Molecular Characteristics of Extended-Spectrum-β-Lactamase-Producing Escherichia coli Isolates Causing Bacteremia in the Calgary Health Region from 2000 to 2007: Emergence of Clone ST131 as a Cause of Community-Acquired Infections

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A study was designed to characterize extended-spectrum-β-lactamase (ESBL)-producing Escherichia coli isolates causing bacteremia over an 8-year period (2000 to 2007) in a large well-defined geographical setting. Molecular characterization was done by using isoelectric focusing; PCR; and sequencing of the blαCTX-M, blαTEM, blαOXA, blαSHV, and plasmid-mediated quinolone resistance determinants. Genetic relatedness was determined by pulsed-field electrophoresis with XbaI and multilocus sequence typing. A total of 67 patients with incident bloodstream infections were identified, and the majority presented with community-acquired infections involving the urinary and biliary tracts. Of the 67 ESBL-producing E. coli isolates recovered, 60 (90%) were positive for blαCTX-M genes; 32 (48%) produced CTX-M-15, 25 (37%) produced CTX-M-14, 1 (2%) produced CTX-M-24, 1 (2%) produced CTX-M-2, and 1 (2%) produced CTX-M-3, while 2 (3%) produced TEM-52 and 5 (7%) produced SHV-2. Twenty-four (36%) isolates were positive for aac(6')-Ib-cr. The majority of isolates were resistant to ciprofloxacin (60 [90%] isolates) and gentamicin (40 [60%] isolates). The occurrence of ESBL-producing isolates was stable during the first 5 years, but there was a substantial increase from 2005 to 2007, mostly due to clone ST131, which produces CTX-M-15 and CTX-M-14, in blood cultures submitted from the community. Our results illustrated that E. coli clone ST131, which coproduces CTX-M-15, OXA-1, TEM-1, and aac(6')-Ib-cr, has emerged as an important cause of community-onset bacteremia caused by ESBL-producing E. coli isolates; and this is the first study to identify CTX-M-14 in E. coli clone ST131.

Organisms (especially Klebsiella spp. and Escherichia coli) producing extended-spectrum β-lactamases (ESBLs) are clinically relevant and are important causes of the failure of therapy with cephalosporins, especially when they are responsible for bloodstream-associated infections (16). Bacteremia caused by ESBL-producing members of the family Enterobacteriaceae was associated with severe adverse outcomes, including higher rates of mortality, increased lengths of hospital stays, delays in the time to the receipt of the appropriate therapy, the discharge of a higher proportion of patients to chronic care, and significantly higher costs, compared to the outcomes for patients with bacteremia caused by non-ESBL producers (28). An Italian study showed that inadequate initial therapy and an unidentified source of the primary infection were predictors of mortality for patients with bloodstream infections caused by ESBL-producing Enterobacteriaceae (32).

E. coli strains producing CTX-M β-lactamases have become prevalent over the last 5 years, especially in certain European and South American countries (4). Infections caused by bacteria producing these enzymes are not limited to the hospital setting, and their potential for spread beyond the hospital environment is an important public health concern (23). CTX-M-producing E. coli strains isolated from hospital and community sites often exhibit coresistance to trimethoprim-sulfamethoxazole, tetracycline, gentamicin, tobramycin, and ciprofloxacin (17). A previous study from the Calgary Health Region in Calgary, Alberta, Canada, demonstrated that CTX-M-producing E. coli is emerging as an important cause of community-onset urinary tract infections (20). That study showed a substantial increase of CTX-M-15-producers from urines that occurred during the latter part of the study period.

Although E. coli is the most common cause of bloodstream infections in our region, with an overall annual incidence of 30.3/100,000 population recently documented, limited data about the molecular epidemiology of ESBL-producing E. coli bacteremia at the population level in large geographical areas are available (12).

