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Characteristics of *Escherichia coli* Sequence Type 131 Isolates That Produce Extended-Spectrum β-Lactamases: Global Distribution of the *H*30-Rx Sublineage

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We designed a study to describe the characteristics of sequence type 131 (ST131) lineages, including the *H*30-Rx sublineage, among a global collection of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from 9 countries collected from 2000 to 2011. A total of 240 nonrepeat isolates from Canada, the United States, Brazil, the Netherlands, France, the United Arab Emirates (UAE), India, South Africa, and New Zealand were included. Established PCR, sequencing, and typing methods were used to define ST131 lineages, *H*30 and *H*30-Rx phylogenetic groups, *gyrA* and *parC* mutations, virotypes, and plasmid-mediated quinolone resistance determinants. The majority of the isolates produced CTX-M-15 with *aac(6′)-lb-cr*, belonged to phylogenetic group B2, and were positive for the *H*30 lineage with the *gyrA1AB* and *parC1aAB* mutations. ST131 showed 15 distinct pulsotypes; 43% of the isolates belonged to four pulsotypes, with a global distribution. Seventy-five percent of the ST131 isolates belonged to *H*30-Rx; this sublineage was present in all the countries and was associated with multidrug resistance, $bla_{CTX\text{-}M\text{-}15}$, *aac(6′)-lb-cr*, and virotypes A and C. The *H*41 lineage was negative for the ST131 *pabB* allele-specific PCR. The multidrug-resistant *H*30-Rx sublineage poses an important public health threat due to its global distribution, association with virotype C, and high prevalence among ST131 isolates that produce CTX-M-15.

Extended-spectrum β-lactamases (ESBLs) belonging to the TEM and SHV families were the predominant types of ESBLs during the 1980s and early 1990s (1). However, since the late 1990s, CTX-M β-lactamases have emerged worldwide among *Enterobacteriaceae*, in particular *Escherichia coli*, and have become the most prevalent and widespread type of ESBL in the world (2). CTX-M-producing *E. coli* organisms are important causes of community-onset urinary tract infections, bacteremia, and intra-abdominal infections (3). Currently, the most widespread and prevalent type of CTX-M enzyme is CTX-M-15 (4).

In 2008, *E. coli* sequence type 131 (ST131) containing CTX-M-15 was simultaneously identified in nine countries, spanning three continents (5, 6). The intercontinental dissemination of this ST has contributed immensely to the worldwide emergence of CTX-M-15-producing *E. coli* (4). Compared to other ESBL-producing *E. coli* organisms, ST131 isolates with CTX-M-β-lactamases are more likely to be resistant to antibiotics and cause community-acquired urinary tract infections (7). Several *in vitro* studies suggest that a unique combination of antimicrobial resistance and virulence factors seem to give ST131 a competitive advantage over other *E. coli* ST, most likely promoting its clonal expansion and dominance over less virulent and/or more susceptible isolates (8, 9).

The global distribution of ST131 reflects the repeated selection of local variants that have acquired certain IncF resistance plasmids carrying genes that encode CTX-Ms (10). However, Johnson and colleagues have shown that the expansion of the *H*30 lineage within ST131 has contributed significantly to the spread of ST131 among fluoroquinolone-resistant *E. coli* isolates in the United States (11).

Blanco and colleagues (12) recently introduced a system for classifying ST131 into 4 groups or virotypes (i.e., A, B, C, and D) that is based on the distribution of four distinctive virulence factors, namely, *afa* FM955459 (Afa/Dr adhesion), *iroN* (catecholate siderophore receptor), *ibeA* (invasion of brain endothelium), and *sat* (secreted autotransporter toxin). They reported that virotypes A and B were associated with multidrug resistance, the presence of CTX-M-15, and the plasmid-mediated quinolone determinant *aac(6′)-lb-cr*. Virotype C had a global distribution (i.e., was present in 8 countries, including Spain, France, Portugal, Switzerland, the United States, Canada, South Korea, and Lebanon) and was statistically associated with a higher frequency of infection (12).

