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Molecular Epidemiology over an 11-Year Period (2000 to 2010) of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Causing Bacteremia in a Centralized Canadian Region

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A study was designed to assess the importance of sequence types among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates causing bacteremia over an 11-year period (2000 to 2010) in a centralized Canadian region. A total of 197 patients with incident infections were identified; the majority presented with community-onset urosepsis, with a significant increase in the prevalence of ESBL-producing *E. coli* during the later part of the study. The majority of *E. coli* isolates produced either CTX-M-15 or CTX-M-14. We identified 7 different major sequence types among 91% of isolates (i.e., the ST10 clonal complex, ST38, ST131, ST315, ST393, ST405, and ST648) and provided insight into their clinical and molecular characteristics. ST38 was the most antimicrobial-susceptible sequence type and predominated during 2000 to 2004 but disappeared after 2008. ST131 was the most antimicrobial-resistant sequence type, and the influx of a single pulsotype of this sequence type was responsible for the significant increase of ESBL-producing *E. coli* strains since 2007. During 2010, 49/63 (78%) of the ESBL-producing *E. coli* isolates belonged to ST131, and this sequence type had established itself as a major drug-resistant pathogen in Calgary, Alberta, Canada, posing an important new public health threat within our region. We urgently need well-designed epidemiological and molecular studies to understand the dynamics of transmission, risk factors, and reservoirs for *E. coli* ST131. This will provide insight into the emergence and spread of this multiresistant sequence type.

The production of extended-spectrum β-lactamases (ESBLs) is one of the most common causes of resistance to the oxyiminocephalosporins (e.g., cefotaxime, ceftriaxone, and ceftazidime) (30). Bacteremia caused by ESBL-producing bacteria is associated with adverse patient outcomes and increased costs compared to those of bacteremia due to non-ESBL-producing *Enterobacteriaceae* (17, 26, 39).

Extended-spectrum β-lactamases belonging to the TEM and SHV families, such as TEM-1, TEM-2, and SHV-1 β-lactamases, which are derivatives of non-ESBLs, were the predominant types of ESBLs during the 1980s and early 1990s (24). However, since the late 1990s, CTX-M β-lactamases have emerged worldwide among *Enterobacteriaceae* species, in particular *Escherichia coli*, and have become the most widespread type of ESBL in the world (30). CTX-M-producing *E. coli* strains are important causes of community-onset urinary tract infections, bacteremia, and intra-abdominal infections (2). Currently, the most widespread and prevalent type of CTX-M enzyme is CTX-M-15, and *E. coli* strains producing this enzyme often belong to the international uropathogenic sequence type named ST131 (25).

Escherichia coli is the most common cause of bloodstream infections in Calgary, Alberta, Canada, with an overall annual population incidence of 30.3/100,000 people (16). Previous studies have shown that ST131 had emerged as an important cause of bloodstream infections due to ESBL-producing *E. coli* in Calgary, especially during 2007 (27, 28). This study was designed to determine if the emergence of ST131 increased during 2008 to 2010, as well as to investigate the role of other sequence types in the molecular epidemiology of bloodstream infections caused by ESBL-producing *E. coli* over an 11-year period in a centralized Canadian region.

MATERIALS AND METHODS

Study population. In Calgary, the Calgary Health Region (CHR) provides all publicly funded health care services to the 1.2 million people residing in the cities of Calgary and Airdrie and numerous adjacent communities covering an area of 37,000 km^2. Acute care is provided principally through one pediatric and three large adult hospitals. A centralized laboratory (Calgary Laboratory Services) performs the routine clinical microbiology services for general practitioners, medical specialists, community clinics, and hospitals within the CHR.

Bacterial isolates and patients. All ESBL-producing *E. coli* isolates recovered from blood between 1 January 2000 and 31 December 2010 were studied. Only nonrepeat isolates from true incident cases were included in this study. A case of ESBL-producing *E. coli* bacteremia was defined as a patient with a systemic inflammatory response (e.g., fever, tachycardia, and leukocytosis) and documented growth of an ESBL-producing isolate in at least one blood culture (34). Patients who developed infections at least 48 h after admission to a health care center were classified as hospital-acquired cases. Patients who visited community-based collection sites or nursing homes or those who developed infections within the first 2 days after admission to an acute-care facility were classified as community-onset cases. The patients were further classified as having either community-acquired or health care-associated community-onset infections (10).