The study described here was designed to determine if the number of ESBL-producing E. coli isolates causing bacteremia increased over an 8-year period (2000 to 2007). We also characterized these isolates to determine if CTX-M-15 producers were also emerging in our region as important causes of bloodstream-associated infections.

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**MATERIALS AND METHODS**

**Study population.** The Calgary Health Region 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 km² in Alberta, Canada. Acute care is provided principally through one pediatric hospital 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.

**Bacterial isolates and patients.** All ESBL-producing *E. coli* isolates recovered from blood between 1 January 2000 and 31 December 2007 were studied. Only nonrepetitive isolates from true incident cases were included in this study. A case of ESBL *E. coli* bacteremia was defined as a patient with a systemic inflammatory response (e.g., fever, tachycardia, and leukocytosis) documented by the growth of an ESBL-producing isolate in at least one blood culture (27). Hospital-acquired cases were classified as patients who developed infections after 48 h from the time of admission to a health care center. Community-onset cases were classified as those patients who visited community-based collection sites or nursing homes or as patients who developed infections within the first 2 days of admission to an acute-care facility. The patients were further classified as having either community-acquired or health care-associated community-onset infections (7). Health care-associated community-onset cases were those that occurred among nursing home residents, hemodialysis patients, or individuals who were either admitted to a hospital for at least 2 days in the preceding 90 days or who received care through a hospital-based clinic in the preceding 30 days.

**Antimicrobial susceptibility testing.** The MIC of the following drugs were determined with the Vitek 2 system (Vitek MS; bioMérieux Vitek Systems Inc., Hazelwood, MO): piperacillin-tazobactam (TZP), imipenem (IPM), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), trimethoprim-sulfamethoxazole (SXT), and ciprofloxacin (CIP). Throughout this study, the results were interpreted by using the criteria of the Clinical and Laboratory Standards Institute (CLSI) for broth dilution (5). The quality control strains used for this part of the study were *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853.

**ESBL screening and confirmation testing.** ESBLs were detected in clinical isolates of *E. coli* by using the CLSI criteria for screening for ESBLs (ceftaxime and ceftazidime at >1 μg/ml) and disk confirmation tests (5). Disks for ESBL confirmation tests were obtained from Oxoid Inc. (Nepean, Ontario, Canada). Klebsiella pneumoniae ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

**β-Lactamase identification.** Isoelectric focusing, which included cephalotaxime hydrolysis and determination of inhibitor profiles on polyacrylamide gels, was performed with freeze-thawed extracts, as described previously (20). PCR amplification of *blaCTX-M*, *blaOXA-1*, *blaOXA-2*, and *blaSHV* from the isolates was carried out with a GeneAmp 7900 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) by using the PCR conditions and primers described previously (20, 22). Automated sequencing of the PCR products was performed with an ABI Prism 3100 genetic analyzer (Applied Biosystems) and Sequence Analysis software. The sequences of the different ampiclons were compared to each other and to homologous sequences by using the Sequence Navigator software. The nucleotide and the deduced protein sequences were analyzed with the software available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/)

**Plasmid-mediated quinolone resistance (PMQR) determinants.** The amplification of the *qnrS*, *qnrB*, and *qnrG* genes was undertaken by multiplex PCR, as described before (24). *aac(6’)-Ib-cr* was amplified in a separate PCR with the primers and conditions described previously (24). The variant *aac(6’)-Ib-cr* was further identified by digestion with BstF11 (New England Biolabs, Ipswich, MA) (24).

**PFGE.** The ESBL-producing *E. coli* isolates were typed by pulsed-field gel electrophoresis (PFGE), following the extraction of genomic DNA and digestion with XbaI, by using the standardized protocol for *E. coli* O157:H7 established by the Centers for Disease Control and Prevention, Atlanta, GA (8). The subsequent PFGE analyses were performed on a CHEF-Mapper apparatus (Bio-Rad Laboratories, Hercules, CA). DNA relatedness was calculated on the basis of the Dice coefficient, and isolates were considered genetically related if the Dice coefficient correlation was 80% or greater, which corresponds to the possibly related criterion (a difference of four to six bands) of Tenover et al. (30).

