Prevalence and characterization of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated in Canadian hospitals: Results from CANWARD 2007

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OBJECTIVE: The purpose of the present study was to determine the prevalence and molecular epidemiology of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae identified from Canadian hospitals in CANWARD 2007. Can J Infect Dis Med Microbiol 2009;20(Suppl A):43A-48A.

METHODS: Clinically significant isolates were collected as part of the Canadian Ward Surveillance Study (CANWARD 2007) from January to December 2007, inclusive, from 12 sentinel hospital centres across Canada. Minimum inhibitory concentrations were determined by broth microdilution, and putative ESBL isolates were confirmed by the Clinical and Laboratory Standards Institute disk diffusion method. Polymerase chain reaction and DNA sequencing were used to detect \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{SHV}}, \text{bla}_{\text{OXA}} \) genes. Strains were typed using pulsed-field gel electrophoresis.

RESULTS: A total of 3.4% and 1.6% of Escherichia coli and Klebsiella pneumoniae, respectively, were identified as ESBL producers. Resistance to fluoroquinolones, doxycycline, trimethoprim/sulfamethoxazole and gentamicin occurred in 92.5%, 71.4%, 75.5% and 71.4%, 67.9% and 57.1%, and 58.5% and 57.1% of ESBL-producing E coli and K pneumoniae, respectively. A total of 90.6% and 71.4% of ESBL-producing E coli and K pneumoniae were identified as multidrug resistant. The CTX-M type was the predominant ESBL, with CTX-M-15 as the predominant genotype. A total of 81.7% of ESBL-producers carried several beta-lactamase genes. Pulsed-field gel electrophoresis revealed that the majority of ESBL producers were not genetically related (less than 80% homology). Similar patient demographics were observed among both ESBL-producing E coli and K pneumoniae.

CONCLUSION: CTX-M has become the most common enzyme among both ESBL-producing E coli and K pneumoniae. The spread of ESBL-producing bacteria across Canada is polyclonal and is not due to the clonal spread of a single strain.

Key Words: Canada; Characterization; Enterobacteriaceae; ESBL; Multidrug resistant; Prevalence

Prevalence et caractérisation des entérobactériaciées productrices de bêta lactamases à spectre élargi isolées dans les hôpitaux canadiens: Résultats de l’étude CANWARD 2007

OBJECTIF: Le but de la présente étude était déterminer la prévalence et l’épidémiologie moléculaire des entérobactériaciées productrices de bêta lactamase à spectre élargi (ou ESBL, pour extended-spectrum beta lactamase) recensées dans les hôpitaux canadiens en 2007.

MÉTHODES : Des isolats cliniquement significatifs ont été recueillis dans le cadre de l’étude CANWARD (Canadian Ward Surveillance Study 2007), entre janvier et décembre 2007, auprès de 12 centres hospitaliers sentinelles des quatre coins du Canada. Les concentrations minimales inhibitrices (CMI) ont été calculées par microdilution en bouillon de culture et les isolats d’ESBL potentiels ont été confirmé au moyen de la méthodes de diffusion sur disque du Clinical and Laboratory Standards Institutes. Des tests de PCR (pour polymérase chain reaction) et de séquençage de l’ADN ont été utilisés pour détecter les gènes \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{SHV}} \) et \( \text{bla}_{\text{OXA}} \). Les souches ont été typée par électrophorèse en champ pulsé.

RÉSULTATS : En tout, 3.4 % et 1.6 % des isolats d’Escherichia coli et de Klebsiella pneumoniae respectivement ont été associé à la fabrication d’ESBL. La résistance aux fluoroquinolones, à la doxycycline, au triméthoprime-sulfaméthoxazole et à la gentamicine a été observée chez 92.5 % et 71.4 %, 75.5 % et 71.4 %, 67.9 % et 57.1 % et 58.5 % et 57.1 % des isolats d’E coli et de K pneumoniae respectivement. En tout, 90.6 % et 71.4 % des isolats d’E coli et de K pneumoniae ont été jugés multirésistants. Le type CTX-M a été l’ESBL prédominant, avec le CTX-M-15 comme gène dominant. En tout, 81.7 % des isolats producteurs d’ESBL étaient porteurs de plusieurs gènes de la bêta lactamase. L’électrophorèse en champ pulsé a révélé que la majorité des souches productrices d’ESBL n’étaient pas génétiquement apparentées (moins de 80 % d’homologie). Pour les isolats d’E coli et de K pneumoniae producteurs d’ESBL, les caractéristiques des patients étaient similaires.