There is limited information regarding the global distribution, virotypes, and characteristics of ST131 lineages that produce ESBLs. We designed a study to describe the characteristics of ST131 lineages, including the *H*30-Rx sublineage, from a global collection of ESBL-producing ST131 isolates from 9 countries collected in 2000 and 2011.

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MATERIALS AND METHODS

Bacterial isolates. We studied a total of 240 nonrepeat ESBL-producing ST131 *E. coli* isolates recovered between 2000 and 2010, from 9 different countries, spanning 4 continents: Canada (Victoria [$n = 5$], Vancouver [$n = 8$], Calgary [$n = 62$], Edmonton [$n = 3$], Medicine Hat [$n = 4$], Regina [$n = 12$], Winnipeg [$n = 11$], Toronto [$n = 10$], Brampton [$n = 13$], Ottawa [$n = 7$], Montreal [$n = 4$]), the United States (Chicago, $n = 16$), Brazil (Rio de Janeiro, $n = 2$), the Netherlands (Rotterdam [$n = 8$], Delft [$n = 1$], Gouda [$n = 5$]), France (Paris, $n = 4$), United Arab Emirates (Abu Dhabi, $n = 8$), India (Vellore, $n = 8$), South Africa (Cape Town, $n = 12$), and New Zealand (Auckland, $n = 37$). Nonrepeat clinical isolates from previous studies (i.e., from Canada [13, 14], the United States [15], Brazil [16], the Netherlands [17], and South Africa [18]), as well as recently acquired isolates from India, France, the United Arab Emirates (UAE), and New Zealand, were included in this study.

Antimicrobial susceptibility testing. MICs were determined with the Vitek 2 instrument (Vitek AMS; bioMérieux Vitek Systems, Inc., Hazelwood, MO) that included the following drugs: amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), ciprofloxacin (CIP), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), imipenem (IPM), meropenem (MEM), and trimethoprim-sulfamethoxazole (SXT). Throughout this study, the results were interpreted using the 2012 CLSI criteria for broth dilution (19).

ESBL screening and confirmation testing. The presence of ESBLs was detected in clinical *E. coli* isolates by using the 2009 CLSI criteria for ESBL screening and confirmation tests (19).

β-Lactamase gene identification. Isoelectric focusing, PCR amplification, and sequencing for $bla_{\text{CTX-M}}$, bla_{OXA}, bla_{TEM}, and bla_{SHV} were carried out on the isolates with a GeneAmp 9700 thermal cycler (Applied Biosystems, Norwalk, CT) using PCR conditions and primers as previously described (20). The isolates were presumed to produce whichever ESBL was encoded by the ESBL gene they were found to contain.

Plasmid-mediated quinolone resistance determinants, phylogenetic groups, and virotypes. Amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken in all ESBL-positive isolates with multiplex PCR (21). *aac(6′)-Ib* and *qepA* were amplified in a separate PCR using primers and under conditions as previously described (22, 23). The variant *aac(6′)-Ib-cr* was further identified by digestion with BstF5I (New England BioLabs, Ipswich, MA). The ESBL-positive isolates were assigned to one of the four main *E. coli* phylogenetic groups (A, B1, B2, or D) by use of a multiplex PCR-based method (24). The 4 main virotypes in ST131 were determined with a multiplex method recently described by Blanco and colleagues (12).

Pulsed-field gel electrophoresis. The genetic relatedness of the ESBL-producing isolates was examined by pulsed-field gel electrophoresis (PFGE) following the extraction of genomic DNA and digestion with XbaI using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA (25). Cluster designation was based on isolates showing approximately ≥80% relatedness, which corresponds to the "possibly related (4 to 6 bands difference)" criterion of Tenover et al. (26).