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Antimicrobial susceptibility testing. MICs were determined with the Vitek 2 instrument (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO) and the Microscan Neg MIC 30 panel (Siemens, Burlington, ON, Canada), which 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, results were interpreted using the 2011 CLSI criteria for broth dilution (6).

ESBL screening and confirmation testing. The presence of ESBLs was detected in clinical isolates of *E. coli* by using the 2009 CLSI criteria for ESBL screening and confirmation tests (5).

β-Lactamase gene identification. Isoelectric focusing (IEF), which included cefotaxime hydrolysis and inhibitor profiles in polyacrylamide gels, was performed on freeze-thaw extracts as previously described (27). PCR amplification and sequencing for the $bla_{\text{CTX-M}}$, bla_{OXA}, bla_{TEM}, and bla_{SHV} genes was carried out on the isolates with a GeneAmp 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) using previously described PCR conditions and primers (27).

Plasmid-mediated quinolone resistance (PMQR) determinants and phylogenetic groups. The amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken in all ESBL-positive isolates with multiplex PCRs (32). *aac*(*6′*)*-Ib* and *qepA* were amplified in a separate PCR using previously described primers and conditions (23, 43). 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, and D) by the use of a multiplex PCR-based method (3).

PFGE. 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 (12). Cluster designation was based on isolates showing approximately 80% or greater relatedness, which corresponds to the criteria of the "possibly related (4 to 6 bands difference)" category of Tenover et al. (38).

Identification of sequence types. Multilocus sequence typing (MLST) was performed using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) (http://mlst.ucc.ie/mlst/dbs/Ecoli). PCR for the ST131 *pabB* allele was also performed as described by Clermont and colleagues (4). For the purpose of this study, we included sequence types only if 5 or more isolates belonged to that specific sequence type.

Statistical methods. χ^2 tests, with Fisher's exact test in the case of small numbers, were used to compare differences for categorical data by using the SPSS statistics (version 17) program (IBM Corporation, NY).

RESULTS

Patients. During 2000 to 2010, a total of 197 CHR residents with incident bloodstream infections due to ESBL-producing *E. coli* isolates were identified; 42 (21%) were classified as hospital-acquired infections, 105 (53%) as health care-associated community-onset infections, and 50 (25%) as community-acquired infections. Health care-associated infections involving the urinary tract increased during the later part of the study. The mean patient age was 66 years (standard deviation [SD], 18.5 years), and 112 (57%) of the patients were males. The majority of the patients (128 [65%]) presented with the clinical syndrome of urosepsis, 32 (16%) presented with intra-abdominal infections (acute cholangitis [$n = 21$] or peritonitis [$n = 11$]), 27 (14%) presented with primary sepsis, 7 (5%) presented with pneumonia, and 3 patients presented with various types of other infections (including cellulitis, osteomyelitis, and septic bursitis). The cases never clustered in an acute-care center or a certain part of Calgary. We have not experienced an outbreak of ESBL-producing *E. coli* in Calgary.

Bacterial isolates and susceptibilities. During 2000 to 2010, 4,698 *E. coli* isolates (1 isolate per patient) were isolated from blood in Calgary; 197 (4%) tested positive for ESBL production (Table 1). The total number of *E. coli* isolates obtained from blood increased steadily over the 11-year period (e.g., 335 in 2000 to 459 in 2010). The number of ESBLs increased from 1/335 (0.3%) in 2000 to 6/372 (2%) in 2001 and then remained stable until 2006 (9/445 [2%]). From 2007, there was a significant increase in the numbers of ESBL producers and 14% (63/459) of *E. coli* isolates obtained from blood in the CHR during 2010 were identified as ESBL producers. Of the 197 isolates included in this study, 150 (76%) were nonsusceptible (i.e., either intermediate or resistant) to SXT, 82 (42%) were nonsusceptible to TZP, 131 (66%) were nonsusceptible to TOB, 104 (53%) were nonsusceptible to GEN, 48 (24%) were nonsusceptible to AMK, and 177 (90%) were nonsusceptible to CIP. No resistance to MEM and IPM was detected using the 2011 CLSI breakpoints (6). The MIC range (μg/ml), MIC_{50} (μg/ml), and MIC_{90} (μg/ml) for other drugs were as follows: for cefotaxime (CTX), 8 to >32, >32, and >32, respectively; for ceftazidime (CAZ), 1 to >16, 16, and >16, respectively; and for cefepime (FEP), 1 to >16, 16, and >16, respectively. MEM and IPM both had MICs of ≤1 μg/ml.

