coli* study isolates, 1314 (20.7%) were FQ-R. The FQ-R isolates represented 45 clonal groups overall (10–28 per site). At each site, the most prevalent clonal group was *H*30 (per-site mean, 45.4%), and the second most prevalent was ST1193 (per-site mean, 23.2%) (Table 1). Although *H*30 was significantly more prevalent than ST1193 at 7 of 9 sites ($P < .05$)—including at least 1 per city—at the remaining 2 sites, each in a different city, the prevalence difference between *H*30 and ST1193 was nonsignificant (Table 1). Collectively, *H*30 and ST1193 accounted on average for 68.6% of FQ-R isolates per site (range, 59.1%–79.8%). By contrast, the next 3 most prevalent 7-SNP clonotypes, corresponding to ST405-*fimH*27, ST10-*fimH*54, and ST131-*fimH*41, each accounted for only approximately 3% of FQ-R isolates and were not all found at some of the study sites (not shown).

Between 2016 and 2017, the prevalence of *H*30 did not change significantly, either overall (means, 45.8% [2016] vs 46.1% [2017]) (Table 1) or at any single site (Supplementary Table 1). By contrast, the prevalence of ST1193 increased both

Table 1. Comparison of Fluoroquinolone-resistant (FQ-R) *Escherichia coli* Clones *H*30 and Sequence Type 1193 by Prevalence Among Other FQ-R *E. coli* by Collection Site, Year, Patients' Age, Clinical Source, and Prevalence of Resistance to Other Antibiotics

Category	Specific Group (Total No. of Isolates)	*H*30[a]	ST1193[a]	*P* Value[b]
Site[b]	Total (N = 1314)[c]	589 (45.5 ± 5.8)	301 (23.2 ± 7.2)	<.001
	KPWA (n = 308)	121 (39.3)	72 (23.4)	<.001
	Harborview Medical Center (n = 183)	86 (47.0)	60 (32.8)	.031
	Seattle Children's Hospital (n = 163)	69 (42.3)	37 (22.7)	.002
	Minneapolis VA Medical Center (n = 147)	74 (50.3)	20 (13.6)	<.001
	Hennepin Medical Center (n = 152)	79 (52.0)	38 (25.0)	<.001
	Keck USC Medical Center (n = 139)	63 (45.3)	21 (15.1)	<.001
	LAC + USC Medical Center (n = 93)	33 (35.5)	22 (23.7)	.14
	NYU Langone Medical Center (n = 80)	42 (52.5)	14 (17.5)	<.001
	NYU Langone Brooklyn Hospital (n = 49)	22 (44.9)	17 (34.7)	.42
Year[b,c,d]	2011 (n = 209)	107 (52.8 ± 5.3)	9 (3.4 ± 1.3)	<.001
	2016–2017 (n = 801)	350 (44.7 ± 2.4)	189 (23.1 ± 3.9)	
	2016 (n = 676)	303 (45.8 ± 2.8)	124 (18.4 ± 1.6)	.018
	2017 (n = 589)	264 (46.1 ± 2.7)	160 (25.9 ± 3.2)	
Age, y[b]	18–40 (n = 70)	28 (40.0)	9 (12.9)	.002
	>40 (n = 185)	116 (62.7)	8 (4.3)	
Specimen[b]	Urine (n = 1259)	552 (43.8)	293 (23.3)	.023
	Blood (n = 47)	31 (66.0)	6 (12.8)	
Resistance to antibiotics[a,b]	Amoxicillin-clavulanate	296 (50.3)	75 (24.9)	<.001
	Cefazolin	256 (43.6)	38 (12.7)	<.001
	TMP-SMX	276 (46.9)	163 (54.2)	.019
	Nitrofurantoin	39 (6.6)	19 (6.3)	.96
	Imipenem	1 (0.2)	1 (0.3)	.78
	Tetracycline	262 (44.7)	157 (52.2)	.016
	ESBL producer	190 (32.4)	24 (8.0)	<.001

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ESBL, extended-spectrum β-lactamase; KPWA, Kaiser Permanente Washington; LAC, Los Angeles County; NYU, New York University; ST, sequence type; TMP-SMX, trimethoprim-sulfamethoxazole; USC, University of Southern California; VA, Veterans Affairs.

[a]For each category except resistance to antibiotics, the percentage of isolates belonging to a specific clone is given in parenthesis; for resistance to antibiotics other than FQ the number of resistant isolates within a specific clone is given with % from all isolates belonging to this clone in parenthesis.