Identification of ST131 and *fimH*, *gyrA*, and *parC* sequencing. Established PCR methods were used to define the ST131 lineages and the *H*30-Rx sublineage among the ESBL isolates (11, 27, 28). *fimH* (type 1 fimbrial adhesion), *gyrA*, and *parC* subtyping were performed as previously described (11). Multilocus sequence typing (MLST) was undertaken on all isolates using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) (see http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

Statistical methods. Analysis was performed using Stata version 10.0 (StataCorp, College Station, TX). Chi-square and Fisher's exact tests were used to compare group categorical data. *P* values of <0.05 were considered significant.

RESULTS

Bacterial isolates and susceptibilities. A total of 240 ESBL-producing *E. coli* ST131 isolates were included in the study; 91 (38%) were recovered from urine samples, 134 (56%) from blood samples, 2 (1%) from intra-abdominal specimens, 4 (2%) from wound swabs, 2 (1%) from respiratory specimens, and 7 (3%) from rectal swabs. One hundred eighty-one (76%) of these specimens were submitted from community collection sites, and 59 (25%) were from hospitals.

Of the 240 study isolates, 173 (72%) were nonsusceptible (i.e., intermediate or resistant) to SXT, 153 (64%) to AMC, 106 (44%) to TZP, 166 (69%) to TOB, 120 (50%) to GEN, 96 (40%) to AMK, and 226 (94%) to CIP. No resistance to ERT or IPM was detected.

β-Lactamases, PMQR determinants, and phylogenetic groups. Of the 240 ST131 ESBL-producing *E. coli* isolates, 193 (80%) produced CTX-M-15, 40 (17%) produced CTX-M-14, 1 (0.5%) produced CTX-M-24, 2 (1%) produced CTX-M-27, 3 (1%) produced CTX-M-3, and 1 (0.5%) produced TEM-52. One hundred fifty-one (63%) of the ESBL *E. coli* isolates were positive for *aac(6′)-lb-cr*, and 6 (3%) were positive for *aac(6′)-lb-cr* and *qnrB1*. None of the other types of plasmid-mediated quinolone resistance (PMQR) determinants were detected. All isolates belonged to phylogenetic group B2.

Pulsed-field gel electrophoresis and virotypes. PFGE identified 15 clusters of *E. coli* ($n = 230$) that were designated pulsotypes A ($n = 8$), B ($n = 20$), C ($n = 11$), D ($n = 22$), E ($n = 5$), F ($n = 7$), G ($n = 11$), H ($n = 20$), I ($n = 10$), J ($n = 17$), K ($n = 29$), L ($n = 38$), M ($n = 14$), N ($n = 4$), and O ($n = 14$) (Table 1). The remaining ESBL-producing isolates ($n = 10$ and referred to as NR [not related]) were not clonally related, i.e., they exhibited PFGE profiles with <60% similarity and did not show patterns similar to those from pulsotypes A to O. Interestingly, certain pulsotypes (e.g., H, J, K, and L) had a global distribution, while other pulsotypes (e.g., A, E, F, and N) tended to be more localized (Table 1). Pulsotypes A to K, N, and O belonged to virotype C, and pulsotypes L and M belonged to virotype A (Table 1). Two isolates from the NR group produced CTX-M-15 and TEM-52 and belonged to virotype D, while the remaining NR isolates belonged to virotype C.

ST131 and *fimH*, *gyrA*, and *parC* sequencing. The majority of ST131 (220/240 [92%]) isolates belonged to the *H*30 lineage, and 181/220 (82%) *H*30 isolates belonged to the *H*30-Rx sublineage (Table 1). The remaining *fimH* types belonged to *H*22 ($n = 2$), *H*35 ($n = 4$), and *H*41 ($n = 14$). The *H*30 lineage comprised 13 different pulsotypes (i.e., A to M), while the *H*35 and *H*41 lineages belonged to pulsotypes N and O, respectively (Table 1). The *H*22 isolates were not clonally related to each other and did not show patterns similar to those from *H*30, *H*35, or *H*41. The *H*30-Rx sublineage was present in 11 pulsotypes (Table 1).