CONCLUSIONS : Le CTX-M est devenu l’enzyme la plus courante parmi les isolats d’E coli et K pneumoniae producteurs d’ESBL. La propagation des bactéries productrices d’ESBL au Canada est polyclonal et n’est pas attribuable à la propagation clonale d’une seule souche.

Extended-spectrum beta-lactams (ESBLs) are the most significant resistance determinants emerging and spreading worldwide among the Enterobacteriaceae (1). They were first reported in 1983 and since then, greater than 300 variants of ESBLs have been identified (http://www.lahey.org/Studies). The majority of ESBLs are Ambler Class A TEM, SHV, and CTX-M variants. Until the late 1990s, TEM and SHV were the predominant ESBL variants and were mainly associated with nosocomial infections caused by Klebsiella pneumoniae (2,3). The epidemiology of ESBLs has undergone a rapid change over the past decade with the emergence and spread of CTX-M ESBLs. CTX-M ESBLs are now the most prevalent genotype and are mainly associated with Escherichia coli infections from both community and nosocomial infections (2,4). ESBLs are enzymes that compromise the efficacy of beta-lactam antibiotics, with the exception of the cephapensins and carbapenems, by hydrolysis of the beta-lactam ring (3,5). Antimicrobial therapy is frequently limited for the treatment of ESBL producers because they are often multidrug resistant (MDR).

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TABLE 1
Demographics, hospital ward and specimen types from patients with infections due to extended-spectrum betalactamase (ESBL)-producing Escherichia coli or Klebsiella pneumoniae in Canadian hospitals

| Parameter          | E. coli, n=53 | K. pneumoniae, n=7 | P*  |
|--------------------|---------------|-------------------|-----|
| Sex                |               |                   |     |
| Male               | 29 (54.7)     | 4 (57.1)          | NS  |
| Female             | 24 (45.3)     | 3 (42.9)          | NS  |
| Age, years         |               |                   |     |
| ≤17                | 19 (35.8)     | 2 (28.6)          | NS  |
| >17                | 34 (64.2)     | 4 (57.1)          | NS  |
| Patient region     |               |                   |     |
| British Columbia/Alberta (n=2)† | 12 (22.7) | 1 (14.3) | NS  |
| Saskatchewan/Manitoba (n=2)† | 5 (9.4)   | 1 (14.3)         | NS  |
| Ontario (n=4)†     | 29 (54.7)     | 3 (42.9)          | NS  |
| Quebec/Maritimes (n=4)† | 7 (13.2) | 2 (28.6)        | NS  |
| Specimen type      |               |                   |     |
| Urine              | 15 (28.3)     | 1 (14.3)          | NS  |
| Blood              | 34 (64.2)     | 4 (57.1)          | NS  |
| Wound              | 1 (1.9)       | 1 (14.3)          | NS  |
| Respiratory tract  | 3 (5.7)       | 1 (14.3)          | NS  |

*Comparison of demographics, hospital wards and specimen types among ESBL-producing E. coli and ESBL-producing K. pneumoniae; †Number of hospitals per region. NS Not significant; P>0.05

The purpose of the present study was to determine the prevalence and molecular epidemiology of ESBL-producing Enterobacteriaceae isolated from Canadian hospitals in 2007.

MATERIALS AND METHODS
Bacterial strains from surveillance study
Bacterial isolates were collected as part of the Canadian Ward Surveillance Study (CANWARD 2007). CANWARD is a laboratory-based surveillance study coordinated by the Health Sciences Centre in Winnipeg, Manitoba. From January 1 through December 31, 2007, inclusive, 12 sentinel hospital centres across Canada submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. Each centre was asked to submit pathogens (consecutive, one per patient/infection site) from blood (n=360), respiratory (n=200), urine (n=100), and wound/intravenous (n=50) infections. If the centres did not collect a minimum of 20 ESBL producers within these objectives, they were asked to continue to collect above the objectives until a minimum of 20 was fulfilled. All isolates were identified at participating sites by routine procedures performed at each laboratory. Isolates were shipped to the reference laboratory at the Winnipeg Health Sciences Centre on Amies charcoal swabs, subcultured onto blood agar, and stocked in skim milk at –80°C until minimum inhibitory concentration (MIC) testing was carried out. In the present study, only the analysis of unique ESBL-producing Enterobacteriaceae isolates that fell within the CANWARD 2007 objectives will be reported.

