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Clinical and Molecular Epidemiology of *Escherichia coli* Sequence Type 131 among Hospitalized Patients Colonized Intestinally with Fluoroquinolone-Resistant *E. coli*

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This study examined molecular and epidemiologic factors associated with *Escherichia coli* sequence type 131 (ST131) among hospitalized patients colonized intestinally with fluoroquinolone (FQ)-resistant *E. coli* between 2002 and 2004. Among 86 patients, 21 (24%) were colonized with ST131. The proportion of ST131 isolates among colonizing isolates increased significantly over time, from 8% in 2002 to 50% in 2004 ($P = 0.003$). Furthermore, all 19 clonally related isolates were ST131. Future studies should identify potential transmissibility differences between ST131 and non-ST131 strains.

The increase in fluoroquinolone (FQ)-resistant *Escherichia coli* (FQREC) over the past decade has been attributed mainly to the widespread emergence of a single disseminated *E. coli* clonal group, sequence type 131 (ST131) (1–4), which frequently exhibits multidrug resistance, most notably to FQs and extended-spectrum cephalosporins (ESCs) (5). *E. coli* isolates within phylogenetic group B2, including specifically *E. coli* ST131, may have a greater capacity for successful and persistent colonization of the gastrointestinal tract (6, 7). However, FQ resistance mechanisms associated with ST131 status have only been characterized in clinical isolates (8). In addition, few data exist on epidemiologic risk factors for colonization or infection with *E. coli* ST131 (8–10). Therefore, we sought to evaluate the association between *E. coli* ST131 and molecular and epidemiologic characteristics among intestine-colonizing FQREC isolates from a previously described population of hospitalized patients (11, 12) that were assessed during the initial rapid rise in prevalence of *E. coli* ST131.

As previously described (11, 12), three annual fecal surveillance surveys were performed hospital wide at two university-affiliated hospitals during 2002, 2003, and 2004. For the present study, each subject could be included only once, with inclusion of only the first sample for each subject. The University of Pennsylvania Institutional Review Board approved the study.

FQREC was defined as isolates exhibiting a levofloxacin MIC of ≥8 μg/ml. Detection of FQREC from fecal samples and evaluation for specific mechanisms of FQ resistance were performed as previously described (11–13). Overexpression of the multidrug efflux pump AcrAB was measured using the organic solvent tolerance (OST) assay (14, 15). Genetic relatedness of *E. coli* isolates was determined by pulsed-field gel electrophoresis (PFGE) analysis (11), with profiles analyzed using Fingerprinting II Informatix software v3.0 (Bio-Rad Laboratories, Inc., Hercules, CA) and interpreted according to established criteria (16). Clonal relatedness was defined as >80% similarity.

The major *E. coli* phylogenetic group was determined by triplex PCR (17). Group B2 isolates were evaluated for ST131 status by detection of ST131-specific single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* (18) and for the O25b *rfb* genotype (19). The *H*30 ST131 subclone was identified by established PCR-based detection of subclone-specific SNPs in *fimH* (5, 8). For the (CTX-M-15-associated) *H*30-Rx subclone within the *H*30 ST131 subclone (20), primers were used that detect an *H*30-Rx-specific SNP (G723A) within the allantoin protein-encoding gene, *ybbW* (21). Isolates were screened for $bla_{\text{CTX-M-15}}$ by PCR (21), tested for susceptibility to antibiotic agents by use of the semiautomated Vitek 2 identification and susceptibility system (bioMérieux, Durham, NC), and interpreted according to Clinical and Laboratory Standards Institute criteria. Clinical data were abstracted as previously described from the Pennsylvania Integrated Clinical and Administrative Research Database (13).

