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EPIDEMIOLOGY AND SURVEILLANCE

Extensive Genetic Commonality among Wildlife, Wastewater, Community, and Nosocomial Isolates of *Escherichia coli* Sequence Type 131 (*H*30R1 and *H*30Rx Subclones) That Carry $bla_{CTX\text{-}M\text{-}27}$ or $bla_{CTX\text{-}M\text{-}15}$

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ABSTRACT *Escherichia coli* sequence type 131 (ST131) is currently one of the leading causes of multidrug-resistant extraintestinal infections globally. Here, we analyzed the phenotypic and genotypic characteristics of 169 ST131 isolates from various sources (wildlife, wastewater, companion animals, community, and hospitals) to determine whether wildlife and the environment share similar strains with humans, supporting transmission of ST131 between different ecological niches. Susceptibility to 32 antimicrobials was tested by disc diffusion and broth microdilution. Antibiotic resistance genes, integrons, plasmid replicons, 52 virulence genes, and *fimH*-based subtypes were detected by PCR and DNA sequencing. Genomic relatedness was determined by pulsed-field gel electrophoresis (PFGE). The genetic context and plasmid versus chromosomal location of extended-spectrum beta-lactamase and AmpC beta-lactamase genes was determined by PCR and probe hybridization, respectively. The 169 ST131 study isolates segregated predominantly into $bla_{CTX\text{-}M\text{-}15}$ *H*30Rx (60%) and $bla_{CTX\text{-}M\text{-}27}$ *H*30R1 (25%) subclones. Within each subclone, isolates from different source groups were categorized into distinct PFGE clusters; genotypic characteristics were fairly well conserved within each major PFGE cluster. Irrespective of source, the $bla_{CTX\text{-}M\text{-}15}$ *H*30Rx isolates typically exhibited virotype A (89%), an F2:A1:B− replicon (84%), and a 1.7-kb class 1 integron (92%) and had diverse structures upstream of the $bla_{CTX\text{-}M}$ region. In contrast, the $bla_{CTX\text{-}M\text{-}27}$ *H*30R1 isolates typically exhibited virotype C (86%), an F1:A2:B20 replicon (76%), and a conserved IS*26*-ΔIS*Ecp1*-$bla_{CTX\text{-}M}$-like structure. Despite considerable overall genetic diversity, our data demonstrate significant commonality between *E. coli* ST131 isolates from diverse environments, supporting transmission between different sources, including humans, environment, and wildlife.

KEYWORDS *Escherichia coli* ST131, ESBL, virulence, nosocomial and community-acquired infections, wildlife, environment

Escherichia coli sequence type 131 (ST131) currently is one of the most important globally disseminated bacterial lineages, causing severe hospital-acquired and community-onset multidrug-resistant (MDR) infections and contributing to the global

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spread of $bla_{CTX\text{-}M\text{-}15}$ and other extended-spectrum beta-lactamase (ESBL) genes (1–3). A substantial level of virulence, combined with carriage of plasmids with transferable antimicrobial resistance-encoding elements, likely has facilitated this clone's pandemic success (1–4).

Discriminatory molecular typing methods have elucidated the fine clonal structure of ST131 by resolving various epidemiologically important subclones. *H*30, the most prevalent subclone and the main driver of the recent worldwide ST131 expansion, was so designated based on its association with allele 30 of the type 1 fimbrial adhesin gene *fimH* (1, 2). Within *H*30, whole-genome sequencing has resolved two main subclones, *H*30R1 and *H*30Rx (also called clades C1 and C2, respectively), which comprise nearly all quinolone-resistant ST131 isolates (1, 5–7). The globally distributed *H*30Rx subset is closely associated with $bla_{CTX\text{-}M\text{-}15}$ and with extensive antimicrobial resistance (5, 8–12). In contrast, although most *H*30R1 subclone isolates are ESBL negative, some of them harbor $bla_{CTX\text{-}M\text{-}27}$ or $bla_{CTX\text{-}M\text{-}14}$ (11).

Although ST131 exhibits a narrower range of virulence factor-encoding genes than does the species overall (3, 13), significant within-ST131 variation in virulence gene content is evident, leading to the delineation of specific ST131 virotypes, designated A to D (13). Virotype C is considered to be the most widespread (3), but virotype A has predominated among *H*30Rx isolates in several recent studies from Europe (8, 10). ST131 also is highly diverse with respect to pulsed-field gel electrophoresis (PFGE) genomic DNA profiles (14). Certain highly prevalent pulsotypes (e.g., 968, 800, and 812) are globally distributed (8, 12, 14).

Mobile genetic elements play a key role in the dissemination of virulence and antibiotic resistance generally, and IncF plasmids in particular likely have contributed to ST131's success (4, 15). IncF plasmids with a complex mosaic structure consisting of multiple plasmid replicons, typically FII, FIA, and/or FIB, dominate within ST131. This is probably due to unique features such as combinations of virulence genes and diverse antibiotic resistance determinants that contribute to bacterial fitness, plus postsegregational killing systems that ensure their maintenance and propagation in the bacterial cell (2–4, 15). IS*Ecp1*-$bla_{CTX\text{-}M}$-*orf477* and IS*Ecp1*-$bla_{CTX\text{-}M}$-IS*903* are two major genetic platforms described in *Enterobacteriaceae* in association with $bla_{CTX\text{-}M}$ genes (5, 11, 15). These platforms can be inserted at both plasmidic and chromosomal sites, and their structure is frequently interrupted by IS*26* elements that are inserted upstream and downstream of the $bla_{CTX\text{-}M}$ gene (4, 5, 15).

Although ST131 is known mainly for causing infections in humans, including urinary tract infections, bloodstream infections, and neonatal sepsis, it has also been identified in companion animals, poultry, livestock, wild animals, and food (3, 16). Its presence in food, water, the environment, and other nonhuman sources suggests a minimally explored complexity of potential transmission routes, whereby migrating birds and wastewater may play an important role in ST131's distribution and circulation. To address these unknowns, we compared the phenotypic, genotypic, and clonal characteristics of 169 *E. coli* ST131 isolates of diverse origins, including human community and nosocomial sources ($n = 130$), wastewater ($n = 19$), dogs ($n = 3$), and wild birds ($n = 17$), to determine whether wild animals and the environment share highly similar strains with humans, implying exchange of ST131 strains between different ecological niches.