β-Lactamases, PMQR determinants, and phylogenetic groups. Of the 197 ESBL-producing *E. coli* isolates, 119 (60%) produced CTX-M-15, 58 (29%) produced CTX-M-14, 5 (3%) produced CTX-M-24, 3 (2%) produced CTX-M-27, 1 (1%) produced CTX-M-3, and 1 (1%) produced CTX-M-2, while 3 (2%) produced TEM-52, 1 (1%) produced SHV-5, 2 (1%) produced SHV-2, and 4 (2%) produced SHV-12. Some of the isolates also produced TEM-1 and OXA-1 in addition to CTX-M-15. Ninety-two (47%) of the ESBL-producing *E. coli* isolates were positive for *aac*(*6′*)*-Ib-cr*, and one isolate was positive for *qepA*. The majority of isolates belonged to phylogenetic group B2 (120 [61%]), 55 (28%) belonged to phylogenetic group D, 17 (9%) belonged to phylogenetic group A, and 5 (3%) belonged to phylogenetic group B1.

Pulsed-field gel electrophoresis. PFGE identified 13 clusters of *E. coli* isolates ($n = 180$) that were designated pulsotypes A1 ($n = 6$), A2 ($n = 4$), A3 ($n = 28$), A4 ($n = 17$), A5 ($n = 40$), A6 ($n = 18$), B ($n = 4$), C ($n = 13$), D ($n = 9$), E ($n = 11$), F ($n = 5$), G ($n = 14$), and H ($n = 11$). The isolates that belonged to pulsotypes A1 to H had PFGE profiles that were >80% similar to those of the other isolates in that pulsotype. Interestingly, isolates that belonged to pulsotypes A1 to A6 exhibited >60% similarity of PFGE profiles, and this suggested that they were related to each other. Pulsotypes B to H were not related to each other or to pulsotypes A1 to A6. The remaining ESBL-producing isolates ($n = 17$) were not clonally related, i.e., they exhibited <60% similarity of PFGE profiles and did not show patterns similar to those from pulsotypes A1 to H.

Multilocus sequence typing. MLST identified pulsotypes A1 to A6 as ST131, B as ST131, C as the ST10 clonal complex (consisting of ST10 and ST617), D as ST315, E as ST38, F as ST393, G as ST405, and H as ST648; overall, 180/197 (91%) of the isolates belonged to one of these sequence types (Table 2). ST315 and ST38 belong to clonal complex ST38. The PCR for the *pabB* allele confirmed pulsotypes A1 to A6 as ST131, while isolates from pulsotype B were negative for the *pabB* allele. The clinical features, location of acquisition, antimicrobial susceptibilities, PMQR determinants, ESBL types, and phylogenetic groups of the ST10

TABLE 1 Numbers of total *Escherichia coli* isolates and of ESBL producers isolated from blood in the Calgary Health Region from 2000 to 2010