**MLST and PCR typing.** Multilocus sequence typing (MLST) was performed with the seven conserved housekeeping genes (*aseC*, *clpX*, *fadD*, *icdA*, *lysP*, *mdh*, and *uidA*). A detailed protocol of the MLST procedure, including allelic type and sequence type (ST) assignment methods, available at the EcMLST website (http://www.shigatext.net/mlst), was used in this study. The DiversiLab semiautomated repetitive sequence-based PCR typing technique was also used to identify ST131, as described previously (18).

**Statistical methods.** The chi-square test and Fisher's exact test were used to compare group categorical data by using the Stata (version 9.0) program (Stata Corp., College Station, TX).

**RESULTS**

**Patients.** During the 8 years of surveillance, a total of 67 Calgary Health Region residents with incident bloodstream infections due to ESBL-producing *E. coli* isolates were identified; 16/67 (24%) were classified as hospital-acquired infections, 23/67 (34%) were classified as health care-associated community-onset infections, and 28/67 (42%) were classified as community-acquired infections. The mean age of the patients was 62.4 years, and 40 (60%) of the patients were males. The majority (44 [66%]) of the patients presented with the clinical syndrome of urosepsis, followed by 14 (21%) with intra-abdominal infections (acute cholecystitis *n* = 9 or cholangitis *n* = 5), and 6 (9%) presented with primary bacteremia or sepsis without a focus. The remaining three (4%) of patients presented with respiratory infections.

**Bacterial isolates and susceptibilities.** During the 8-year study period, 3,060 *E. coli* isolates (1 isolate per patient) were isolated from blood at Calgary Laboratory Services, and 67 (2%) tested positive for ESBL production (Table 1). The total number of *E. coli* isolates recovered from blood remained stable over the 8-year period, while the number of ESBL producers decreased from 1/335 (0.3%) in 2000 to 22/436 (5%) in 2007 (Table 1). Of the 67 isolates included in this study, 44 (66%) were resistant to SXT, 31 (47%) were resistant to TZP, 47 (70%) were resistant to TOB, 40 (60%) were resistant to GEN, and 60 (90%) were resistant to CIP. No isolate with resistance to AMK and IPM was detected.

**β-Lactamases and PMQR determinants.** Of the 67 ESBL-producing *E. coli* isolates recovered from blood, 60 (90%) were positive for *blaCTX-M* genes; 32 (48%) produced CTX-M-15, 25 (37%) produced CTX-M-14, 1 (2%) produced CTX-M-24, 1 (2%) produced CTX-M-2, and 1 (2%) produced CTX-M-3, while 2 (3%) produced TEM-52, and 5 (7%) produced SHV-2 (Table 1). Some of the CTX-M-producing isolates also produced the TEM-1 (CTX-M-2, CTX-M-14, and CTX-M-15) and the OXA-1 (CTX-M-15) β-lactamases. Of the 16 *E. coli* isolates responsible for nosocomial infections, 1 produced TEM-52, 2 produced SHV-2, and 13 produced the CTX-M types (including CTX-M-14 and CTX-M-15) of ESBLs, while 26/28 isolates responsible for community-acquired infections were positive for *blaCTX-M* β-lactamases (included CTX-M-3, CTX-M-14, CTX-M-15, and CTX-M-24). The remaining two isolates produced SHV-2. Of the 23 *E. coli* isolates involved in health care-associated community-onset infections, 1 produced SHV-2, 1 produced TEM-52, and the remaining 22 produced CTX-M types (including CTX-M-2, CTX-M-14, and CTX-M-15) of ESBLs.

