ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2010, p. 1327–1330 Vol. 54, No. 3
0066-4804/10/$12.00 doi:10.1128/AAC.01338-09

High Prevalence of ST131 Isolates Producing CTX-M-15 and CTX-M-14 among Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Isolates from Canada▽

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Received 23 September 2009/Returned for modification 13 December 2009/Accepted 27 December 2009

Phenotypic and genotypic methods were used to characterize extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* isolated in 2007 from 11 different Canadian medical centers. Of the 209 ESBL-producing *E. coli* isolates tested, 148 (71%) produced CTX-M-15, 17 (8%) produced CTX-M-14, 5 (2%) produced CTX-M-3, and 1 produced CTX-M-27. Overall, 96 (46%) of the ESBL producers belonged to clonal complex ST131, with the highest prevalence in Brampton, Calgary, and Winnipeg. ST131 is an important cause of community onset urinary tract infections due to ESBL-producing *E. coli* across Canada.

Since 2000, *Escherichia coli* producing CTX-M enzymes has emerged worldwide as an important cause of community onset urinary tract infections (UTIs), and this has been called "the CTX-M pandemic" (3). This phenomenon accelerated rapidly, especially during the past 5 years, and today organisms producing these enzymes are the most common type of extended-spectrum β-lactamase (ESBL) producers found in most areas of the world (24). Although several members of the family *Enterobacteriaceae* that produce CTX-M β-lactamases have been involved in hospital-acquired infections, *E. coli* producing these enzymes is more likely to be responsible for community onset infections (21).

Currently, the most widely distributed CTX-M enzyme is CTX-M-15, which was first detected in *E. coli* from India in 2001 (10). Multidrug-resistant, CTX-M-15-producing *E. coli* is emerging worldwide, especially since 2003, as an important pathogen causing both community onset and hospital-acquired infections (6, 14, 20).

Two recent studies using multilocus sequencing typing (MLST) identified a single clone of CTX-M-15-producing *E. coli*, named ST131, in isolates from several countries, including Spain, France, Canada, Portugal, Switzerland, Lebanon, India, Kuwait, and Korea (6, 14). This clone is associated with serogroup O25, belongs to highly virulent phylogenetic group B2, and harbors multidrug-resistant IncFII plasmids. Since those initial studies, isolates of clonal complex ST131 that produce CTX-M-15 have also been reported in several countries, including the United Kingdom (11), Italy (2), Turkey (27), Croatia (12), Japan (25), the United States (8), and Norway (13). Isolates of clonal complex ST131 have also been associated with other types of β-lactamases, as well as ciprofloxacin-resistant *E. coli* isolates that do not have ESBLs (4, 9, 12, 15).

Due to the worldwide emergence of clone ST131 isolates that produce CTX-M β-lactamases, we designed a study to investigate the prevalence and characteristics of this clone in ESBL-producing *E. coli* isolated from community and hospital settings during 2007 from 11 different Canadian medical centers.

(This study was presented at the 26th International Congress of Chemotherapy and Infection in Toronto, Ontario, Canada, 2009 [abstract P179].)

Nonrepeat ESBL-producing *E. coli* was collected over a 1-month period in 2007 from different Canadian medical centers representing 11 cities in six provinces (Table 1). ESBL production was confirmed phenotypically by using the Clinical and Laboratory Standards Institute [CLSI] criteria for ESBL screening and disk confirmation tests (5).

MICs determined by using AST-N121 susceptibility cards were determined by Vitek 2 (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO). Throughout this study, results were interpreted by using CLSI criteria 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.

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 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) by using PCR conditions and primers previously described (18, 19).

Amplification of the *qnrA*, *qnrS*, and *qnrB* genes was done by multiplex PCR as described before (23). *aac*(*6′*)-*Ib* was ampli-

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▽ Published ahead of print on 4 January 2010.