Antimicrobial susceptibility testing
Following two subcultures from frozen stock, the in vitro activities of various antimicrobials were determined in triplicate by microbroth dilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (6). Colistin (polymyxin E) MICs were determined using the E-test method. Food and Drug Administration (USA) interpretation breakpoints were used for tigecycline (susceptible: 2 µg/mL or less, intermediate: 4 µg/mL and resistant: 8 µg/mL or greater) and colistin (susceptible: 2 µg/mL or less and resistant: 4 µg/mL or greater). Strains concomitantly resistant to three or more different antimicrobial classes were defined as MDR.

Any E. coli, K. pneumoniae, Klebsiella oxytoca or Proteus mirabilis with a ceftriaxone MIC of 1 µg/mL or greater was identified as a putative ESBL and underwent further analysis. The putative ESBL phenotype was confirmed by the disk diffusion method as described by CLSI. E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were the control strains used in the study.

Characterization of ESBL genes
Genotypic characterization of ESBLs was performed by polymerase chain reaction and sequencing of blaTEM, blaSHV, and blaOXA-1 genes as previously described (7-9). An Basic Local Alignment Search Tool search of the DNA sequence was conducted to determine specific genotypes.

Genetic relationships
Genetic relationships of the ESBL-producing Enterobacteriaceae were assessed by pulsed-field gel electrophoresis following digestion with XbaI as previously described (7).

Statistical analysis
χ² analysis was used to evaluate statistical significance, as appropriate, using Graphpad Quickcalcs (Graphpad Software Inc).

RESULTS
Epidemiology of ESBL-producing Enterobacteriaceae
A total of 7881 clinical isolates were collected as part of the CANWARD 2007 surveillance study. E. coli, K. pneumoniae, K. oxytoca and P. mirabilis ranked first (n=1702, 21.6%), fifth (n=457, 5.8%), 11th (n=119, 1.5%) and 14th (n=100, 1.3%), respectively, among all pathogens collected. Of those that fell within the CANWARD objectives, 93 of 1560 (6.0%) E. coli, 12 of 442 (2.7%) K. pneumoniae, seven of 119 (5.9%) K. oxytoca and three of 111 (2.7%) P. mirabilis had a ceftriaxone MIC of 1 µg/mL or greater and were identified as putative ESBL producers. Of those with a ceftriaxone MIC of 1 µg/mL or greater, 53 E. coli and seven K. pneumoniae were identified phenotypically as unique ESBL producers and were further analyzed. The prevalence of ESBL-producing E. coli was 3.4% (53 of 1560) and ranged from 1.1% in emergency rooms, 1.9% in intensive care units, 3.3% in hospital clinics, 6.2% in medical wards to...
7.9% in surgical wards. ESBL-producing E coli was identified at 11 of the 12 sites and the prevalence ranged from 0% to 9.3% among participating hospitals. The prevalence of ESBL-producing K pneumoniae was 1.6% and ranged from 0% in emergency rooms, 1.3% in intensive care units, 2.1% in surgical wards, 2.2% in hospital clinics to 2.8% in medical wards. ESBL-producing K pneumoniae was identified at five of the 12 sites with the prevalence ranging from 0% to 4.3% among participating hospitals. Patient demographics for ESBL-producing E coli and K pneumoniae are summarized in Table 1. The demographics for patients with ESBL-producing E coli and K pneumoniae infections were similar (P>0.05, not significant [NS]). The majority of patients were older than 66 years of age and were hospitalized in medical wards. No significant differences in the proportion of E coli isolates identified as ESBL producers were obtained from blood (34 of 788; 4.4%), respiratory tract (three of 100; 3.0%) and wound (one of 37; 2.7%) compared with urine (15 of 635; 2.4%) specimens (P>0.05; NS). Similar observations were made for K pneumoniae, where no significant differences in the proportion of ESBL producers were obtained from wound (one of 17; 5.9%), respiratory tract (one of 59; 1.7%) and blood (four of 266; 1.5%) compared with urine (one of 100; 1.0%) specimens (P>0.05; NS).