Associations between *E. coli* ST131 and molecular and clinical

TABLE 1 Microbiologic characteristics of 86 ST131 and non-ST131 *E. coli* fecal isolates from patients colonized with fluoroquinolone-resistant *E. coli*[a]

Variable	Result for: ST131 ($n = 21$)	Non-ST131 ($n = 65$)	*P* value
No. (%) with *H*30	21 (100)	NA	
No. (%) with *H*30-Rx	7 (33)	NA	
Median levofloxacin MIC (IQR)	32 (32–32)	32 (32–32)	0.64
Median no. of *gyrA* mutations (IQR)	2 (2–2)	2 (2–2)	0.42
Median no. of *parC* mutations (IQR)	2 (2–2)	1 (1–1)	<0.001
No. (%) with OST-positive status	3 (14)	38 (59)	<0.001
No. (%) with $bla_{\text{CTX-M-15}}$	0 (0)	1 (2)	>0.99

[a] Data are presented as number (percentage) of isolates unless noted otherwise. NA, not applicable; IQR, interquartile range; OST, organic solvent tolerance.

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TABLE 2 Antibiotic resistance in relation to ST131 status among 86 fluoroquinolone-resistant fecal *Escherichia coli* isolates from colonized hospital inpatients

Antibiotic	Prevalence of resistance, no. of isolates (column %)		
	ST131 ($n = 21$)	Non-ST131 ($n = 65$)	*P* value
Ampicillin-sulbactam	19 (92)	46 (72)	0.08
Cefazolin	6 (29)	20 (32)	>0.99
Ceftazidime	1 (5)	12 (19)	0.17
Ceftriaxone	1 (5)	11 (17)	0.28
Gentamicin[a]	7 (33)	22 (34)	>0.99
Imipenem	1 (5)	0 (0)	0.24
Piperacillin-tazobactam	3 (14)	4 (6)	0.35
Trimethoprim-sulfamethoxazole	12 (57)	39 (60)	>0.99

[a] Isolate resistance profiles were the same for tobramycin as for gentamicin.

characteristics were determined. Categorical variables were compared using the Fisher exact test, and continuous variables were compared using the Wilcoxon rank sum test. Multivariable analyses were performed using multiple logistic regression (22), with calculation of adjusted odds ratios (ORs) with 95% confidence intervals (CIs). A stepwise selection procedure was used, with variables with *P* values of <0.20 on bivariable analyses considered candidate variables and maintained in the final model if their inclusion was statistically significant on likelihood ratio testing (23). For all calculations, a two-tailed *P* value of <0.05 was considered significant. All calculations were performed using STATA v13.0 (StataCorp LP, College Station, TX).

Over the 3-year study period, a total of 89 (11.5%) of 774 unique subjects were colonized with FQREC (12, 13). For the present study, 86 FQREC isolates (each representing a unique study patient) constituted the study population and were characterized further. Of these, 21 (24%) were identified as ST131 by dual-SNP PCR (Table 1). All 21 ST131 isolates represented the *H*30 *fimH*-based subclone within ST131, and 7 (33%) of these belonged specifically to the (CTX-M-15-associated) *H*30-Rx subset within *H*30.

ST131 and non-ST131 isolates did not differ significantly for the levofloxacin MIC or for the number of mutations in *gyrA* (Table 1). However, these groups differed for specific *gyrA* mutations; all 21 ST131 isolates, but only 51 (79%) of non-ST131 isolates, exhibited Ser83→Leu and Asp87→Asn mutations ($P = 0.02$). ST131 isolates had a greater number of replacement *parC* mutations compared to non-ST131 isolates. The most common combination of replacement *parC* mutations among ST131 isolates (88%) was Ser80→Ile and Glu84→Val. Results of antibiotic susceptibility testing are shown in Table 2. ST131 isolates demonstrated a low prevalence of ESC resistance, with the single ESC-resistant isolate belonging to the *H*30-Rx subgroup within *H*30.