[b]*P* values indicate significance of the difference between *H*30 and ST1193 characteristics, estimated in likelihood ratio test for prevalence by site; in logistic regression with adjustment by site for time-dependent changes; in χ^2 test for association with specific age group or specific specimen; in χ^2 or, if appropriate, 2-tailed Fisher exact test for prevalence of resistance to other antibiotic classes.

[c]For the total prevalence, the mean ± standard error of the by-site prevalence (%) is given in parentheses.

[d]Prevalence by year is calculated for 4 sites when 2011 is compared to combined 2016–2017, and for 8 sites when 2016 is compared to 2017.

overall (from 18.4% [2016] to 25.9% [2017]; $P < .001$) and at 6 of 8 sites.

At the 4 sites that provided both historical (2011) and recent (2016–2017) FQ-R isolates (see Methods), between 2011 and 2016–2017 the prevalence of *H*30 did not change significantly, either overall (average by-site prevalence 52.8% in 2011 vs 44.7% in 2016–2017; $P = .06$) (Table 1) or at individual sites (Supplementary Table 1). By contrast, during this interval ST1193 exhibited a 7-fold overall prevalence increase, from negligible in 2011 (3.4%) to 23.4% in 2016–2017 ($P < .001$), and a significant increase at each of the 4 sites ($P < .05$).

According to the retrospective analysis of 2 previous studies (see Methods), patients with ST1193 were significantly younger on average than patients with *H*30 ($P = .034$). The largest K-S statistic (D = .339) was around age 40, with ST1193 isolated significantly more frequently from patients aged 18–40 years than *H*30 ($P = .002$; Table 1) or other FQ-R isolates combined (61 of 238 patients [34.5%]; $P = .015$). With multivariable adjustment by the study, ST1193 remained highly associated with younger age in comparison to *H*30 (odds ratio, 4.3; $P = .007$).

Among the present 1306 FQ-R isolates, we compared the frequency of *H*30 and ST1193 in relation to urine vs blood source. Whereas *H*30 was 1.5 times more prevalent among blood isolates than urine isolates, ST1193 exhibited an opposite association, being twice as prevalent among urine isolates as blood isolates (Table 1). (Due to the 2016–2017 study design, clinical source could not be adjusted for age or other patient's conditions.)

As compared with *H*30 isolates, ST1193 isolates were less frequently resistant to amoxicillin-clavulanate and cefazolin or produced ESBLs, but more frequently were resistant to trimethoprim-sulfamethoxazole and tetracycline (Table 1). Despite these differences, both clonal groups exhibited a similarly high prevalence of multidrug resistance, a similarly low prevalence of nitrofurantoin resistance, and nearly universal susceptibility to imipenem.

Sequence analysis of *gyrA* and *parC* among 102 randomly selected ST1193 isolates revealed QRDR mutations in both genes (S83L and D87N in *gyrA*, S80I in *parC*), indicating that, as with *H*30, FQ resistance within ST1193 is of chromosomal origin. Furthermore, according to the MLST database, ST1193 belongs to the ST14 clonal complex within *E. coli* phylogroup B2 and, across the 7 MLST loci, differs from ST14 by only 1 SNP (a nonsynonymous g551a mutation in *icd*, producing a G184D amino acid substitution), indicating that ST1193 is a recent evolutionary derivative of ST14. Members of ST14, in contrast to ST1193, are almost all fluoroquinolone susceptible (data not shown). Furthermore, according to *fumC*/*fimH* sequence typing, ST1193 carries *fimH* allele number 64, which differs from allele number 27 of ST14 by only 1 SNP (c311t, producing a P104L substitution). The designed *fimH*64-specific PCR test (see Methods) was positive for all 75 randomly selected ST1193 isolates, but negative for 623 randomly selected FQ-susceptible isolates from the current study. Thus, *fimH*64 could be a highly specific marker for ST1193, a clonal group that might be entirely FQ-R.

DISCUSSION

This multicenter molecular surveillance study of FQ-R *E. coli* clinical isolates establishes the widespread occurrence of ST1193 across the United States as an important and growing contributor to the FQ-R *E. coli* population. As there are already reports of ST1193 being isolated in hospitals in Europe and Asia, it is likely to be a pandemic clonal group similar to *H*30.

The emergence of ST1193 represents the only known large clonal expansion of FQ-R *E. coli* isolates apart from the global spread of ST131 *H*30 over the last 2 decades. The rise of ST1193 apparently began more recently than that of *H*30, and in contrast to the *H*30 expansion, which appears to have plateaued, could still be ongoing rapidly.