**Antimicrobial susceptibilities**

Susceptibility testing demonstrated that resistance to fluoroquinolones, doxycycline, trimethoprim/sulfamethoxazole and gentamicin occurred in 92.5% and 71.4%, 75.5% and 71.4%, 67.9% and 57.1%, and 58.5% and 57.1% of ESBL-producing E coli and K pneumoniae, respectively (Table 2). Forty-eight of

### Table 2

**Comparison of antimicrobial susceptibilities of extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing isolates (Escherichia coli and Klebsiella pneumoniae)**

| Antibiotic       | MIC50 | MIC90 | Range | % S | % I | % R | MIC50 | MIC90 | Range | % S | % I | % R |
|------------------|-------|-------|-------|-----|-----|-----|-------|-------|-------|-----|-----|-----|
| Amox/Clav†       | 8     | 10    | 2–32  | 60  | 38  | 12  | 4     | 6     | 0.5–32| 93  | 5.6 | 1.2 |
| Cefazolin        | 128   | >128  | >64   | 64  | 100 | 0   | 2     | 8     | ≤0.5–>128| 92  | 3.5 | 4.3 |
| Ceftriaxone      | >64   | >64   | 2–64  | 3.8 | 15  | 81  | ≤1    | ≤1    | ≤1–64| 98  | 1.0 | 0.8 |
| Cefepime         | 16    | >32   | ≤1–32 | 45  | 30  | 24  | ≤1    | ≤1    | ≤1–32| 99  | 0.3 | 0.1 |
| TZP              | 4     | 16    | ≤1–512| 92  | 5.7 | 1.9 | ≤1    | ≤1    | ≤1–512| 98  | 0.8 | 1.2 |
| Cefoxitin†       | 8     | 8     | 0.5–32| 92  | 5.7 | 1.9 | 4     | 8     | ≤0.06–32| 95  | 2.4 | 2.2 |
| Ertapenem†       | ≤0.06 | 0.12  | ≤0.06–0.25| 100 |     |     | ≤0.06 | ≤0.06 | ≤0.06–0.25| 100 |     |     |
| Meropenem        | ≤0.12 | ≤0.12 | ≤0.12 | 100 |     |     | ≤0.12 | ≤0.12 | ≤0.12–0.5 | 100 |     |     |
| Ciprofloxacin    | >16   | >16   | ≤0.06–>16| 7.5 | 92.5| 0   | ≤0.06 | >16   | ≤0.06–>16| 81.7| 3.0 | 18.0 |
| Levofloxacin     | 16    | 32    | ≤0.06–>32| 7.5 | 92.5| 0   | ≤0.06 | 16    | ≤0.06–>32| 82.2| 0.8 | 17.0 |
| Amikacin         | 4     | 8     | ≤2–64 | 94  | 3.8 | 96  | ≤2    | 4     | ≤2–64  | 99  | 0.9 | 0.1 |
| Gentamicin       | 32    | >32   | ≤0.5–32| 41.5| 58  | 5  | ≤0.5  | 1     | ≤0.5–32 | 93.1| 0.5 | 6.4 |
| Doxycycline      | 32    | >256  | 2–256 | 22  | 75.5| 2.5 | –     | –     | –     | –   | –   | –   |
| Tigecycline      | 0.5   | 1     | 0.25–2| 100 |     |     | 0.25  | 0.5   | 0.06–4 | 99  | 0.1 | NS  |
| TMP/SMX          | >8    | >8    | ≤0.12–>8| 32  | 67.9| 30  | ≤0.12 | >8    | ≤0.12–>8| 77.2| 22.8| 0.001 |
| Nitrofurantoin†  | 16    | 32    | 1–64  | 96.2| 3.8 | 1.9 | 16    | 32    | ≤0.5–128 | 96  | 6.2 | 2.6 |
| Colistin†        | 1     | 1     | ≤0.06–4| 98.1| 1.9 | 1.9 | 0.5   | 1     | ≤0.06–16 | 99  | 1.0 | NS  |