Twenty-four (36%) of the ESBL-producing *E. coli* isolates (i.e., producers of CTX-M-15 [n = 23] and CTX-M-14 [n = 1]) were positive for *aac(6’)-Ib-cr*. The distribution of the different ESBL-producing *E. coli* isolates over the study period is
shown in Fig. 1. *E. coli* isolates producing CTX-M-14 were first isolated in 2000, those producing SHV-2 were first isolated in 2001, those producing CTX-M-3 and CTX-M-15 were first isolated in 2002, those producing TEM-52 were first isolated in 2004, and those producing CTX-M-2 were first isolated in 2005, while CTX-M-24 appeared in 2006 (Fig. 1).

**Plasmid profiles.** The restriction profiles obtained with HpaI showed that plasmids isolated from clones B, BR, and C were identical (plasmid size range, 110 to 120 kb) and were related to plasmid isolated from NR strains (plasmid size range, 130 to 140 kb).

**PFGE.** PFGE identified four closely related groups of *E. coli* isolates producing ESBLs. These were designated clone A (which produced CTX-M-14 \[n = 10\]), clone B (which produced CTX-M-14 \[n = 4\] and CTX-M-15 \[n = 14\], BR (i.e., related to B, which produced CTX-M-15 \[n = 3\]), and a separate clone named C (which produced CTX-M-15 \[n = 5\]). The group A, B, BR, and C isolates formed separate clusters with PFGE profiles that were 80% similar. The profiles of the group BR isolates exhibited 60% similarity to the profiles of the group B isolates, which suggests that group BR is related to group B. These clones were previously reported in a molecular epidemiology study (20).

The remaining ESBL-producing isolates demonstrated the following PFGE patterns: isolates producing CTX-M-14 \(n = 11\), CTX-M-15 \(n = 10\), CTX-M-3 \(n = 1\), TEM-52 \(n = 2\), and SHV-2 \(n = 5\) were not clonally related; i.e., they exhibited PFGE profiles that were >80% similar and did not show patterns similar to those from clones in groups A, B, BR, and C. The isolates producing CTX-M-2 and CTX-M-24 did not provide DNA bands by the PFGE method used in this study (most likely due to nondigestion).

**MLST and PCR typing.** MLST and repetitive sequence-based PCR typing of the CTX-M producing isolates identified PFGE clones B and BR as ST131. Overall, 21 isolates (i.e., PFGE clones B \[n = 18\] and BR \[n = 3\]) were identified to be clone ST131. The distribution of clone ST131 over the study period is illustrated in Fig. 2. ST131 isolates producing CTX-M-15 first appeared in blood in 2003 and became especially predominant from 2005 to 2007.

**Characteristics of MLST clone ST131.** The characteristics of clone ST131 compared to those of non-ST131 ESBL-producing *E. coli* are shown in Table 2.
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TABLE 2. Characteristics of MLST clone ST131 and non-ST131 ESBL-producing Escherichia coli isolates

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of isolates/ total no. tested (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST131</td>
<td>Non-ST131</td>
</tr>
<tr>
<td>Antimicrobial susceptibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEN resistant</td>
<td>18/21 (86)</td>
<td>22/46 (48)</td>
</tr>
<tr>
<td>TOB resistant</td>
<td>18/21 (86)</td>
<td>29/46 (63)</td>
</tr>
<tr>
<td>SXT resistant</td>
<td>14/21 (67)</td>
<td>30/46 (65)</td>
</tr>
<tr>
<td>TZP resistant</td>
<td>17/21 (81)</td>
<td>14/46 (30)</td>
</tr>
<tr>
<td>CIP resistant</td>
<td>21/21 (100)</td>
<td>39/46 (85)</td>
</tr>
<tr>
<td>Presence of aac(6’)-Ib-cr</td>
<td>18/21 (86)</td>
<td>6/46 (13)</td>
</tr>
<tr>
<td>PMQR determinant</td>
<td></td>
<td></td>
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<tr>
<td>Location of acquisition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>0/21 (0)</td>
<td>16/46 (35)</td>
</tr>
<tr>
<td>Health care associated</td>
<td>8/21 (38)</td>
<td>15/46 (33)</td>
</tr>
<tr>
<td>Community</td>
<td>13/21 (62)</td>
<td>15/46 (33)</td>
</tr>
<tr>
<td>Focus</td>
<td></td>
<td></td>
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<tr>
<td>Urosepsis</td>
<td>18/21 (86)</td>
<td>26/46 (57)</td>
</tr>
<tr>
<td>Intra-abdominal infection</td>
<td>0/21 (0)</td>
<td>14/46 (30)</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>1/21 (5)</td>
<td>2/46 (4)</td>
</tr>
<tr>
<td>Primary bacteremia</td>
<td>2/21 (10)</td>
<td>4/46 (9)</td>
</tr>
</tbody>
</table>