Yr	Total no. of *E. coli* isolates	No. of isolates producing ESBLs (% of total)	Sequence type (*n*)[a]	ST131 pulsotype (*n*)
2000	335	1 (0.3%)	ST648 (1)	
2001	372	6 (2%)	ST38 (4)	A3 (1)
			ST131 (1)	
			Non-STs (1)	
2002	335	6 (2%)	ST10CC (2)	
			ST38 (2)	
			ST405 (1)	
			Non-STs (1)	
2003	377	5 (1%)	ST38 (2)	A1 (1)
			ST131 (2)	A3 (1)
			ST648 (1)	
2004	375	5 (1%)	ST10CC (1)	
			ST38 (1)	
			Non-STs (3)	
2005	385	13 (3%)	ST10CC (1)	A1 (1)
			ST131 (6)	A3 (2)
			ST315 (1)	A4 (2)
			ST405 (2)	A5 (1)
			ST648 (1)	
			Non-STs (2)	
2006	445	9 (2%)	ST10CC (1)	A3 (3)
			ST131 (3)	
			ST315 (1)	
			ST405 (2)	
			Non-STs (2)	
2007	436	18 (4%)[b]	ST10CC (1)	A1 (1)
			ST38 (1)	A2 (1)
			ST131 (8)	A3 (1)
			ST315 (1)	A5 (3)
			ST393 (1)	A6 (2)
			ST405 (3)	
			ST648 (2)	
			Non-STs (1)	
2008	484	27 (6%)	ST10CC (1)	A1 (1)
			ST38 (1)	A3 (3)
			ST131 (14)	A4 (3)
			ST315 (2)	A5 (4)
			ST393 (2)	A6 (2)
			ST405 (4)	B (1)
			Non-STs (3)	
2009	498	44 (9%)	ST10CC (2)	A1 (1)
			ST131 (34)	A3 (8)
			ST315 (2)	A4 (5)
			ST393 (1)	A5 (12)
			ST405 (1)	A6 (5)
			ST648 (3)	B (3)
			Non-ST (1)	
2010	459	63 (14%)	ST10CC (4)	A1 (1)
			ST131 (49)	A2 (3)
			ST315 (2)	A3 (9)
			ST393 (1)	A4 (7)
			ST405 (1)	A5 (20)
			ST648 (3)	A6 (9)
			Non-STs (3)	
Total	4,698	197 (4%)		

[a] ST10CC, ST10 clonal complex consisting of ST10 and ST617; non-STs, isolates that did not belong to a major sequence type. In a previous study (26), 3 isolates of ST131 (1 each in 2001, 2003, and 2005) were missed using the Diversilab technique.

[b] In a previous study (26), we described 22 ESBL-producing *E. coli* isolates during 2007; however, in this study, we only included true incident cases and 4 isolates were found to be repeats.

clonal complex, ST38, ST131, ST315, ST393, ST405, ST648, and those that did not belong to a major sequence type (referred to as non-STs) are shown in Table 2. These sequence types belonged to phylogenetic groups A (i.e., ST10), B2 (i.e., ST131), and D (i.e., ST38, ST315, ST315, ST393, and most of ST405 and ST648) and often produced either CTX-M-14 or CTX-M-15. ST131 was the most resistant sequence type, while ST38 was the most susceptible (Table 2).

DISCUSSION

Several worldwide reports have shown the emergence of CTX-M-producing *E. coli* as a significant pathogen since the turn of the 21st century and have shown that these bacteria are important causes of bloodstream infections, with the urinary tract as the most frequent source (33). Our study investigated the clinical and molecular characteristics of ESBL-producing *E. coli* isolates associated with bacteremia over an 11-year period (2000 until 2010) in a centralized health region in North America (specifically the Calgary Health Region) and showed that these isolates significantly increased during the later part of the study period (i.e., 45/2,624 [2%] of *E. coli* isolates obtained from blood during 2000 to 2006 were ESBL producers, as opposed to 152/1,877 [8%] isolated during 2007 to 2010 [$P < 0.001$]).

Multilocus sequence typing, which uses sequence variation in a number of housekeeping genes to define types or clones, is an excellent tool for evolutionary studies to show common ancestry lineages among bacteria (36). It has led to the definition of major sequence types and the recognition of successful widespread international clones, such as ST131 (25). We have identified 7 different "major" MLST sequence types, the ST10 clonal complex, ST38, ST131, ST315, ST393, ST405, and ST648, among 91% of the ESBL-producing *E. coli* isolates included in this study.

The ST10 clonal complex with CTX-M-14 or -15 had previously been described in clinical isolates from Egypt (9) and Spain (40), as well as in poultry from Spain (8) and enteroaggregative *E. coli* from Nigeria (22). ST38 with CTX-M-9, -14, and -15 has previously been described in clinical isolates from Japan (37), the Netherlands (41), South Korea (15), and Tanzania (20). This sequence type is also associated with OXA-48 (31) and NDM-1 (42). ST393 with CTX-M-14 and -15 had mostly been described in urinary isolates from Spain (1) and South Korea (18). ST405 with various types of CTX-Ms has a worldwide distribution (7, 14, 19, 35), while ST648 with CTX-M-15 and -32 is present in poultry from Spain (8) and humans in China (44) and the Netherlands (41). ST405 and ST648 are also associated with NDM β-lactamases (11, 21).