TABLE 1. ESBL-producing *E. coli* isolated at various medical centers in Canada

Medical center	City	Province	No. of ESBL producers	β-Lactamases (no. of isolates)	No. (%) of clonal complex ST131 members
William Osler Health Centre (community-based hospital)	Brampton	Ontario	20	CTX-M-15 (19), CTX-M-14 (1)	13 (65)
Dynacare Kasper Medical Laboratories (community-based laboratory)	Edmonton	Alberta	12	CTX-M-15 (7), SHV-2 (5)	3 (25)
Mount Sinai Hospital (tertiary hospital)	Toronto	Ontario	21	CTX-M-15 (19), SHV-12 (2)	10 (48)
Montreal General Hospital (tertiary hospital)	Montreal	Quebec	18	CTX-M-15 (12), CTX-M-14 (1), CTX-M-3 (1), SHV-12 (2), TEM-52 (2)	4 (22)
Regina Department of Laboratories (community-based laboratory)	Regina	Saskatchewan	23	CTX-M-15 (14), CTX-M-14 (2), CTX-M-3 (1), SHV-12 (5), SHV-2 (1)	12 (52)
The Ottawa Hospital (tertiary hospital)	Ottawa	Ontario	18	CTX-M-15 (15), SHV-2 (3)	7 (39)
St. Boniface General Hospital (community-based hospital)	Winnipeg	Manitoba	18	CTX-M-15 (13), CTX-M-14 (2), SHV-2 (2), SHV-12 (1)	11 (61)
Royal Jubilee Hospital (Tertiary hospital)	Victoria	British Columbia	19	CTX-M-15 (7), CTX-M-3 (1), SHV-2 (8), SHV-12 (2), TEM-52 (1)	4 (21)
Medicine Hat General Hospital (community-based hospital)	Medicine Hat	Alberta	7	CTX-M-15 (6), SHV-2 (1)	4 (57)
Calgary Laboratory Services (centralized laboratory)	Calgary	Alberta	33	CTX-M-15 (18), CTX-M-14 (11), CTX-M-3 (1), CTX-M-27 (1), SHV-2 (1), SHV-12 (1)	20 (61)
Total			209	CTX-M-15 (148), CTX-M-14 (17), CTX-M-3 (5) CTX-M-27 (1), SHV-2 (22), SHV-12 (13), TEM-52 (3)	96 (46)

fied in a separate PCR using primers and conditions previously described (22). The variant *aac*(6′)-*Ib-cr* was further identified by digestion with BstF5I (New England BioLabs, Ipswich, MA) (16).

The ESBL-producing *E. coli* isolates were typed 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 (7). DNA relatedness was calculated on the basis of the Dice coefficient, and isolates were considered to be genetically related if the Dice coefficient correlation was 80% or greater, which corresponds to the "possibly related (4 to 6 bands difference)" criterion of Tenover et al. (26).

The DiversiLab semiautomated repetitive-sequence-based PCR typing technique was used to identify members of clonal complex ST131 as previously described (17). ST131 was further confirmed by using PCR detection of the *pabB* allele recently described by Clermont and colleagues (4). Fisher's exact tests were used to compare group categorical data using Stata 9.0 (Stata Corp., College Station, TX).

During November 2007, 209 ESBL-producing *E. coli* strains were isolated at the various medical centers (Table 1). The majority of the ESBL-producing isolates ($n = 164$ [78%]) were recovered from urine, 31 (15%) were from blood, 6 (3%) were from intra-abdominal specimens, 5 (2%) were from wounds, and 3 (1%) were from respiratory specimens. One hundred thirty (62%) of these specimens were submitted from community collection sites, 63 (30%) were from hospitals, and 16 (8%) were from nursing homes. Of the 209 isolates included in this study, 187 (89%) were nonsusceptible (i.e., intermediate or resistant) to ciprofloxacin, 151 (72%) were nonsusceptible to amoxicillin-clavulanate, 144 (69%) were nonsusceptible to tobramycin (TOB), 143 (68%) were nonsusceptible to trimethoprim-sulfamethoxazole, 104 (50%) were nonsusceptible to gentamicin (GEN), 82 (39%) were nonsusceptible to amikacin (AMK), 56 (27%) were nonsusceptible to piperacillin-tazobactam (TZP), and 17 (8%) were nonsusceptible to nitrofurantoin (NIT). No resistance to imipenem was detected.

Of the 209 ESBL-producing *E. coli* isolates, 171 (82%) were positive for $bla_{\text{CTX-M}}$ genes; 148 (71%) produced CTX-M-15, 17 (8%) produced CTX-M-14, 5 (2%) produced CTX-M-3, and 1 produced CTX-M-27, while 22 (11%) produced SHV-2, 13 (6%) produced SHV-12, and 3 (1%) produced TEM-52 (Table 1). Some of the CTX-M-producing isolates also produced TEM-1 (i.e., those with CTX-M-3, -14, and -15) and OXA-1 (only those with CTX-M-15) β-lactamases. One hundred twelve (54%) of the ESBL-producing *E. coli* isolates (CTX-M-15, $n = 111$; CTX-M-3, $n = 1$) were positive for *aac*(6′)-*Ib-cr*, and one (CTX-M-15) was positive for both *aac*(6′)-*Ib-cr* and *qnrB*. None of the CTX-M-14-, TEM-, or SHV-producing *E. coli* strains were positive for plasmid-mediated quinolone resistance (PMQR) determinants.