Among the 71 FQREC isolates that could be successfully characterized by PFGE (13), 19 demonstrated clonal relatedness; all of these were ST131. Thus, 19 (90%) of 21 ST131 isolates were clonally related, compared to 0 (0%) of 50 non-ST131 isolates ($P < 0.001$). The 21 ST131 study isolates' PFGE profiles were subse-

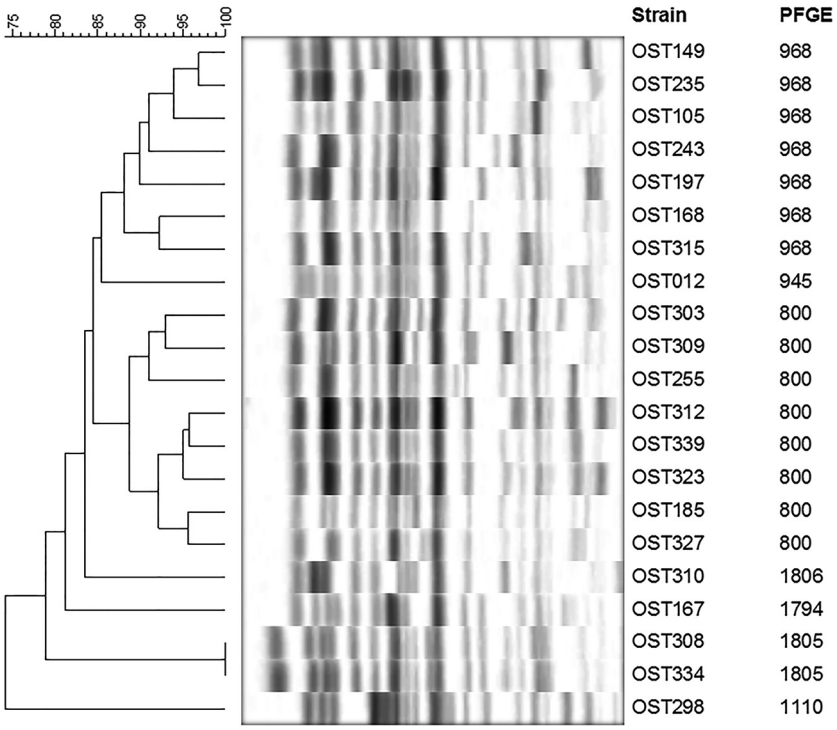

FIG 1 Pulsed-field gel electrophoresis (PFGE) profiles of 21 ST131 fluoroquinolone-resistant fecal *Escherichia coli* isolates. Data columns to the right of the PFGE profiles show the isolate number (left) and pulsotype (right). Pulsotypes were assigned by comparison to an existing large private PFGE profile reference library (24). Pulsotypes 968, 945, 800, and 1110 ($n = 17$ isolates) were already established within the reference library; pulsotypes 1806, 1794, and 1805 ($n = 4$ isolates) were newly assigned here. Note that since profiles were classified as to pulsotype based on ≥94% profile similarity to an index isolate's profile, within the same pulsotype, certain profiles could exhibit as little as 88% similarity.

TABLE 3 Bivariable analyses of risk factors for ST131 among hospital inpatients colonized intestinally with fluoroquinolone-resistant *E. coli*[a]

Variable	Result for: ST131 ($n = 21$)	Non-ST131 ($n = 65$)	OR (95% CI)	*P* value
Yr of culture:				
2002	2 (8)	23 (92)		
2003	7 (19)	30 (81)		0.003
2004	12 (50)	12 (50)		
Mean age (SD), yr	61.0 (15)	63.8 (16)		0.53
No. (%) female	6 (29)	28 (43)	0.53 (0.15–1.69)	0.31
No. (%) nonwhite	10 (48)	39 (60)	0.61 (0.20–1.84)	0.45
No. (%) in surgical service	9 (43)	17 (26)	2.12 (0.66–6.60)	0.18
No. (%) with nosocomial onset	14 (74)	41 (71)	1.16 (0.33–4.77)	>0.99
No. (%) with admission to hospital 2	5 (24)	20 (31)	0.70 (0.18–2.40)	0.59
Mean (SD) hospital day of sampling	14.4 (14)	13.4 (24)		0.15
No. (%) in ICU on culture date	3 (14)	11 (17)	0.82 (0.13–3.60)	>0.99
No. (%) with diabetes mellitus	5 (24)	26 (40)	0.47 (0.12–1.57)	0.20
No. (%) with malignancy	5 (24)	21 (32)	0.65 (0.17–2.22)	0.59
No. (%) with renal insufficiency	2 (10)	13 (20)	0.42 (0.04–2.16)	0.34
Mean (SD) Charlson comorbidity score	2.2 (2)	3.9 (4)		0.09
No. (%) with chemotherapy ≤30 days prior to sampling	3 (14)	5 (8)	2.00 (0.28–11.4)	0.40
No. (%) with immunosuppression ≤30 days prior to sampling	3 (14)	10 (15)	0.92 (0.15–4.12)	>0.99
No. (%) taking antibiotics ≤30 days prior to sampling[b]				
Any antibiotic	13 (62)	35 (54)	1.39 (0.46–4.42)	0.62
1st-generation cephalosporin[c]	7 (33)	12 (19)	2.21 (0.61–7.47)	0.22
Levofloxacin	10 (48)	13 (20)	3.64 (1.11–11.7)	0.02