DISCUSSION

Two recent reports, one from Israel (1) and one from Spain (25), revealed that CTX-M-producing E. coli is emerging as an important cause of community-onset bloodstream infections. A report from Italy has shown that these organisms are also important causes of nosocomially acquired bloodstream infections (31). The study from Tel-Aviv in Israel investigated patients with community-onset bacteremia admitted to a hospital and found that 14% of the cases were due to ESBL-producing organisms (most often E. coli producing CTX-M-2). These bacteria were multiresistant, and nursing home residency and male sex were independent risk factors (1). Rodriguez-Bano et al. reported on 43 prospectively observed cases of ESBL-producing E. coli bloodstream infections over a 4-year period in Seville, Spain; 51% were community-onset infections most often caused by CTX-M-9- and CTX-M-14-producing isolates (25). These bacteria were also multiresistant, and the most frequent origin of infection was the urinary and the biliary tracts. In our study, bacteremia caused by ESBL-producing E. coli isolates predominantly occurred in males presenting with urosepsis or intra-abdominal infections. In addition, these organisms were multiresistant and showed high levels of resistance to CIP, GEN, TZP, and SXT. We found that 42% of the cases were community acquired, which is markedly different from the 19% incidence of community-acquired cases found in the Spanish study (25). This could be because our study included all cases in a large well-defined geographical region. The Spanish study was performed at a single 950-bed teaching hospital (25).

This study described the molecular epidemiology of ESBL-producing E. coli isolates recovered from blood over an 8-year period and showed that the incidence of these types of isolates increased in the Calgary Health Region during the second half of the study period (i.e., 18/1,419 [1%] of E. coli isolates recovered from blood to 2000 to 2003 were ESBL producers, whereas 49/1,641 [3%] isolates recovered from 2004 to 2007 were ESBL producers) (Table 1). This was due to an increase in the incidence of CTX-M-15 producers (i.e., 5/18 [28%] of ESBLs isolated from 2000 to 2003 were CTX-M-15, whereas 27/49 [55%] isolated from 2004 to 2007 were CTX-M-15) (Fig. 1). The increase in the number of CTX-M-15 producers recovered during the second half of the study was mostly due to an increase in the incidence of PFGE clones B and BR (which were subsequently identified as MLST clone ST131) (i.e., 2/5 [40%] of CTX-M-15 producers isolated from 2000 to 2003 belonged to clones B and BR, whereas 15/27 [56%] isolated from 2004 to 2007 belonged to clones B and BR). Tumbarello and colleagues noticed a threefold increase in the incidence of nosocomially acquired bloodstream infections due to ESBL-producing E. coli isolates over a 7-year period (1999 to 2005) at a hospital in Rome, Italy (31). However, it is unclear if that increase was due to a single type of ESBL-producing isolate or a specific clone.