Clinically, ST1193 differs from *H*30 in targeting younger patients and seeming to have less tendency to be isolated from blood; this, however, requires further investigation. In addition to being FQ-R, ST1193 isolates are often co-resistant to trimethoprim-sulfamethoxazole and tetracycline, but currently (unlike *H*30) remain susceptible to most β-lactam antibiotics. Discovery of the basis for the global expansion of ST1193 could provide insights into how successful clonal groups of multidrug-resistant *E. coli* emerge and what interventions could limit their spread.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Professor Steve Moseley and Dr Dagmara Kisiela for scientific advice and critical proofreading of the manuscript; clinical microbiological laboratories personnel in Seattle, Los Angeles, New York, and Minneapolis for assistance in collection of isolates; and Matthew Radey, Huxley Smart, Hovhaness Avagyan, Inga Avagyan, and Thalia Solyanik for technical assistance at the University of Washington laboratory and ID Genomics.

Financial support. This work was supported by National Institutes of Health (grant numbers R01AI106007 to E. V. S., and R41AI116114 and R42 AI116114-02 to ID Genomics and E. V. S.); the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (grant number 1 I01 CX000192 01 to J. R. J.); the Partnership for Innovation Award, Group Health Foundation, and Development Fund Award, Group Health Research Institute; and the Gerber Foundation (to E. V. S.).

Potential conflicts of interest. J. R. J. has received research funding from Achaogen, Allergan, Merck, Syntiron, and Tetraphase; is a consultant for Janssen/Crucell and Syntiron; and has patent applications for tests to detect *E. coli* strains. V. L. T. and E. V. S. have patent applications to detect *E. coli* strains. E. V. S. is a major shareholder in ID Genomics. B. S. has received grants and nonfinancial support from Nabriva, Pfizer, Cempra, Bayer, Forge, Shionogi, Alexion, Synthetic Biologics, Paratek, Ovagene, Bioversys, Acurx, Motif, BioAIM, Synthetic Biologics, Mycomed, and

ExBaq. F. C. F. has received grants, personal fees, and nonfinancial support from Cepheid and BioFire; grants and nonfinancial support from ELITech; nonfinancial support from Luminex; and personal fees from the Infectious Diseases Society of America. 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.

References

1. 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.
2. Jørgensen S, Sunde M, Berg E, et al. Fluoroquinolone resistant *Escherichia coli* ST1193 – another global successful clone? 27th ECCMID **2017**; P0204.
3. Kim Y, Oh T, Nam YS, Cho SY, Lee HJ. Prevalence of ST131 and ST1193 among bloodstream isolates of *Escherichia coli* not susceptible to ciprofloxacin in a tertiary care university hospital in Korea, 2013–2014. Clin Lab **2017**; 63:1541–3.
4. Platell JL, Trott DJ, Johnson JR, et al. Prominence of an O75 clonal group (clonal complex 14) among non-ST131 fluoroquinolone-resistant *Escherichia coli* causing extraintestinal infections in humans and dogs in Australia. Antimicrob Agents Chemother **2012**; 56:3898–904.
5. Wu J, Lan F, Lu Y, He Q, Li B. Molecular characteristics of ST1193 clone among phylogenetic group B2 non-ST131 fluoroquinolone-resistant *Escherichia coli*. Front Microbiol **2017**; 8:2294.
6. Xia L, Liu Y, Xia S, et al. Prevalence of ST1193 clone and IncI1/ST16 plasmid in *E. coli* isolates carrying blaCTX-M-55 gene from urinary tract infections patients in China. Sci Rep **2017**; 7:44866.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 20th information supplement. Vol 30. Wayne, PA: CLSI, **2010**.
8. Tchesnokova V, Avagyan H, Billig M, et al. A novel 7-single nucleotide polymorphism-based clonotyping test allows rapid prediction of antimicrobial susceptibility of extraintestinal *Escherichia coli* directly from urine specimens. Open Forum Infect Dis **2016**; 3:ofw002.
9. Weissman SJ, Johnson JR, Tchesnokova V, et al. High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. Appl Environ Microbiol **2012**; 78:1353–60.
10. Tchesnokova V, Billig M, Chattopadhyay S, et al. Predictive diagnostics for *Escherichia coli* infections based on the clonal association of antimicrobial resistance and clinical outcome. J Clin Microbiol **2013**; 51:2991–9.
11. Tchesnokova V, Avagyan H, Rechkina E, et al. Bacterial clonal diagnostics as a tool for evidence-based empiric antibiotic selection. PLoS One **2017**; 12:e0174132.
12. Nakas CT, Alonzo TA, Yiannoutsos CT. Accuracy and cut-off point selection in three-class classification problems using a generalization of the Youden index. Stat Med **2010**; 29:2946–55.