RESULTS

Plasmid-mediated resistance genes, integrons, replicons, *fimH*30 subtyping, and antimicrobial resistance. In total, 169 ST131 *E. coli* isolates were analyzed, including 151 ESBL-positive, one AmpC beta-lactamase-positive, and 17 ESBL-negative, AmpC-negative isolates. All but three (166/169, 98%) were type O25b, and nearly all of the ESBL-positive isolates carried either $bla_{CTX\text{-}M\text{-}15}$ (106/169, 63%) or $bla_{CTX\text{-}M\text{-}27}$ (42/169, 25%) (Fig. 1; see also Fig. S1 in the supplemental material). $bla_{CTX\text{-}M\text{-}15}$-positive isolates frequently also contained $bla_{OXA\text{-}1}$ (92/106, 87%) and *aac(6′)-Ib-cr* (93/106, 88%), in some instances accompanied by $bla_{TEM\text{-}1}$ (26/106,

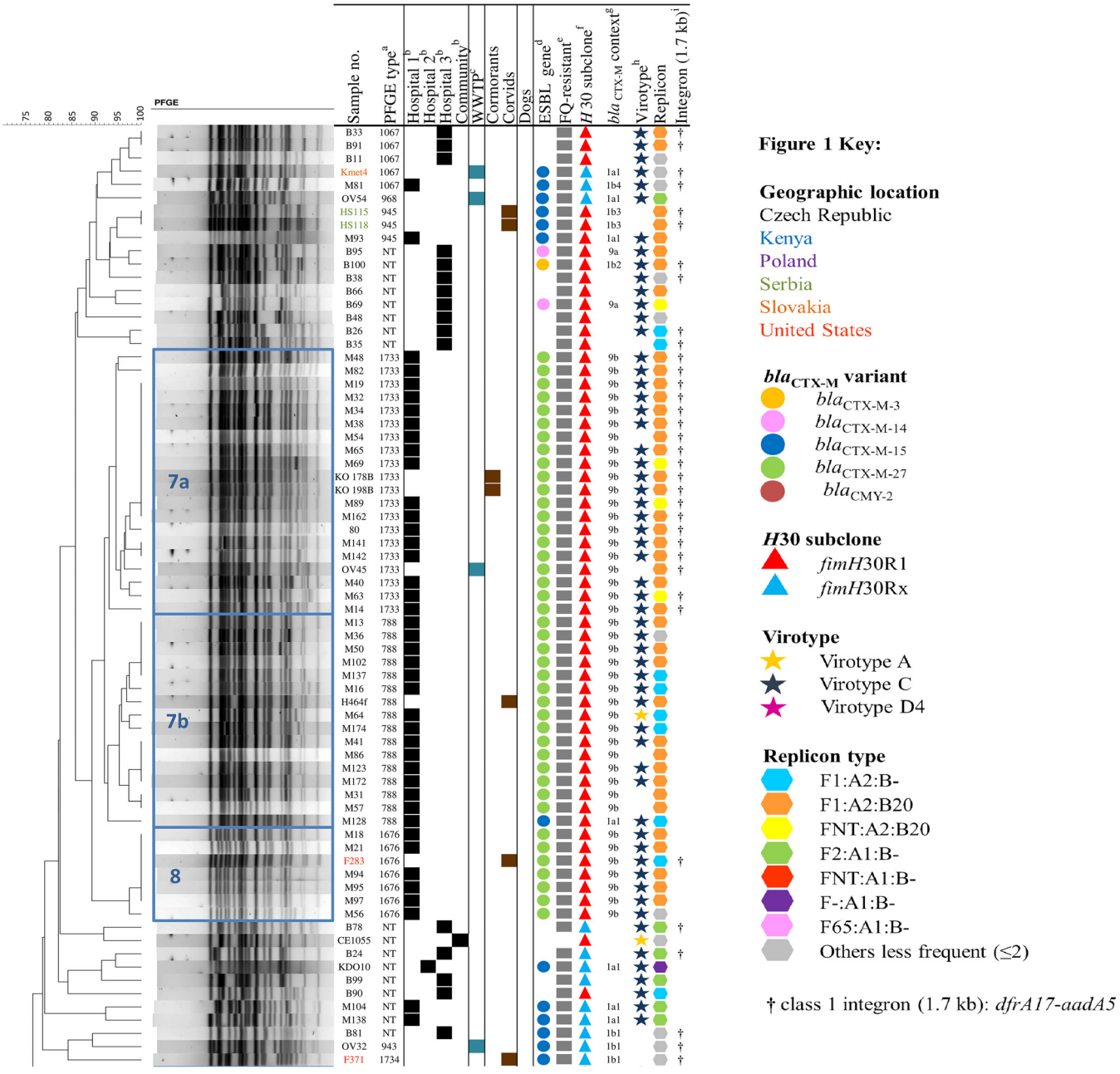

FIG 1 The clonality and common characteristics of representatives of 169 *Escherichia coli* ST131 from various sources. The superscript notations in the figure are defined as follows. [a]NT, not typeable using reference pulsotypes 788, 797, 800, 805, 806, 812, 837, 842, 903, 905, 906, 943, 945, 968, 987, 1067, 1140, 1676, 1733, 1734, 1735, 1737, 1738, and 1739. [b]Human clinical isolates: hospital 1, St. Anne's Faculty Hospital, Brno, Czech Republic (CZ); hospital 2, Children's Medical Center, Brno, CZ; hospital 3, University Hospital Motol, Prague, CZ; Community: human community isolate, Karvina, CZ. [c]WWTP, wastewater treatment plant. [d,g,i]An empty box indicates that no corresponding gene was detected. [e]FQ, fluoroquinolones (an empty box indicates that the isolate was not resistant to fluoroquinolone antibiotics). [f,h]An empty box indicates that the isolate could not be assigned to particular subclone or virotype. [g]The structure of bla_{CTX-M} and bla_{CMY} genetic environment is displayed in Fig. 2; ND, not determined.

25%) and/or the quinolone resistance gene *qnrB1* (5/106, 5%), whereas most $bla_{CTX-M-27}$-positive isolates (33/42, 79%) carried no additional beta-lactam or quinolone resistance genes (see Fig. S1 in the supplemental material). Likewise, a 1.7-kb class 1 integron that contained *dfrA17* and *aadA5* gene cassettes was significantly more frequent among $bla_{CTX-M-15}$-positive isolates than those with $bla_{CTX-M-27}$ (96/106, 91%, versus 20/42, 48%: $P < 0.001$) (Fig. 1).

All but one of the study isolates (168/169, 99%) belonged to the ST131-*H*30 subclone and were accounted for by its *H*30Rx (109/169, 64%) and *H*30R1 (59/169, 35%)