Among the 240 ST131 isolates, sequence analysis of the *gyrA* and *parC* genes identified 2 *gyrA* alleles and 3 *parC* alleles (Table 1). The CIP-susceptible isolates ($n = 14$) were negative for PMQR determinants and belonged to the *H*41 ($n = 13$) and *H*22 ($n = 1$) *fimH* lineages. Seven of the CIP-susceptible isolates contained the putative ancestor allele of *gyrA* (i.e., *gyrA1*) and the *parC* variant with 1 silent mutation (i.e., *parC1b*). The remaining 7 CIP-susceptible isolates (MICs, 0.12 to 0.5 μg/ml) contained the *gyrA1A* allele (i.e., one amino acid replacement mutation [Ser-83-Leu]) combined with the *parC1b* allele.

TABLE 1 The molecular characteristics of different pulsotypes among a global collection of ST131 isolates that produce extended-spectrum β-lactamases

Pulsotype (*n*)[a]	Isolate origin(s)	β-Lactamase(s) (*n*)	PMQR[b] determinant(s) (*n*)	Virotype	*fimH* lineage	*H*30-Rx (*n*)	*gyrA* allele(s)	*parC* allele(s)
A (8)	Canada, New Zealand	CTX-M-15 (8)	*aac(6′)-lb-cr* (6)	C	*H*30	8	*gyrA1AB*	*parC1aAB*
B (20)	Canada, France, South Africa, New Zealand	CTX-M-14 (20)	*aac(6′)-lb-cr* (2)	C	*H*30	0	*gyrA1AB*	*parC1aAB*
C (11)	Canada, India, South Africa	CTX-M-15 (11)	*aac(6′)-lb-cr* (10)	C	*H*30	11	*gyrA1AB*	*parC1aAB*
D (22)	Canada, New Zealand	CTX-M-15 (22)	*aac(6′)-lb-cr* (22)	C	*H*30	22	*gyrA1AB*	*parC1aAB*
E (5)	Canada	CTX-M-14 (2) CTX-M-24 (1) CTX-M-27 (2)	Neg[c]	C	*H*30	0	*gyrA1AB*	*parC1aAB*
F (7)	Canada, New Zealand	CTX-M-15 (7)	*aac(6′)-lb-cr* (4)	C	*H*30	7	*gyrA1AB*	*parC1aAB*
G (11)	Canada, United States, South Africa	CTX-M-15 (11)	*aac(6′)-lb-cr* (8)	C	*H*30	11	*gyrA1AB*	*parC1aAB*
H (20)	Canada, United States, South Africa, New Zealand, UAE	CTX-M-15 (20)	*aac(6′)-lb-cr* (19)	C	*H*30	20	*gyrA1AB*	*parC1aAB*
I (10)	Canada, United States, New Zealand	CTX-M-15 (10)	*aac(6′)-lb-cr* (4)	C	*H*30	10	*gyrA1AB*	*parC1aAB*
J (17)	Canada, United States, South Africa, UAE, New Zealand, the Netherlands	CTX-M-15 (12), CTX-M-14 (5)	*aac(6′)-lb-cr* (10) *aac(6′)-lb-cr* + *qnrB* (3)	C	*H*30	12	*gyrA1AB*	*parC1aAB*
K (29)	Canada, United States, UAE, South Africa, New Zealand, India	CTX-M-15 (28), CTX-M-14 (1)	*aac(6′)-lb-cr* (23)	C	*H*30	28	*gyrA1AB*	*parC1aAB*
L (38)	Canada, United States, UAE, South Africa, Brazil, the Netherlands, India	CTX-M-15 (36), CTX-M-3 (2)	*aac(6′)-lb-cr* (30)	A	*H*30	38	*gyrA1AB*	*parC1aAB*
M (14)	Canada, India, South Africa, the Netherlands	CTX-M-15 (14)	*aac(6′)-lb-cr* (8) *aac(6′)-lb-cr* + *qnrB* (2)	A	*H*30	14	*gyrA1AB*	*parC1aAB*
N (4)	Canada	CTX-M-15 (4)	*aac(6′)-lb-cr* (3)	C	*H*35	0	*gyrA1AB*	*parC1aAB*
O (14)	Canada, France, the Netherlands, New Zealand	CTX-M-15 (4), CTX-M-3 (1), CTX-M-14 (9)	Neg	C	*H*41	0	*gyrA1AB*, *gyrA1A*	*parC3A*, *parC1b*
NR (10)	Canada, Brazil, United States, South Africa, New Zealand	CTX-M-15 (6), CTX-M-14 (3), TEM-52 (1)	*aac(6′)-lb-cr* (2), *aac(6′)-lb-cr* + *qnrB* (1)	C, D	*H*30, *H*22	0	*gyrA1AB* *gyrA1A*	*parC1aAB*, *parC1b*