There was a predominance of ST38 during the first 4 years of the study period, but this sequence type disappeared from ESBL-producing *E. coli* strains associated with bloodstream infections in our region after 2008 (i.e., 9/23 [40%] of ESBL-producing *E. coli* isolates obtained during 2000 to 2004 were ST38, compared to 2/174 [1%] of ESBL-producing *E. coli* isolates obtained during 2005 to 2010 [$P < 0.001$]). Interestingly, ST38 was responsible for community-wide outbreaks of upper and lower urinary tract infections in Alberta, Canada, in 2001 to 2002, and at that time, it was known as clones 14A and 14AR (29).

ST131 was first described in ESBL-producing *E. coli* from blood in 2001, and its numbers remained stable until 2006 (Table 1). However, since 2007, the numbers of ST131 isolates increased significantly (i.e., 12/45 [27%] of ESBL-producing *E. coli* isolates

TABLE 2 Characteristics of sequence types ST10, ST38, ST131, ST315, ST393, ST405, and ST648 and non-STs for ESBL-producing *E. coli* isolates in the Calgary Health Region, Calgary, Alberta, Canada, 2000 to 2010

Characteristic	No. of isolates (%) of each sequence type (*n*)[a]								
	ST10CC (13)	ST38 (11)	ST131 (117)	ST315 (9)	ST393 (5)	ST405 (14)	ST648 (11)	Non-STs (17)	*P* value
Location of acquisition									0.69
Hospital	3 (23)	3 (27)	20 (17)	2 (22)	1 (20)	5 (36)	3 (27)	5 (29)	
Health care-associated area	6 (46)	4 (36)	71 (61)	4 (44)	3 (60)	4 (28)	5 (45)	8 (47)	
Community	4 (31)	4 (36)	26 (22)	3 (33)	1 (20)	5 (36)	3 (27)	4 (24)	
Clinical presentation									NA[c]
Urosepsis	5 (38)	6 (55)	85 (73)	2 (22)	5 (100)	11 (79)	7 (64)	7 (41)	
Intra-abdominal infection	4 (31)	4 (36)	9 (8)	5 (56)	0	1 (7)	4 (36)	5 (29)	
Primary sepsis	3 (23)	0	17 (15)	2 (22)	0	1 (7)	0	4 (24)	
Other	1 (8)	1 (9)	6 (5)	0	0	1 (7)	0	1 (6)	
Antimicrobial nonsusceptibility[b]									
AMC	10 (77)	5 (45)	96 (82)	8 (89)	2 (40)	11 (79)	9 (82)	10 (59)	0.03
TZP	5 (38)	0	60 (51)	5 (56)	1 (20)	5 (36)	3 (27)	2 (12)	0.007
CIP	13 (100)	10 (91)	114 (97)	5 (56)	4 (80)	14 (100)	11 (100)	6 (35)	<0.001
SXT	11 (85)	3 (27)	94 (80)	8 (89)	4 (80)	12 (86)	8 (73)	10 (59)	0.008
GEN	12 (92)	2 (18)	63 (54)	5 (56)	2 (40)	10 (71)	4 (36)	6 (35)	0.005
TOB	12 (92)	2 (18)	85 (73)	7 (78)	2 (40)	11 (79)	6 (55)	6 (35)	<0.001
AMK	2 (15)	0	40 (34)	2 (22)	0	1 (7)	1 (9)	2 (12)	0.02
PMQR determinants									<0.001
aac(6′)-*Ib-cr*	6 (46)	0	69 (59)	2 (22)	1 (20)	7 (50)	5 (46)	3 (18)	
qepA	1 (8)	0	0	0	0	0	0	0	
Type of ESBL									NA
CTX-M-14	0	11 (100)	29 (24)	4 (44)	2 (40)	5 (36)	3 (27)	4 (24)	
CTX-M-15	11 (85)	0	83 (71)	3 (33)	1 (20)	9 (64)	8 (72)	4 (24)	
Other CTX-M	2 (15)	0	4 (3)	1 (11)	1 (20)	0	0	2 (12)	
TEM-52	0	0	0	1 (11)	1 (20)	0	0	4 (24)	
SHV-2, SHV-5, or SHV-12	0	0	1 (1)	0	0	0	0	3 (18)	
Phylogenetic group									NA
A	13 (100)	0	0	0	0	3 (21)	0	1 (6)	
B1	0	0	0	0	0	1 (7)	0	3 (18)	
B2	0	0	117 (100)	0	0	0	0	4 (24)	
D	0	11 (100)	0	9 (100)	5 (100)	10 (71)	11 (100)	9 (53)	