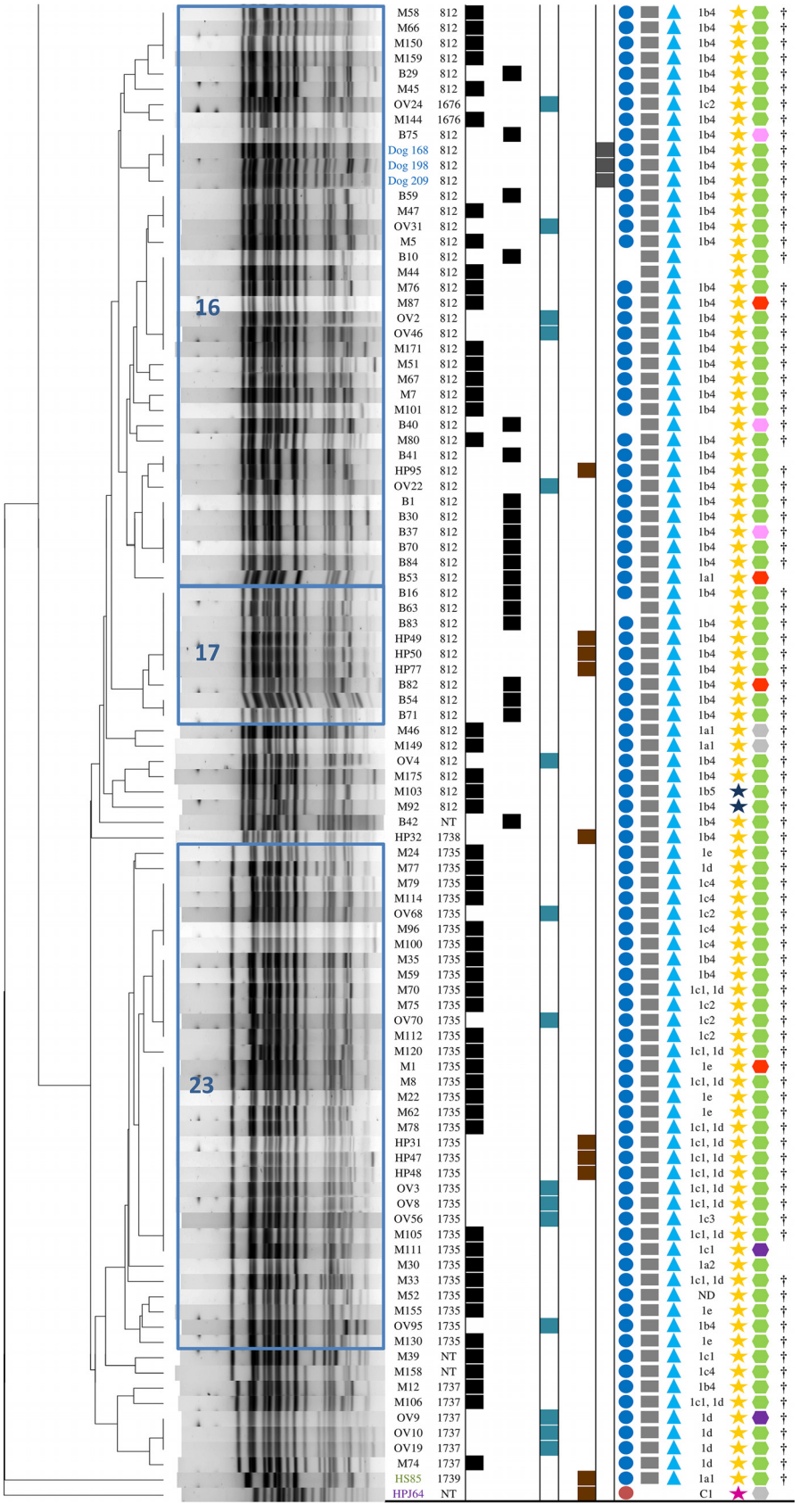

FIG 1 (Continued)

TABLE 1 Characteristics of two major subgroups among 144 diverse-source bla_{CTX-M}-positive *E. coli* ST131 isolates

Category	Specific characteristic	Prevalence of characteristics, no. of isolates (column %)		
		$bla_{CTX-M-15}$, *H*30Rx (*n* = 102)	$bla_{CTX-M-27}$, *H*30R1 (*n* = 42)	*P*[a] ($bla_{CTX-M-15}$ vs $bla_{CTX-M-27}$)
Antimicrobial resistance[b]	Ampicillin	102 (100)	42 (100)	
	Piperacillin	101 (99)	42 (100)	
	Cefazolin	101 (99)	42 (100)	
	Cefuroxime	102 (100)	42 (100)	
	Cefoxitin	4 (4)	0 (0)	
	Ceftazidime	58 (57)	4 (10)	<0.001
	Cefotaxime	102 (100)	42 (100)	
	Cefoperazone	77 (75)	31 (74)	
	Cefepime	71 (70)	4 (10)	<0.001
	Aztreonam	88 (86)	15 (36)	<0.001
	Amoxicillin-clavulanate	93 (91)	2 (5)	<0.001
	Ampicillin-sulbactam	79 (77)	2 (5)	<0.001
	Piperacillin-tazobactam	32 (31)	0 (0)	<0.001
	Nalidixic acid	102 (100)	42 (100)	
	Ciprofloxacin	101 (99)	42 (100)	
	Ofloxacin	102 (100)	42 (100)	
	Trimethoprim-sulfamethoxazole	92 (90)	21 (50)	<0.001
	Tetracycline	82 (80)	20 (48)	<0.001
	Doxycycline	80 (78)	20 (48)	<0.001
	Sulfonamides	95 (93)	22 (52)	<0.001
	Trimethoprim	95 (93)	21 (50)	<0.001
	Azithromycin	95 (93)	21 (50)	<0.001
	Streptomycin	95 (93)	21 (50)	<0.001
	Tobramycin	86 (84)	0 (0)	<0.001
	Gentamicin	5 (5)	0 (0)	
	Amikacin	26 (25)	0 (0)	<0.001
	Nitrofurantoin	4 (4)	0 (0)	
	Tigecycline	5 (5)	2 (5)	
	Colistin	1 (1)	0 (0)	
Other resistance genes	bla_{OXA-1}	88 (86)	0 (0)	<0.001
	bla_{TEM-1}	22 (22)	4 (10)	
	aac(6′)-Ib-cr	89 (87)	6 (14)	<0.001
	qnrB1	1 (1)	0 (0)	
Integrons[c]	IntIA (1.7 kb)	94 (92)	20 (48)	<0.001
	IntIB (0.8 kb)	0 (0)	1 (2)	
Replicons	F2:A1:B−	86 (84)	0 (0)	<0.001
	F1:A2:B20	0 (0)	32 (76)	<0.001
	F1:A2:B−	0 (0)	5 (12)	<0.001
Virulence genotype	Virotype A	91 (89)	1 (2)	<0.001
	Virotype C	8 (8)	36 (86)	<0.001
	Virotype undefined	3 (3)	5 (12)	0.03
	ExPEC[d]	102 (100)	37 (88)	<0.001

[a]*P* values (determined by chi-square test) are shown where $P < 0.05$.
[b]No isolate was resistant to meropenem, ertapenem, or chloramphenicol.
[c]That is, IntI1A, class 1 integron (1.7 kb), *dfrA17-aadA5*, and IntI1B (0.8 kb), class 1 integron, *dfrA5*.
[d]ExPEC, extraintestinal pathogenic *E. coli*.

subsets. $bla_{CTX-M-15}$ was disproportionately prevalent among *H*30Rx isolates (102/109 [94%], *H*30Rx, versus 7 [6%], others: $P < 0.001$), whereas $bla_{CTX-M-27}$ was disproportionately prevalent among *H*30R1 isolates (42/59 [71%], *H*30R1, versus 17 [29%], others: $P < 0.001$) (Fig. 1).