[a] The isolates that belonged to pulsotypes A to O had >80% similar PFGE profiles among the isolates of each pulsotype. The remaining ESBL-producing isolates referred to as NR (not related) were not clonally related, i.e., they exhibited <60% similar PFGE profiles and did not show patterns similar to those from pulsotypes A to O.
[b] PMQR, plasmid-mediated quinolone resistance.
[c] Neg, negative.

In contrast, the majority of CIP-nonsusceptible isolates were positive for *aac(6′)-lb-cr* (156/226 [69%]), belonged to the *H*30 (*n* = 220), *H*35 (*n* = 4), *H*41 (*n* = 1), and *H*22 (*n* = 1) *fimH* lineages, and possessed the *gyrA* allele named *gyrA1AB* (i.e., differed from *gyrA1A* by the distinct secondary mutation Asp-87-Asn) and the *parC* variant named *parC1aAB* (i.e., *parC1a* plus the replacement mutations Ser-80-Ile and Glu-85-Val) (11). The *H*41 CIP nonsusceptible isolate contained the *gyrA1AB* and *parC3A* alleles (11).

The antimicrobial susceptibilities, PMQR determinants, ESBL types, and virotypes of ST131, *H*30, and *H*30-Rx are shown in Table 2. ST131 was divided into 3 groups according to *H*30 and *H*30-Rx status: group 1 was isolates that were negative for *H*30 (non-*H*30), group 2 was isolates that were positive for *H*30 but negative for *H*30-Rx (*H*30 non-Rx), and group 3 was isolates that were positive for both *H*30 and *H*30-Rx (*H*30-Rx). *H*30-Rx isolates were significantly more likely to be resistant to various antimicrobial agents, to be positive for *aac(6′)-lb-cr* and $bla_{\text{CTX-M-15}}$, and to belong to virotype A (Table 2). *H*30 non-Rx isolates were more likely to be positive for $bla_{\text{CTX-M-14}}$ and belong to virotype C.

DISCUSSION

E. coli ST131 is known to cause extraintestinal infections, as its isolates are fluoroquinolone resistant (FQ-R) and associated with ESBL production most often due to CTX-M-15 (14, 27, 29). ST131 is not a single entity and can be subdivided into different clusters by other typing methods. Previous studies have shown that ST131 isolates with similar pulsotypes (i.e., >80% relatedness) can be present in different regions or countries (6, 10, 30). However, the same studies have also shown that ST131 isolates with different pulsotypes (<80% relatedness) can be present in the same location (6, 30). These studies have included relatively few isolates with limited geographical origins and time periods. Johnson and colleagues (31) addressed these issues when they studied 579 ST131 isolates from various countries and diverse sources (humans, animals, and the environment) that spanned from 1967 to 2009. They concluded that ST131 is highly diverse at the pulsotype level, but a small number of pulsotypes predominate internationally, and these high-frequency pulsotypes had emerged within the last decade (31). A previous study from Cal-