[a] ST10CC, ST10 clonal complex consisting of ST10 and ST617; non-STs, isolates that did not belong to a major sequence type.
[b] Number of isolates either intermediate or resistant to indicated drug. AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SXT, trimethoprim-sulfamethoxazole.
[c] NA, not available (too many cells with fewer than 5 isolates).

obtained during 2000 to 2006 belonged to ST131, compared to 105/152 [69%] isolated during 2007 to 2010 [$P < 0.001$]). It is clear that ST131 was responsible for the significant increase of bloodstream infections caused by ESBL-producing *E. coli* since 2007.

Pulsed-field gel electrophoresis, following the restriction of genomic DNA with an appropriate rare-cutting enzyme, provides excellent discrimination between isolates and is especially appropriate for outbreak analysis (38). It was interesting to note that ST131 consisted of 6 distinct but related PFGE clones named A1 to A6 and a different pulsotype named B. Pulsotype A5 was the most prevalent clone, and overall, 40/117 (34%) of the ST131 isolates belonged to this pulsotype (Table 1). Pulsotype A5 was first identified in 2005, but since 2007, its number has increased drastically (Table 1) (i.e., 1/12 [8%] of ST131 isolates obtained during 2000 to 2006 belonged to pulsotype A5, compared to 39/105 [37%] of ST131 isolates obtained during 2007 to 2010 [$P = 0.056$]). It is clear that the increase of ST131 since 2007 was mostly due to pulsotype A5.

MLST identified pulsotype B ($n = 4$) as being ST131; however, the PCR for the *pabB* allele, as described by Clermont and colleagues (4), was negative for these 4 isolates. Pulsotype B was identified in 2008 ($n = 1$) and 2009 ($n = 3$) but disappeared in 2010. The discrepancies observed between the *pabB* allele-specific PCR and MLST for the ST131 identification are noteworthy and merit further investigation of this pulsotype. However, this is outside the scope of our study.

Johnson and colleagues recently investigated the presence and virulence properties of ST131 among 127 extracellular pathogenic *E. coli* (ExPEC) isolates from the 2007 SENTRY and Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance programs across the United States (13). Overall, 54

(17%) isolates belonged to ST131, but interestingly, this sequence type was responsible for 52% of isolates that showed resistance to ≥3 antimicrobial classes. Johnson et al. indicated that ST131 had distinctive virulence and resistance profiles and concluded that the combination of antimicrobial resistance and virulence may be responsible for the epidemiologic success of this sequence type. This combination seems to give ST131 a competitive advantage over other isolates of *E. coli*, most likely promoting its clonal expansion and dominance over less virulent and/or more susceptible *E. coli* clones (13). The molecular epidemiology results of our study support the laboratory findings of Johnson and colleagues. ST131 was the most antimicrobial-resistant sequence type in our study and practically invaded our region starting in 2007; 49/63 (78%) of ESBL-producing *E. coli* isolates obtained from blood during 2010 were ST131. Of particular interest was that during the same year, 41% (20/49) of ST131 isolates belonged to pulsotype A5. Our results suggest that certain pulsotypes of ST131 might be responsible for the success of this sequence type. Johnson and colleagues also noted that the different pulsotypes among ST131 isolates were present in their study but did not specifically address the virulence factors associated with different pulsotypes.

ST131 is a major drug-resistant pathogen among ESBL-producing *E. coli* strains in different parts of the world, including Calgary, and poses an important new public health threat within our region. We urgently need well-designed epidemiological and molecular studies to understand the dynamics of transmission, risk factors, and reservoirs for *E. coli* ST131. This will provide insight into the emergence and spread of this multiresistant sequence type that will hopefully lead to information essential for preventing the spread of ST131.

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