*Comparison of resistant (R) with nonresistant (susceptible [S] and intermediate [I]) isolates among ESBL-producing and non-ESBL-producing isolates. †Tested against fewer isolates of non-ESBL-producing isolates (E coli n=504; K pneumoniae n=185). Amox/Clav Amoxicillin/clavulanate; Colistin (polymyxin E); MIC50/90 Minimum inhibitory concentration (in µg/mL) required to inhibit the growth of 50%/90% of organisms; N/A Not available; NS Not significant, P>0.05; TMP/SMX Trimethoprim/sulfamethoxazole; TZP Piperacillin-tazobactam
53 (90.6%) and five of seven (71.4%) ESBL-producing E. coli and K. pneumoniae were identified as MDR. All ESBL producers remained susceptible to the carbapenems. A comparison of antimicrobial susceptibilities between ESBL-producing and non-ESBL-producing isolates to various antibiotics are summarized in Table 2. Not surprisingly, the ESBL producers displayed higher resistance rates to all antimicrobials tested with the exception of the carbapenems and tigecycline in comparison to non-ESBL producing isolates.

### Distribution of beta-lactamase genes among extended-spectrum beta-lactamase (ESBL) producers

| Genotype* | Organism         | Number of isolates |
|-----------|------------------|--------------------|
| CTX-M-2, SHV-11 | K. pneumoniae | 1 |
| CTX-M-3, TEM-1, OXA-1 | E. coli | 1 |
| CTX-M-14 | E. coli | 3 |
| CTX-M-14, TEM-1 | E. coli | 14 |
| CTX-M-15 | E. coli | 3 |
| CTX-M-15, TEM-1 | E. coli | 2 |
| CTX-M-15, OXA-1 | E. coli | 16 |
| CTX-M-15, SHV-1, OXA-1 | K. pneumoniae | 1 |
| CTX-M-15, SHV-28, OXA-1 | K. pneumoniae | 1 |
| CTX-M-15, TEM-1, OXA-1 | E. coli | 8 |
| CTX-M-15, TEM-1, SHV-5, OXA-1 | K. pneumoniae | 1 |
| CTX-M-24, TEM-1 | E. coli | 1 |
| CTX-M-27 | E. coli | 2 |
| CTX-M-65 | E. coli | 1 |
| SHV2a | E. coli | 1 |
| Unknown, SHV-1 | K. pneumoniae | 3 |
| Unknown, TEM-1 | E. coli | 1 |

*TEM-1, SHV-1, SHV-11 and OXA-1 are not ESBLs.

Molecular typing using pulsed-field gel electrophoresis was conducted to study clonal relationships among ESBL-producing and K. pneumoniae. A dendrogram depicting the genetic relationships among ESBL-producing E. coli and K. pneumoniae isolates from Canadian hospitals is shown in Figure 1. The majority of ESBL-producing E. coli were determined to be genetically unrelated (less than 80% homology). Several small clusters (two to five isolates) of genetically related ESBL-producing E. coli carrying the same type of CTX-M were observed. However, there were two clusters of two genetically related E. coli from different regions of Canada with CTX-M from different CTX-M groups (CTX-M-1 and CTX-M-9) suggesting two different acquisition events among the same strain.

Clonal spread was suggested to have occurred at one of the participating sites on two separate occasions involving two ESBL-producing E. coli. The ESBL-producing K. pneumoniae were determined to be genetically unrelated. A fingerprint was not obtained for one ESBL-producing K. pneumoniae due to autodigestion of the DNA.

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**Figure 1: Pulsed-field gel electrophoresis dendrogram depicting the genetic relationships among extended-spectrum beta-lactamase (ESBL) producers. A. ESBL-producing Escherichia coli; B. ESBL-producing Klebsiella pneumoniae.**
DISCUSSION

The prevalence and molecular epidemiology of ESBL-producing *E. coli* and *Klebsiella* species in Canada has been monitored since the late 1990s (7-12). Throughout these studies, we have observed increases in the prevalence and changing trends in the molecular epidemiology of ESBL producers. The present study updates the prevalence and molecular epidemiology of ESBL producers among Canadian hospitals.