TABLE 2 The antimicrobial susceptibilities, plasmid-mediated quinolone resistance determinants, extended-spectrum β-lactamases, and different virotypes of ST131 and its *H*30 and *H*30-Rx sublineages

Isolate characteristic[a]	No. (%) of isolates in sublineage[b]: Non-*H*30 ($n = 20$)	*H*30 non-Rx ($n = 39$)	*H*30-Rx ($n = 181$)	*P*
Collection site				0.5
Hospital	6 (30)	9 (23)	44 (24)	
Community	14 (70)	30 (77)	137 (76)	
Antimicrobial NS				
AMC	4 (20)	16 (41)	133 (73)	<0.001
TZP	2 (10)	10 (44)	94 (52)	0.002
CIP	10 (50)	35 (90)	181 (100)	<0.001
SXT	1 (5)	19 (49)	153 (85)	<0.001
GEN	2 (10)	20 (51)	98 (54)	0.4
TOB	3 (15)	23 (59)	140 (77)	0.01
AMK	1 (5)	11 (28)	84 (46)	0.02
PMQR determinant				
aac(6′)-lb-cr	3 (15)	6 (15)	142 (78)	<0.001
aac(6′)-lb-cr + *qnrB*	1 (5)	1 (3)	4 (2)	NA[c]
Virotype				
A	0	0	52 (29)	<0.001
B	0	0	0	
C	18 (90)	39 (100)	129 (71)	<0.001
D	2 (10)	0	0	NA
Type of ESBL				
CTX-M-14	9 (45)	31 (79)	0	<0.001
CTX-M-15	9 (45)	5 (13)	179 (99)	<0.001
Other CTX-M	1 (5)	3 (8)	2 (1)	NA
Other ESBLs	1 (5)	0	0	NA

[a] NS, nonsusceptible, i.e., either intermediate or resistant; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; PMQR, plasmid-mediated quinolone resistance; ESBLs, extended-spectrum β-lactamases.
[b] Non-*H*30, ST131 *E. coli* isolate that was negative for *H*30; *H*30 non-Rx, ST131 *E. coli* isolate that was positive for *H*30 but negative for *H*30-Rx; *H*30-Rx, *E. coli* isolate that was positive for ST131, *H*30, and *H*30-Rx.
[c] NA, not available (too many cells with numbers of <5).

gary, Canada, over an 11-year period (2000 to 2010) showed that a distinct pulsotype of ST131 (named A5 in that study) was responsible for the significant increase in ESBL-producing *E. coli* isolates that had been causing bloodstream infections since 2007 (14).

Our current study identified 15 pulsotypes among 240 human ESBL-producing ST131 isolates recovered from 9 different countries, spanning 4 continents, between 2000 and 2010. Our results were similar to the pulsotype results reported by Johnson et al. (31): 43% of the isolates from our study belonged to four distinct pulsotypes (e.g., H, J, K, and L), with a global distribution (i.e., were present in ≥5 different countries and named international pulsotypes). The remaining 11 pulsotypes tended to be more localized to certain countries and were named localized pulsotypes. We do acknowledge that the localized pulsotypes may actually be more widely disseminated internationally due to sampling bias, as small numbers of isolates were sampled from Brazil, India, France, and UAE. One of the international pulsotypes (i.e., pulsotype L) was identical to the Calgary A5 pulsotype and was present in Canada (i.e., Victoria, Vancouver, Edmonton, Medicine Hat, Regina, Winnipeg, Toronto, Ottawa, Brampton, and Montreal), the United States, UAE, South Africa, Brazil, the Netherlands, and India (Table 1).