[a] Data are presented as numbers (percentages) except where noted. OR, odds ratio; CI, confidence interval; SD, standard deviation; ICU, intensive care unit.
[b] Only results for antibiotics with a *P* value of <0.30 are shown.
[c] Cefazolin or cephalexin.

quently compared to an existing reference library comprising PFGE profiles from 1,292 ST131 isolates (mostly human clinical isolates) (24). The predominant pulsotypes (defined as having ≥94% similarity to an index isolate) represented among the present ST131 colonizing isolates were 968 and 800 (33% and 38% of isolates, respectively) (Fig. 1). Pulsotypes 968 and 800 were also the two most common pulsotypes in the reference library (29% and 12% of isolates, respectively).

The by-year prevalence of ST131 rose steadily across the study period (8% in 2002, 19% in 2003, and 50% in 2004; $P = 0.003$, chi-square test for trend) (Table 3). In multivariable analyses (Table 4), only study year was an independent risk factor for having an ST131 isolate (OR, 3.89; 95% CI, 1.71 to 8.81; $P = 0.001$). The dramatically increasing proportion of ST131 colonizing isolates over time, which resulted in ST131 comprising half of FQREC isolates during the final year, is in concordance with the increasing prevalence of *E. coli* ST131 among clinical isolates over the past decade (1–3). This suggests that ST131 has emerged as both a highly prevalent pathogen causing clinical infections and a successful colonizer in the hospital setting.

TABLE 4 Multivariable model of risk factors for recovery of ST131 isolates among hospital inpatients colonized with fluoroquinolone-resistant *E. coli*

Variable	OR (95% CI)[a]	*P* value
Receipt of levofloxacin ≤30 days prior to sampling	2.28 (0.68–7.68)	0.18
Yr of culture	3.89 (1.71–8.81)	0.001
Charlson comorbidity score	0.80 (0.64–1.01)	0.055

[a] OR, odds ratio; CI, confidence interval.

A majority of ST131 isolates exhibited a specific combination of *gyrA* and *parC* replacement mutations, which confirms a close association of the *H*30 ST131 subclone with a distinctive *gyrA* and *parC* allele combination among FQREC clinical isolates, consistent with the largely single-strain origin of FQ resistance within ST131 *E. coli* isolates (8). Furthermore, all 19 clonally related study isolates were ST131, suggesting that ST131 isolates, or those of the particular pulsotypes observed here, may be characterized by increased transmissibility compared with other *E. coli* strains. However, future studies are needed to evaluate the potential increased risk of dissemination in the clinical setting. In addition, the predominance of pulsotype 968 (which is associated specifically with FQ resistance) among both colonizing and clinical isolates (24) suggests that this particular pulsotype may exhibit greater intestinal fitness than others. This possible intestinal fitness advantage of pulsotype 968, which has become predominant in the later years in the emergence of ST131 may have contributed to the recent expansion of ST131 in the clinical setting (24). However, further research is needed to assess this hypothesized association.

In conclusion, our findings demonstrate a dramatically increasing prevalence of ST131 over time (2002 to 2004) among hospitalized patients with FQREC gastrointestinal tract colonization. Our results highlight the importance of evaluation for potentially increased transmissibility of ST131 isolates, including specifically in the hospital setting, and of the development and implementation of infection control interventions to reduce such spread if it is documented.

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