Resistance was detected to all individual agents except meropenem and ertapenem, and 140 (83%) of the 169 isolates were MDR (see Fig. S1 in the supplemental material). $bla_{CTX-M-15}$-positive *H*30Rx isolates exhibited a significantly higher prevalence of resistance to several antimicrobial agents than did the $bla_{CTX-M-27}$ *H*30R1 isolates (Table 1; see also Fig. S1 in the supplemental material).

Genetic environment of bla_{CTX-M} and bla_{CMY}. According to PCR mapping and sequencing, the genetic context of the region upstream of bla_{CTX-M} and bla_{CMY} could be divided into several groups (Fig. 1 and 2).

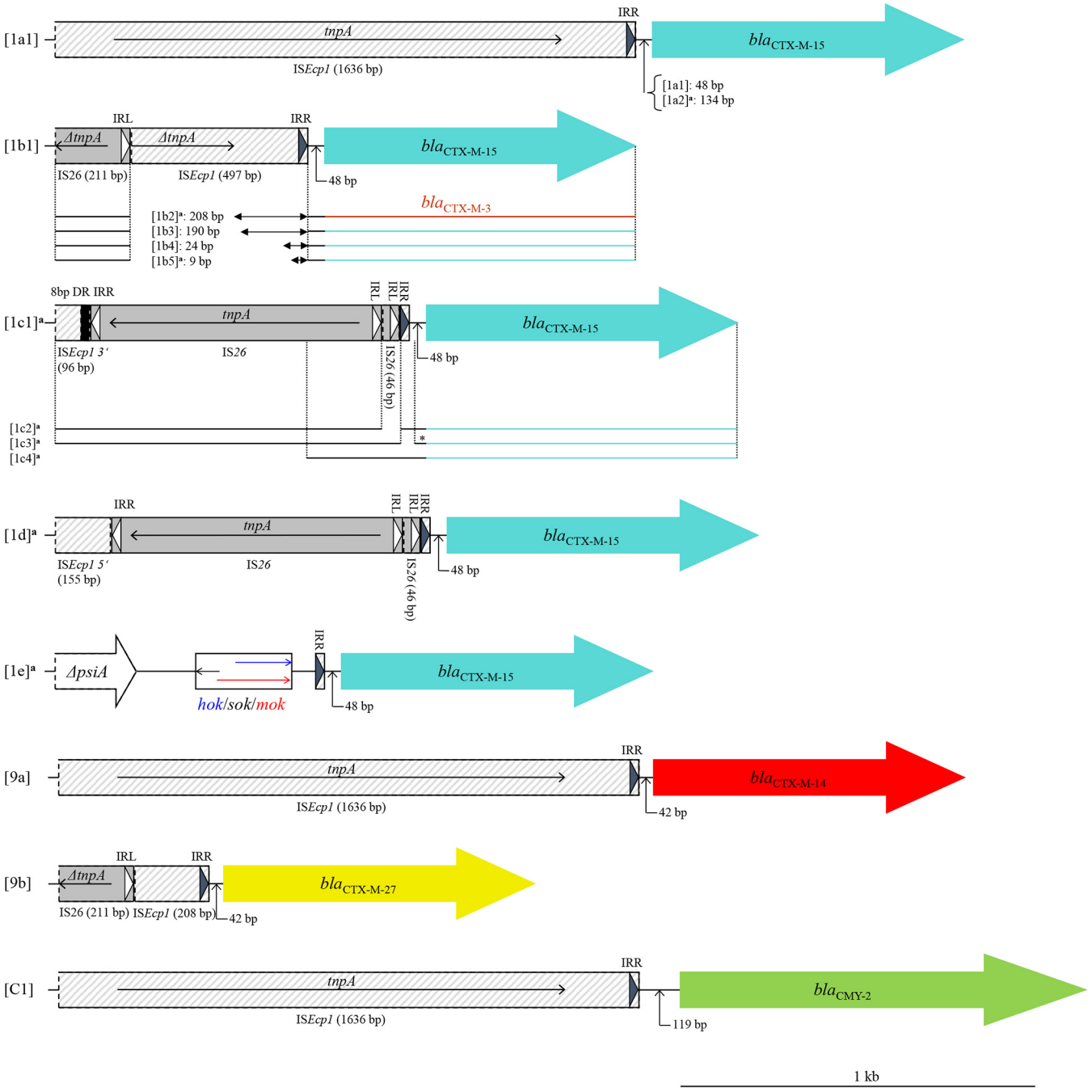

FIG 2 Genetic environment of bla_{CTX-M} and bla_{CMY-2} groups among *E. coli* ST131 isolates. A dotted line in a gene box indicates truncation. IRR, right-hand inverted repeat; IRL, left-hand inverted repeat; DR, direct repeat of partial IS*Ecp1* inverted repeat; *, shortened spacer (32 bp) in variant 1c3; white triangle, IS*26* inverted repeat; black triangle, IS*Ecp1* inverted repeat. [a]Novel genetic environment structure found in this study (no identical sequence was deposited in GenBank).

The great majority of $bla_{CTX-M-15}$-positive isolates (87/106, 82%) had IS*Ecp1* truncated by IS*26* in different positions, with remnant parts of various lengths (Fig. 2). The most prevalent organization, formed by IS*26* upstream of a 24-bp remnant of IS*Ecp1* (group 1b4 in Fig. 2), occurred in 51/106 (48%) isolates (Fig. 1).

Here, the IS*26*-ΔIS*Ecp1*-bla_{CTX-M}-like platform was also very common among $bla_{CTX-M-27}$-positive isolates. The dominant arrangement consisted of 208-bp remnant of IS*Ecp1* flanked by IS*26* upstream of $bla_{CTX-M-27}$ (Fig. 1; group 9b in Fig. 2).

The one AmpC-positive ST131 isolate contained an intact IS*Ecp1* 119-bp upstream of bla_{CMY-2} (Fig. 2).

Plasmid analysis and hybridization of ESBL genes. All isolates contained at least one IncF replicon, most commonly F2:A1:B− (92/169, 54%), followed by F1:A2:B20 (40/169, 24%) and F1:A2:B− (9/169, 5%). The F2:A1:B− replicon was associated with $bla_{CTX\text{-}M\text{-}15}$ (86/92, 93%) and *H*30Rx (92/92, 100%), whereas the F1:A2:B20 and F1:A2:B− replicons were associated with *H*30R1 (49/49, 100%) and $bla_{CTX\text{-}M\text{-}27}$ (32/40, 80%, and 5/9, 56%, respectively) (Fig. 1).

In total, 49 isolates, representing different sources, subclones, molecular characteristics, plasmid groups, and PFGE clusters, were subjected to S1 PFGE and probe hybridization to determine the plasmidic versus chromosomal location of $bla_{CTX\text{-}M}$ (see Table S1 and Fig. S1 in the supplemental material). $bla_{CTX\text{-}M}$ was located on an IncF plasmid in 33 isolates, on plasmids other than IncF in 4, on the chromosome in 10, and on both plasmid and chromosome in 2 (see Table S1 and Fig. S1 in the supplemental material). Overall, $bla_{CTX\text{-}M\text{-}15}$ was located mostly on F2:A1:B− plasmids in *H*30Rx strains, while $bla_{CTX\text{-}M\text{-}27}$ with F1:A2:B20 plasmids in *H*30R1 strains (see Table S1 and Fig. S1 in the supplemental material).