As expected, there was a predominance of CTX-M-producing organisms mostly isolated from urine specimens submitted from the community. There was uniformity of genotypes among the different medical centers across Canada, with $bla_{\text{CTX-M-15}}$ representing over 70% of the ESBLs isolated. Five centers had only two different types of ESBLs (the combination of CTX-M-15 and SHV-2 being the most prevalent), while the greatest variety of ESBLs was present in Calgary, with six different types identified (Table 1). CTX-M-15-producing *E.*

TABLE 2. Characteristics of MLST clonal complex ST131 members (n = 96) compared to those of non-ST131 (n = 113) ESBL-producing *E. coli* strains

Characteristic	No. of isolates/total (%)		*P* value
	Clonal complex ST131	Non-ST131	
Antimicrobial susceptibilities (n = 209):			
GEN nonsusceptible	64/96 (67)	40/113 (35)	0.0001
TOB nonsusceptible	89/96 (93)	55/113 (49)	<0.0001
AMK nonsusceptible	43/96 (45)	39/113 (35)	0.2
TZP nonsusceptible	33/96 (34)	23/113 (20)	0.03
NIT nonsusceptible	4/96 (4)	13/113 (12)	0.07
PMQR determinants (n = 209):			
aac(6′)-Ib-cr	66/96 (69)	46/113 (41)	0.0001
aac(6′)-Ib-cr and *qnrB*	1/96 (1)	0/113	
Collection sites (n = 209):			
Community	55/96 (57)	75/113 (66)	0.2
Hospital	29/96 (30)	34/113 (30)	1.0
Nursing home	12/96 (13)	4/113 (4)	0.02
Specimens (n = 209):			
Urine	70/96 (73)	94/113 (83)	0.4
Blood	20/96 (21)	11/113 (10)	0.03
Other	6/96 (6)	8/113 (7)	1

coli was the most common type of ESBL in all of the medical centers included in this survey (overall prevalence of 71%, ranging from 7/19 [37%] in Victoria to 19/20 [95%] in Brampton [Table 1]).

PFGE identified four closely related groups of *E. coli* isolates producing ESBLs (data not shown). These were designated cluster A (n = 26 isolates producing CTX-M-15), cluster AR (i.e., related to A; n = 41 isolates producing CTX-M-14 [n = 8] and CTX-M-15 [n = 33]), cluster ARR (i.e., related to AR; n = 29 isolates producing CTX-M-15), and a separate cluster named B (n = 5 isolates producing CTX-M-15). Similar clusters were previously reported in a molecular epidemiology study (18). The repetitive-sequence-based PCR typing and PCR for the *pabB* allele performed on the ESBL-producing isolates identified PFGE clusters A, AR, and ARR as members of MLST clonal complex ST131. Overall, 96/209 (46%) of the ESBL-producing isolates of CTX-M producers were identified as members of clonal complex ST131, which were present in all of the medical centers across Canada with prevalences ranging from 4/19 (21%) in Victoria to 13/20 (65%) in Brampton (Table 1). In contrast to our findings, the 2007 CANWARD study concluded that the spread of CTX-M-15-producing *E. coli* across Canadian hospitals is polyclonal and not due to a single strain (1). The characteristics of clonal complex ST131 are illustrated in Table 2. Clonal complex ST131 in our study (compared to other ESBL-producing *E. coli* strains) was more likely to be resistant to GEN, TOB, and TZP (but less likely to be resistant to NIT), more likely to be isolated from blood, and more likely to be present in specimens submitted from nursing homes (Table 2). Molecular characterization of clonal complex ST131 showed that the majority (91%) of the strains produced CTX-M-15 and 69% were positive for *aac(6′)-Ib-cr*. The nine strains of ST131 that produce CTX-M-14 were not as widespread across Canada as the CTX-M-15-producing isolates and were isolated from medical centers in Calgary, Regina, and Winnipeg.

In summary, clonal complex ST131 is an important cause of community onset UTIs due to ESBL-producing *E. coli* across Canada. This study highlights the need for monitoring the spread of this multidrug-resistant clonal complex throughout the world and provides better understanding of the contribution of clonal dissemination among Gram-negative resistant pathogens.

Gisele Peirano was supported by a postdoctoral research fellowship from Foreign Affairs and International Trade Canada (DFAIT). This work was supported by research grants from Calgary Laboratory Services (73-4063) and from Merck (Canada).

We thank Barbara Willey for her technical assistance and Elisabeth Thomas for providing isolates.

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