Most of the pulsotypes from our study belonged to virotype C (Table 1). Our study supported some of the results reported by Blanco et al. (12); e.g., virotype A from our study was associated with the presence of CTX-M-15 and *aac(6′)-lb-cr*. However, virotype C was associated with both CTX-M-15 and CTX-M-14 and the *H*35 and *H*41 lineages (Table 1). Interestingly, the international pulsotype L, which was responsible for the significant increase in bloodstream infections in Calgary since 2007, belonged to virotype A and was positive for *H*30-Rx and *aac(6′)-lb-cr*.

Whole-genome sequencing of 105 ST131 isolates from 5 countries revealed that fluoroquinolone resistance was confined to a single rapidly expanding sublineage designated *H*30-R (32). Interestingly, *H*30-R with CTX-M-15 belonged to a single well-defined clade nested within other *H*30-R isolates, which was named *H*30-Rx due to its more extensive resistance to antimicrobial agents. A study by Banerjee and colleagues (27) determined the prevalences of ST131 and its sublineages among 267 *E. coli* isolates from the Chicago region. The authors were able to show that *H*30-R and *H*30-Rx were more antimicrobial resistant than and had distinctive virulence profiles compared to those of non-*H*30 ST131 isolates. Interestingly, the *H*30-Rx sublineage had the highest virulence scores, implying greater virulence potential and possibly explaining its high prevalence (27).

We believe this is the first study to explore the associations of the *H*30 lineage and the *H*30-Rx sublineage of ST131 with different virotypes, resistance phenotypes, ESBL types, and the presence of plasmid-mediated quinolone resistance determinants in a global collection of *E. coli* ST131 isolates that produce ESBLs. We confirmed the association of the *H*30-Rx sublineage with multidrug resistance and the presence of CTX-M-15 (Table 2). We uniquely identified the association of *H*30-Rx with virotypes A and C and the presence of *aac(6′)-lb-cr* (Table 2). Isolates of the *H*30 lineage that tested negative for Rx (i.e., *H*30 non-Rx) were associated with virotype C and the presence of CTX-M-14 (Table 2). The *H*35 and *H*41 lineages were linked with virotype C and the presence of CTX-M-14 and CTX-M-15 (Table 2). However, our study did not include a sufficient number of non-virotype C isolates to reasonably conclude that *H*30-Rx isolates were associated with virotype C. To our knowledge, this is the first report that shows the association of non-*H*30 with CTX-M-14 due to the *H*41 lineage.

MLST identified pulsotype O ($n = 14$) as being from ST131; however, the PCR for the *pabB* allele, as described by Clermont and colleagues (28), was negative for these isolates. Pulsotype O belonged to the *H*41 lineage, was positive for virotype C, negative for plasmid-mediated quinolone resistance determinants, and present in Canada, France, the Netherlands, and New Zealand (Table 1). These results were similar to those from a recent study that determined the virulence properties of the *H*41 lineage and also found an association of the *H*41 lineage with virotype C (33). The discrepancies observed between the *pabB* allele-specific PCR and MLST for the *H*41 lineage of ST131 are noteworthy. The *H*41 lineage belongs to serotype O16:H5, and a PCR method specific for the detection of this subgroup was recently published (34).

The *H*30-Rx sublineage of ST131 is a major drug-resistant

pathogen among fluoroquinolone-resistant *E. coli* isolates in the United States (11, 27, 29) and is associated with sepsis (32). Our study shows that *H*30-Rx also poses an important public health threat due to its global distribution, association with virotype C, and high prevalence among ST131 isolates that produce CTX-M-15. We urgently need well-designed epidemiological and molecular studies to understand the dynamics of the transmission, risk factors, and reservoirs for the *E. coli* ST131 *H*30-Rx sublineage. This will provide insight into the emergence and spread of this multidrug-resistant sequence type that will hopefully lead to information essential for preventing the spread of ST131.

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