Virulence genes. Based on the presence or absence of specific virulence genes, isolates were classified into established ST131 virotypes. Of these, virotype A was the most common, occurring in 97 isolates (57% of 169), followed by virotype C (60/169, 36%) and virotype D4 (1/169, 1%). Virotype A was associated mainly with *H*30Rx (95/97, 98%), and virotype C with *H*30R1 (49/60, 82%) (Fig. 1). Based on the criteria of Dahbi et al. (10), 11 isolates could not be categorized into any of the established virotypes.

In addition, cluster analysis was used to identify associations among all 52 detected virulence genes. Based on unique combinations of virulence genes, the isolates were divided into 20 clusters (according to 100% similarity). The largest such cluster included 74 isolates (44% of 169) with a unique set of 11 virulence genes (*afa/dra*, *iha*, *fimH*, *sat*, *fyuA*, *iutA*, *kpsII*, *traT*, *usp*, *ompT*, and *malX*) that corresponded to virotype A. These isolates carried almost exclusively $bla_{CTX\text{-}M\text{-}15}$ (71/74; 96%) or were ESBL negative (3/74; 4%), and all represented the *H*30Rx subclone. In addition, 70 (95% of 74) and 68 (92%) also harbored a 1.7-kb class 1 integron and the F2:A1:B− replicon, respectively (see Fig. S2 in the supplemental material).

The second largest cluster, which comprised 44 *H*30R1 isolates (26% of 169), contained mainly $bla_{CTX\text{-}M\text{-}27}$-positive isolates (34/44, 77%) with a different unique set of 11 virulence genes (*iha*, *fimH*, *sat*, *fyuA*, *iutA*, *kpsII*, *kpsM II-K5*, *traT*, *usp*, *ompT*, and *malX*), that corresponded to virotype C. Of these isolates, 27 (61% of 44) harbored an F1:A2:B20 replicon, and 19 (43%) harbored a 1.7-kb class 1 integron (see Fig. S2 in the supplemental material).

Isolates classified as extraintestinal pathogenic *E. coli* (ExPEC; 153/169, 91%) were distributed in all source groups (see Fig. S1 in the supplemental material), but predominantly among $bla_{CTX\text{-}M\text{-}15}$ *H*30Rx isolates (Table 1; see also Fig. S1 in the supplemental material).

PFGE and distribution of pulsotypes by source group. PFGE was used for genomic comparison between isolates in relation to source group. Among the 169 study isolates, PFGE analysis yielded 27 unique pulsotypes at the 91% similarity level (Fig. 1). The PFGE profiles of 148 (88%) of the 169 study isolates corresponded with established human-associated international pulsotypes (14), including, in order of descending prevalence (no., percentage of 169), pulsotypes 812 (51, 30%), 1735 (33, 20%), 1733 (20, 12%), 788 (16, 9%), 1676 (9, 5%), 1737 (6, 4%), 1067 (5, 3%), and 945 (3, 2%) comprising >1 isolate (Table 2).

In the PFGE dendrogram, 123 isolates (73% of 169) were grouped within the five main clusters, arbitrarily labeled 7, 8, 16, 17, and 23. Of these, clusters 16, 17, and 23 accounted collectively for 80 (47% of 169) isolates, all from the *H*30Rx subclone (Fig. 1). These three clusters had in common multiple accessory traits, including $bla_{CTX\text{-}M\text{-}15}$ (76/80, 95%), 1.7-kb class 1 integrons (75/80, 94%), F2:A1:B− replicons (72/80, 90%), and virulence genes that corresponded to virotype A (80/80, 100%) (Fig. 1). The main genetic

TABLE 2 Distribution of 169 *E. coli* ST131 isolates by pulsotype, ecological source, and location

Pulsotype	Total (%)	Source[a]							
		Humans (CZ)[b]					Wild birds		Companion animals (dogs)
		H1	H2	H3	CO	WWTP[c]	Cormorants[d]	Corvids	
788	16 (9)	15						1 (CZ-1)	
812	51 (30)	21		18		5 (CZ)		4 (CZ-2)	3 (KE)
943	1 (1)					1 (CZ)			
945	3 (2)	1						2 (SB)	
968	1 (1)					1 (CZ)			
1067	5 (3)	1		3		1 (SL)			
1676	9 (5)	7				1 (CZ)		1 (US)	
1733	20 (12)	17				1 (CZ)	2 (CZ)		
1734	1 (1)							1 (US)	
1735	33 (20)	24				6 (CZ)		3 (CZ-2)	
1737	6 (4)	3				3 (CZ)			
1738	1 (1)							1 (CZ-2)	
1739	1 (1)							1 (SB)	
NT[e]	21 (12)	4	1	14	1			1 (PL)	
Total	169	93	1	35	1	19	2	15	3

[a]CZ, Czech Republic; KE, Kenya; PL, Poland; SB, Serbia; SL, Slovakia; US, United States. CZ-1, Zidlochovice, 17 km from Brno, CZ; CZ-2, Přerov, 67 km from Brno, CZ.
[b]H, Human clinical isolates, all from the Czech Republic: H1, hospital 1, St. Anne's Faculty Hospital, Brno, CZ; H2, hospital 2, Children's Medical Center, Brno, CZ; H3, hospital 3, University Hospital Motol, Prague, CZ; CO, human community isolate, Karvina, CZ.
[c]WWTP, wastewater treatment plant (municipal), Brno-Modrice, CZ.
[d]Nature Reserve Oskovec, Straznice, CZ, 58 km from Brno, CZ.
[e]NT, not typeable using reference pulsotypes 788, 797, 800, 805, 806, 812, 837, 842, 903, 905, 906, 943, 945, 968, 987, 1067, 1140, 1676, 1733, 1734, 1735, 1737, 1738, and 1739.

difference distinguishing clusters 16 and 17 from cluster 23 was in the region upstream of $bla_{CTX\text{-}M\text{-}15}$. Specifically, the most common genetic environment for $bla_{CTX\text{-}M\text{-}15}$ in clusters 16 and 17 included group 1b4 (41/43, 95%) (Fig. 1 and 2), while in cluster 23 various structures were identified (Fig. 1). In addition, the consensus PFGE profile of clusters 16 and 17 corresponded with international pulsotype 812 (45/47, 96%), and that of cluster 23 with pulsotype 1735 (33/33, 100%). All three clusters contain isolates from multiple sources, including three canine isolates from Kenya, with considerable overlap of sources among clusters (Fig. 1).