CTX-M-14 and CTX-M-15 producers are the major players as important causes of nosocomial (13/16 [81%]), health care-associated community-onset (20/23 [87%]), and community-acquired (24/28 [86%]) bloodstream infections due to ESBL-producing E. coli isolates in our region. We had a predominance of CTX-M-14 producers during the first 4 years of the study period: 10 of 18 (55%) ESBL-producing E. coli isolates recovered from 2000 to 2003 were CTX-M-14 producers, whereas 15/49 (31%) isolates recovered from 2004 to 2007 were CTX-M-14 producers (Fig. 1). This was associated with community outbreaks due to PFGE clone A from 2000 to 2003. Clone A accounted for 6/10 (60%) of CTX-M-14-producing isolates in the first half of the study, whereas it accounted for 2/15 (13%) of CTX-M-14-producing isolates from 2004 to 2007. This clone had previously been described to be causing outbreaks of community-acquired urinary tract infections among older females in 2000 and 2001 (21).

Multidrug-resistant CTX-M-15-producing E. coli is emerging worldwide as an important pathogen causing community-onset and hospital-acquired infections (26). An identical clone named ST131 has been identified by using MLST among CTX-M-15-producing E. coli isolates recovered from 2000 to 2006 from several countries, including Spain, France, Canada, Portugal, Switzerland, Lebanon, India, Kuwait, and South Korea (6, 15). This clone belongs to highly virulent phylogenetic group B2 and has often been found to harbor multidrug resistance plasmids of the IncFII group. Clone ST131 producing CTX-M-15 has also recently been described in the United Kingdom (10), Italy (3), Turkey (33), Croatia (14), Japan (29), and the United States (9). E. coli isolates that belong to clone ST131 but that do not produce CTX-M β-lactamases have been isolated from the stools of healthy volunteers in Paris, France (13). A previous study from the Calgary Health Region demonstrated that travel to the Indian subcontinent, Africa, and the Middle East was associated with a high risk of urinary tract infection (including urosepsis) caused by ESBL-producing E. coli in returning travelers (11), and this was mostly due to clone ST131 isolates producing CTX-M-15 (19).

This study has shown that clone ST131 isolates coproducing CTX-M-15, OXA-1, and TEM-1 first appeared in 2003 and are emerging as important causes of bloodstream infections due to
ESBL-producing E. coli, especially during the latter part of the study (i.e., 1/18 [5%] of ESBL-producing E. coli isolates recovered from blood from 2000 to 2003 were ST131, whereas 20/49 [41%] of ESBL-producing E. coli isolates recovered from blood from 2004 to 2007 were ST131). An interesting finding from our study was the identification of CTX-M-14-producing E. coli isolates as clone ST131. To our knowledge, this is the first study to describe the presence of CTX-M-14 in E. coli ST131. Clone ST131 producing CTX-M-14 did not produce OXA-1 or TEM-1 (as clone ST131 isolates producing CTX-M-15 do).

The features of clone ST131 isolates compared to those of non-ST131 ESBL-producing E. coli isolates are summarized in Table 2. Clone ST131 was more likely to be resistant to GEN, TOB, CIP, and TZP; more likely to produce the aminoglycoside-modifying enzymeaac(6′)I-ib-cr; and more likely to cause community-acquired infections and urosepsis. However, clone ST131 was less likely to cause hospital-acquired and intra-abdominal types of infections.

In conclusion, our study illustrates that multidrug-resistant E. coli clone ST131 coproducing CTX-M-15, OXA-1, TEM-1, and aac(6′)I-ib-cr has emerged as an important cause of community-onset bacteremia. We suspect that this ESBL-producing E. coli isolate was most likely introduced into our region via foreign travel to the Indian subcontinent, Africa, and the Middle East. This is the first study to identify CTX-M-14 in E. coli clone ST131. There is a serious need to monitor the spread of this multidrug-resistant clone throughout the world. Empirical antibiotic coverage for these resistant organisms should be considered in patients in the community presenting with sepsis involving the urinary and biliary tracts, especially in areas with a high prevalence of ESBL-producing E. coli isolates.

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