Our study found the prevalence of ESBL-producing *E. coli*, *K pneumoniae*, *K oxytoca* and *P mirabilis* isolated from Canadian hospitals in 2007 to be 3.4%, 1.6%, 0% and 0%, respectively. These results are similar to the prevalence rates we observed from our Canadian Intensive Care Unit (CAN-ICU) Surveillance study from 2005 to 2006, where the prevalences of ESBL-producing *E. coli* and *Klebsiella* species were determined to be 3.7% and 1.8%, respectively (9,12). However, in contrast to the CAN-ICU study, the majority of ESBL producers from the CANWARD 2007 study were obtained from medical wards (30 of 60 ESBL-producers; 50.0%) with very few coming from intensive care units (four of 60 ESBL-producers; 6.7%) (P=0.0001).

CTX-M ESBLs were the most common variants observed among both ESBL-producing *E. coli* and *K pneumoniae*, with CTX-M-15 being the most common genotype among both as well as CTX-M-14 among *E. coli*. This is not novel among ESBL-producing *E. coli* (9,10,12,13). However, this is the first report of CTXM becoming the predominant enzyme among ESBL-producing *K pneumoniae* in Canada, replacing SHV (7,11). In addition, we describe the first CTX-M-65-producing *E. coli* in Canada. CTX-M-65, a variant of CTX-M-14, was first reported and identified in the United States in 2008 (14).

The majority of ESBL-producing *E. coli* also carried the narrow-spectrum *blaTEM-1* gene, whereas ESBL-producing *K pneumoniae* more commonly carried a narrow-spectrum *blaSHV* gene, whereas ESBL-producing *K pneumoniae* more commonly carried a narrow-spectrum *blaSHV* gene.

*E. coli* has become the predominant organism producing ESBLs in Canada over the past decade with the spread of CTX-M (9,12). However, with the emergence and increase of CTX-M-producing *K pneumoniae*, we may observe increases in their prevalence if CTX-M spreads as efficiently among *K pneumoniae* as it did among *E. coli*.

Surprisingly, ESBL-producing *E. coli* and *K pneumoniae* showed a trend to be more commonly isolated from blood, respiratory tract and wound specimens in comparison with urine specimens. This suggests that ESBL producers may be becoming more invasive; however, other factors that were not assessed by the present study could also be contributing to this observation, such as previous antimicrobial therapy, length of hospital stay and underlying disease.

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Therapeutic options for ESBL producers are severely limited because they are often MDR. Both ESBL-producing *E. coli* and *K pneumoniae* displayed high coreistance rates to the fluoroquinolones, trimethoprim/sulfamethoxazole, doxycycline and gentamicin, with a high percentage exhibiting a MDR phenotype. Although resistance rates were high, ESBL-producing *E. coli* and *K pneumoniae* still remained susceptible to the carbapenems and ESBL-producing *E. coli* remained susceptible to tigecycline. Alternative therapies such as nitrofurantoin (urinary tract infection only) and colistin (polymyxin E) still remain potential options for ESBL-producing *E. coli* because they were 98.0% and 96.0% susceptible, respectively.

Molecular typing of the isolates revealed that the majority of ESBL producers were genetically unrelated. This suggests that horizontal transfer of plasmids bearing the ESBL gene plays a larger role in the spread of ESBLs across Canada compared with clonal spread of an epidemic strain.

In summary, we update the prevalence and molecular epidemiology of ESBL-producing Enterobacteriaceae from hospitals across Canada in 2007. The prevalence of ESBL-producing *E. coli*, *K pneumoniae*, *K oxytoca* and *P mirabilis* in Canadian hospitals is 3.4%, 1.6%, 0% and 0%, respectively. CTX-M has become the most prevalent enzyme among both ESBL-producing *E. coli* and *K pneumoniae*. Coreistance rates were high among ESBL producers to many non-beta-lactam agents, with a high percentage exhibiting a MDR phenotype. The spread of ESBL producers across Canada is polyclonal and is not due to the clonal spread of a single strain.

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