In contrast, clusters 7 and 8 comprised collectively 43 isolates, all from to *H*30R1 subclone (43/43, 100%). These two clusters were characterized by multiple shared traits, including $bla_{CTX\text{-}M\text{-}27}$ (42/43, 98%), the F1:A2:B20 replicon (32/43, 74%), a conserved set of virulence genes that corresponded with virotype C (37/43, 86%), and a conserved region upstream of $bla_{CTX\text{-}M}$ that represented group 9b (42/43, 98%) (Fig. 1 and 2). Cluster 7 was divided into subclusters 7a and 7b, which had consensus PFGE profiles corresponding with international pulsotypes 1733 (20/20, 100%) and 788 (16/16, 100%), respectively. In contrast to the cluster 7a isolates, which frequently carried 1.7-kb class 1 integron (19/20, 95%) and were MDR, most cluster 7b and 8 isolates carried no integron and were less extensively resistant (Fig. 1; see also Fig. S1 in the supplemental material). All three clusters showed a broad and overlapping source distribution.

DISCUSSION

E. coli ST131 represents one of the most medically important and globally widespread clonal lineages and is a major challenge to public health worldwide. However, it remains unknown to what extent and through which pathways these pathogenic bacteria are transmitted between different ecological niches. Here, accessory traits, including virulence gene profiles, plasmid replicons, integron content, and antimicrobial resistance profiles, were fairly well conserved within each major PFGE cluster but differed between clusters. Importantly, genetic and phylogenetic structure of studied ST131 population did not segregate according to source group (Fig. 1 and 3 and Table 2). These broad genetic commonalities across sources strongly suggest an exchange of highly genetically related strains between

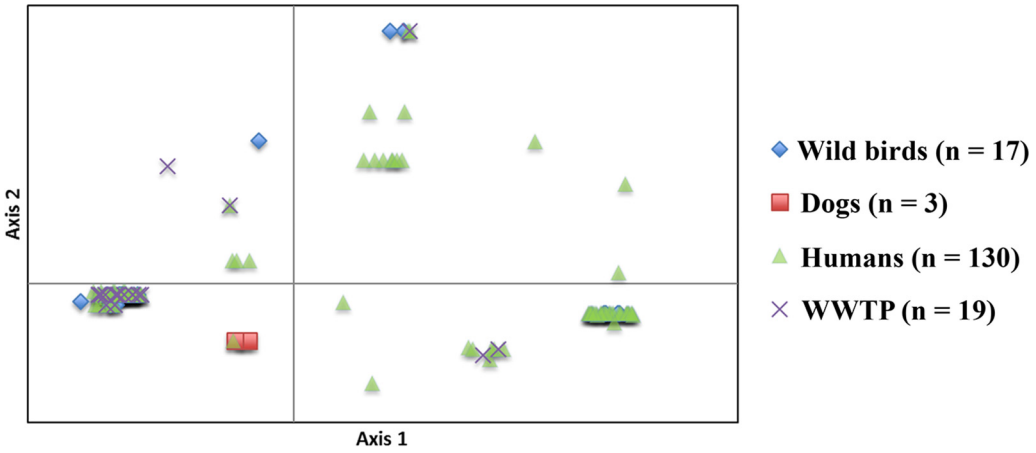

FIG 3 Principal-coordinate analysis (PCoA) of virulence genes, O-type, and *H*30 subclone designation among 169 *E. coli* ST131 isolates. The PCoA was based on results of all 52 virulence genes, O25b, and *H*30R1 and *H*30Rx status. Each isolate is plotted according to its value for PCoA coordinates 1 (horizontal axis) and 2 (vertical axis), where coordinates 1 and 2 capture 55.6% and 18.1% of the total variation, respectively. Based on PCoA, the genotypes and phylogeny structure of ST131 overlap extensively across the source groups.

sources groups (Fig. 3), consistent with the existence of environmental (wastewater) reservoirs and animal (wildlife) vectors for strains of human health importance. Furthermore, the presence of the *H*30R1 and *H*30Rx subclones among environmental and animal isolates, which are the most highly prevalent subclones of ST131 among human-source isolates (5), strongly supports our hypothesis of transmission of this clone between different ecological niches.

In addition, isolates obtained from different hospitals (H1 and H3) in different regions of Czech Republic showed indistinguishable pulsotypes and represented the internationally important *H*30R1 and *H*30Rx subclones, suggesting the successful spread of these lineages within Czech health care facilities. Here, $bla_{CTX\text{-}M\text{-}15}$-positive *H*30Rx ST131 isolates predominated, as also reported in multiple studies of human-source isolates from the community and health care facilities in different continents (5, 8–10, 12). In contrast, $bla_{CTX\text{-}M\text{-}27}$-containing *H*30R1 isolates, the second largest ST131 subset in our study population, were detected in just one of three sampled Czech hospital. This hospital contributed multiple independent isolates of what proved to be the same strain (hospital H1; 37 *H*30R1 isolates among 93 total ST131 isolates, each from a different patient), suggesting local spread within this facility. Furthermore, our $bla_{CTX\text{-}M\text{-}27}$-containing *H*30R1 isolates shared extensive genetic commonalities with isolates previously reported from Japanese and Korean hospitals (11, 17), suggesting that Asia might be the original source of this clone.

In the PFGE dendrogram, although the *H*30Rx and *H*30R1 isolates generally clustered by subclone, this segregation was incomplete, and certain isolates clustered with different subclones, indicating the limitation of PFGE for strain typing (5). Whole-genome sequence analysis should allow more accurate resolution of phylogenetic relationships (5, 18).

The strong associations we found of $bla_{CTX\text{-}M\text{-}15}$ with the F2:A1:B− multireplicon (19, 20) and the *H*30Rx subclone (4), and of $bla_{CTX\text{-}M\text{-}27}$ with the F1:A2:B20 replicon (19, 20) and the *H*30R1 subclone (4), have been documented in multiple previous studies. This indicates strong association of particular plasmid types and certain ST131 subclones. Since IncF plasmids likely contribute to ST131's success, further plasmid characterization may help to elucidate the relationship between particular plasmids and the epidemic success of high-risk clonal lineages.

In *Enterobacteriaceae*, $bla_{CTX\text{-}M\text{-}15}$ has been reported typically as a part of an IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477* transposition unit, nested within a Tn*2* element (15), whereas $bla_{CTX\text{-}M\text{-}27}$ typically occurs within IS*Ecp1*-$bla_{CTX\text{-}M}$-IS*903* (11, 15). The intact IS*Ecp1* 48-bp upstream of

bla$_{CTX-M-15}$ is considered the most common structure distributed globally (11, 15). However, we identified this arrangement only sporadically among our isolates; most *bla*$_{CTX-M-15}$-positive isolates had IS*Ecp1* truncated by IS*26* in different positions, with remnant parts of various lengths (Fig. 2). The most prevalent organization was formed by IS*26* upstream of a 24-bp remnant of IS*Ecp1* (group 1b4 in Fig. 2), as identified previously in clinical isolates and in the UK epidemic ST131 "strain A" (20), in *E. coli* isolates from travelers returning from India (21), and in *H*30Rx isolates from Japanese hospitals (11).

The IS*26*-ΔIS*Ecp1*-*bla*$_{CTX-M}$-like platform was also prevalent among *bla*$_{CTX-M-27}$-positive isolates. The dominant organization consisted of 208-bp remnant of IS*Ecp1* flanked by IS*26* upstream of *bla*$_{CTX-M-27}$ (group 9b in Fig. 2), as reported previously in most *bla*$_{CTX-M-27}$-positive *H*30 isolates from Japan (11).

Virotype C is considered to be the most broadly distributed virotype within ST131, occurring in both the *H*30R1 and *H*30Rx subclones (3, 9, 13). Here, virotype C predominated among *bla*$_{CTX-M-27}$-positive *H*30R1 isolates, as also reported recently from Japan (11). In contrast, virotype A was found by several European studies to predominate among *H*30Rx isolates in association with human-associated pulsotype 812, corresponding with the UK epidemic "strain A" (8, 12, 13). These findings, in conjunction with our data, suggest that virotype A might be highly prevalent in Europe among *bla*$_{CTX-M-15}$-*H*30Rx isolates, most likely due to the wide dissemination of international pulsotype 812. Here, pulsotype 812 was the most common, observed in isolates from all investigated sources in the Czech Republic. Moreover, we uniquely identified it in stray dogs from Africa and corvids from the United States (Fig. 1 and Table 2), indicating its broad geographical and host species distribution.

Collectively, these findings indicate that ST131 may spread successfully via different pathways from humans to wildlife and the environment, pointing out the capability of ST131 to survive and thrive in diverse hosts and ecological niches without direct antimicrobial selective pressure. Several potential transmission routes can be considered. Surface waters such as rivers and lakes, which contain water originating from diverse sources (e.g., wastewater treatment plants [WWTPs], urban or industrial effluents, and agricultural activities), may represent important reservoirs of antibiotic-resistant bacteria that might further disseminate to diverse ecological niches (22). It was estimated that each day a single WWTP can release into surface waters staggering numbers (>600 billion cells) of ESBL-producing *E. coli*, including epidemiologically important clones (23). The presence of ST131 isolates containing *bla*$_{CTX-M-27}$ has been reported in Europe from diverse aquatic environments such as rivers and lakes (23, 24), and the intestines of freshwater fish (24).

Here, similar PFGE profiles were found among *bla*$_{CTX-M-27}$-positive *H*30R1 isolates from hospital H1, WWTPs, and wild waterfowl (great cormorants). The foraging behavior of such waterfowl is strongly associated with aquatic environments, and all of these sources are located in close proximity to each other within the South Moravian Region in the Czech Republic. Although the direction of transmission is conjectural, these findings support that WWTP effluents contaminated by isolates of human origin may influence the commensal microbiota of water-associated animals such as waterfowl that live, breed, and forage in or near such contaminated aquatic environments.

We also found that corvids, which are synanthropic omnivorous birds that live in close contact with humans in habitats highly influenced by human activities (25, 26), were colonized by the (human-associated) ST131-*H*30Rx subclone (5). This could result from such birds' foraging behavior, which includes seeking for food in settings that may contain abundant resistant bacteria, e.g., fields that have been fertilized by sewage sludge or different types of animal waste, or various human or animal waste depots such as landfills (25, 26).

Related to this, in France, 7,500 metric tons of sewage sludge containing 2.6×10^5 ESBL-producing *E. coli* per gram is produced annually by one WWTP and is used as fertilizer on agricultural fields (23). ST131 has been reported from food-producing and companion animals (3, 16). Several studies recovered ESBL/AmpC-producing strains from food-producing animal feces, their housing environment, manure slurry, and

fertilized fields near the poultry, pig, and cattle farms (27–29). This application of slurry to farm fields as a fertilizer raised major biosafety concerns, since slurry exhibited the highest detection rate of ESBL/AmpC-producing *E. coli* among the investigated samples (27, 28), and bacteria with PFGE profiles indistinguishable from those of slurry-derived isolates could be detected on the field surfaces for several months after slurry application (27, 29). Therefore, such a contaminated environment might become a source of MDR bacteria for wildlife that feed in these habitats.

In summary, we found that our ST131 isolates from diverse ecological sources exhibited broad genetic commonality for multiple accessory traits and phylogenetic background, providing strong evidence that this pandemic clone spreads between different ecological niches, including humans, the environment, and wildlife. This presumably creates additional environmental reservoirs and vectors for strains of human medical importance, e.g., *H*30R1 and *H*30Rx, and may contribute to the global dissemination of these MDR pathogens. Because the expansion of *H*30R1 and *H*30Rx subclones has had a major impact on human health, interruption of such dissemination and elimination of relevant reservoirs should be a public health priority.

MATERIALS AND METHODS

Isolates. The study population was a convenience sample of 169 *E. coli* ST131 isolates, some previously published (25, 26, 30–34), as obtained from diverse sources and geographical locations. We compared isolates of human origin ($n = 130$) with isolates from synanthropic (avian symbionts of humans) wild bird species ($n = 17$) and companion animals ($n = 3$), which live in close contact with humans and thus have an elevated likelihood of colonization by human-source bacteria. We also compared them with isolates from WWTP effluent ($n = 19$) that was discharged into surface waters, which could serve as a connecting link between humans, the environment, and wildlife. Most of the isolates (73%, 123/169) were collected within the South Moravian Region in the Czech Republic; however, international isolates also were included. Most study isolates were obtained by selective cultivation on media supplemented with cefotaxime (2 mg/liter) and/or were identified as ESBL producers. To analyze the commonalities between different ST131 populations, quinolone-resistant or fully susceptible isolates were included. Detailed isolate characteristics (e.g., source, sampling location, isolation method, sampling date, etc.) are listed in Table 3 and also Table S2 in the supplemental material.

Molecular typing methods. ST131 status was verified for all isolates by PCR-based detection of ST131-specific SNPs in *mdh* and *gyrB* (35), and for selected representatives (43 of 169) of particular PFGE clusters, by multilocus sequence typing (MLST) based on sequence analysis of seven housekeeping genes (http://enterobase.warwick.ac.uk/species/index/ecoli). ST131 clonal subsets were identified by subclone-specific PCR (5, 36). The O25b and O16 *rfb* variants were detected by O-type-specific PCR (37).

Data regarding antimicrobial resistance-associated phenotypic and genotypic characteristics were available for some isolates, but all characteristics were newly generated here. Genes encoding ESBLs (bla_{CTX-M}, bla_{TEM}, bla_{OXA}, and bla_{SHV}), AmpC beta-lactamases (bla_{DHA}, bla_{ACC-1}, bla_{ACC-2}, bla_{MOX}, bla_{CMY}, and bla_{FOX}), and plasmid-mediated quinolone resistance [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB*] were detected by PCR and DNA sequencing (25). The presence and structure of class 1 and class 2 integrons was determined by restriction fragment length polymorphism mapping or sequencing of PCR amplicons (26). Plasmid incompatibility groups were determined by PCR-based replicon typing (38), and IncF plasmids were further analyzed by replicon sequence typing (39).

The genetic environment upstream of bla_{CMY} and bla_{CTX-M} variants representing CTX-M-1 ($bla_{CTX-M-3}$ and $bla_{CTX-M-15}$) and CTX-M-9 ($bla_{CTX-M-14}$ and $bla_{CTX-M-27}$) groups was mapped by PCR and sequencing by using published primers (30). The novel reverse primers CMY-mapIS (5′-CGATCCTAGCTCAAACAGC-3′) and CTX-M-9-mapIS (5′-CTTTTGCTGCACCGCACTC-3′) were used in combination with previously published forward primers (30) to examine the upstream region of bla_{CMY} and CTX-M group 9 variants, respectively. Isolates were grouped based on identical upstream region PCR results, and selected representatives of each such PCR group underwent Sanger sequencing of the PCR products to define their exact composition.

Virulence genes. Using multiplex PCR, the isolates were tested for 52 virulence genes associated with ExPEC (40). Isolates were classified as ExPEC if positive for ≥2 of the following genes: *papA* and/or *papC* (P fimbriae; counted as one), *sfa* or *focDE* (S and F1C fimbriae), *afa* or *draBC* (Dr-binding adhesins), *kpsMII* (group 2 capsule synthesis), and *iutA* (aerobactin system) (41). The virulence score was estimated as the number of extraintestinal virulence genes detected, adjusted for multiple detection of the *pap*, *sfa*, *foc*, and *kps* operons. Virulence gene patterns were calculated using BioNumerics (v6.6). Virotypes and subtypes were determined according to the presence of specific gene combinations (10).

Antimicrobial susceptibility. Susceptibility to 10 and 22 antimicrobial agents was assessed by disc diffusion and broth microdilution, respectively (42, 43). Interpretation of antimicrobial susceptibility was based on inhibition zone diameters or MIC values. Antimicrobial agents and reference strains used for susceptibility testing are listed in Fig. S1 in the supplemental material. Breakpoints were as specified by the Clinical and Laboratory Standards Institute (CLSI) for all antimicrobials excepting colistin and tigecycline (42) (CLSI breakpoints undefined), for which EUCAST breakpoints were used (43), and

TABLE 3 Source characteristics of the 169 *E. coli* ST131 study isolates

Origin	Location	Type of study[a]	Sampling period	No. of isolates (n = 169)	Source or reference
Czech Republic				159	
Humans[c]					
Hospital 1 (H1)	South Moravian Region: Brno	ESBL *E. coli* from patients with UTIs	2008–2011	93	33
Hospital 2 (H2)	South Moravian Region: Brno	ESBL *E. coli* from patient with febrile neutropenia	2009	1	31
Hospital 3 (H3)	Prague Region: Prague	FQ-R *E. coli* from urine samples	2011–2012	35	NP[b]
Community (CO)	Moravian-Silesian Region: Karvina	*E. coli* from cultivation on MCA	2012	1	NP
WWTP[d]					
Treated municipal wastewaters	South Moravian Region: Brno	*E. coli* from selective cultivation on MCA_{CTX}	2008–2009	18	30
Wildlife					
Rooks	South Moravian Region: Prerov	*E. coli* from selective cultivation on MCA_{CTX}	2010	8	25
Rooks	South Moravian Region: Zidlochovice	*E. coli* from selective cultivation on MCA_{CTX}	2012	1	NP
Great cormorants	South Moravian Region: Straznice[e]	*E. coli* from selective cultivation on MCA_{CTX}	2008	2	32
International isolates				10	
WWTP					
Treated municipal wastewaters	Slovakia	*E. coli* from cultivation on MCA	2011–2013	1	NP
Wildlife					
Rooks	Poland	*E. coli* from selective cultivation on MCA_{CTX}	2011	1	25
Rooks	Serbia	*E. coli* from selective cultivation on MCA_{CTX}	2011	3	25
American crows	United States	*E. coli* from selective cultivation on MCA_{CTX}	2012	2	26
Companion animals					
Dogs	Kenya	*E. coli* from selective cultivation on MCA_{CTX}	2009	3	34

[a]MCA, MacConkey agar; MCA_{CTX}, MacConkey agar supplemented with cefotaxime (2 mg/liter); ESBL, extended-spectrum beta-lactamases; UTI, urinary tract infection; FQ-R, fluoroquinolone resistant.
[b]NP, not published.
[c]H, human clinical isolates: H1, St. Anne's Faculty Hospital, Brno, CZ; H2, Children's Medical Center, Brno, CZ; H3, University Hospital Motol, Prague, CZ; CO: human community isolate, Karvina, CZ.
[d]WWTP, wastewater treatment plant.
[e]Nature Reserve Oskovec.

azithromycin (no defined *E. coli* breakpoints), for which *Streptococcus pneumoniae* breakpoints were used (42). Intermediate results were interpreted as resistant. Isolates resistant to at least one representative of ≥3 antimicrobial classes were interpreted as multidrug resistant.

PFGE and hybridization experiments. Genomic relatedness was defined by XbaI PFGE (25). Macrorestriction patterns were analyzed using BioNumerics v6.6 software (Applied Maths, Ghent, Belgium). Cluster analysis of the Dice similarity indices according to the unweighted-pair group method was used to infer a dendrogram describing the similarity relationships among PFGE profiles. Clusters were defined at an arbitrary 91% similarity level. Macrorestriction profiles of study isolates were compared with international pulsotypes (types 788, 797, 800, 805, 806, 812, 837, 842, 903, 905, 906, 943, 945, 968, 987, 1067, 1140, 1676, 1733, 1734, 1735, 1737, 1738, and 1739) within a large private PFGE profile library (14). In representative isolates, the plasmid versus chromosomal location of bla_{CTX} gene was determined by S1 PFGE and hybridization with DIG-11-dUTP-labeled probes specific for $bla_{CTX\text{-}15}$, $bla_{CTX\text{-}27}$, and IncF replicons (44). The isolates for S1 PFGE were selected to provide broad diversity with respect to source groups, ST131 subclones, molecular characteristics, plasmid types, and PFGE clusters.

Statistical analysis. Comparisons of proportions were tested for significance by a chi-square test, using MS Excel (Microsoft, Redmond, WA). The significance criterion was $P < 0.05$. Principal-coordinate analysis (PCoA) was used to reduce the dimensionality of the molecular data set for simplified comparisons (45).

Accession number(s). The nucleotide sequences of the $bla_{CTX\text{-}M}$ genetic environment (variant designation) from strains M46 (1a1), M30 (1a2), B81 (1b1), B100 (1b2), HS115 (1b3), M81 (1b4), M103

(1b5), M111 (1c1), M75 (1c2), OV56 (1c3), M79 (1c4), M77 (1d), M24 (1e), B95 (9a), and M57 (9b) have been deposited in GenBank under accession numbers MH357356, MH357357, MH357358, MH357359, MH357360, MH357361, MH357362, MH357363, MH357364, MH357365, MH357366, MH357367, MH357368, MH357369, and MH357370, respectively. The nucleotide sequence of the bla_{CMY-2} genetic environment from strain HPJ64 (C1) has been deposited in GenBank under accession number MH357371.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00519-18.

SUPPLEMENTAL FILE 1, XLSX file, 2.6 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 4, XLSX file, 0.1 MB.

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