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

Patricia J Baudry BSc[1,2], Melissa McCracken MSc[3], Philippe Lagacé-Wiens MD[2], Michael R Mulvey PhD[1,3], George G Zhanel PhD[1,2], Daryl J Hoban PhD[1,2]

PJ Baudry, M McCracken, P Lagacé-Wiens, MR Mulvey, GG Zhanel, DJ Hoban. Prevalence and characterization of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated in Canadian hospitals: Results from CANWARD 2007. Can J Infect Dis Med Microbiol 2009;20(Suppl A):43A-48A.

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 2007.

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 bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ and $bla_{OXA\text{-}like}$ 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% 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. 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% 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*

Prévalence et caractérisation des entérobatériacées productrices de bêtalactamases à spectre élargi isolées dans les hôpitaux canadiens : Résultats de l'étude CANWARD 2007

OBJECTIF : Le but de la présente étude était de déterminer la prévalence et l'épidémiologie moléculaire des entérobactériacées productrices de bêtalactamase 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 confirmés au moyen de la méthode de diffusion sur disque du *Clinical and Laboratory Standards Institutes*. Des tests de PCR (pour *polymerase chain reaction*) et de séquençage de l'ADN ont été utilisés pour détecter les gènes bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ et $bla_{type\ OXA}$. Les souches ont été typées par électrophorèse en champ pulsé.

RÉSULTATS : En tout, 3,4 % et 1,6 % des isolats d'*Escherichia coli* et de *Klebsiella pneumoniæ* respectivement ont été associés 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 pneumoniæ* producteurs d'ESBL, respectivement. En tout, 90,6 % et 71,4 % des isolats d'*E coli* et de *K pneumoniæ* ont été jugés multirésistants. Le type CTX-M a été l'ESBL prédominant, avec le CTX-M-15 comme génotype A prédominant. En tout, 81,7 % des isolats producteurs d'ESBL étaient porteurs de plusieurs gènes de la bêtalactamase. 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 pneumoniæ* 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 pneumoniæ* producteurs d'ESBL. La propagation des bactéries productrices d'ESBL au Canada est polyclonale et n'est pas attribuable à la propagation clonale d'une seule souche.

Extended-spectrum beta-lactamases (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 cephamycins 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).

[1]*Department of Medical Microbiology, Faculty of Medicine, University of Manitoba;* [2]*Department of Clinical Microbiology, Health Sciences Centre;* [3]*National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba*

Correspondence: Patricia J Baudry, Health Sciences Centre, Department of Clinical Microbiology, MS673-820 Sherbrook Street, Winnipeg, Manitoba, R3A 1R9. Telephone 204-787-4684, fax 204-787-4699, e-mail trishbaudry@hotmail.com

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

	Patients, n (%)		
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	0 (0)	1 (14.3)	NS
18–65	19 (35.8)	2 (28.6)	NS
≥66	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
Patient type			
Inpatient	38 (71.7)	6 (85.7)	NS
Outpatient	15 (28.3)	1 (14.3)	NS
Hospital ward			
Outpatient clinic	8 (15.0)	1 (14.3)	NS
Emergency room	7 (13.2)	0 (0)	NS
Intensive care unit	3 (5.7)	1 (14.3)	NS
Medical ward	26 (49.1)	4 (57.1)	NS
Surgical ward	9 (17.0)	1 (14.3)	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 bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ and $bla_{OXA\text{-}like}$ genes as previously described (7-9). A 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 *Xba*I as previously described (7).

Statistical analysis

χ^2 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

TABLE 2
Comparison of antimicrobial susceptibilities of extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing isolates (*Escherichia coli* and *Klebsiella pneumoniae*)

	ESBL-producing *E coli* (n=53)						Non-ESBL-producing *E coli* (n=1507, †n=504)						
Antibiotic	**MIC_{50}**	**MIC_{90}**	**Range**	**% S**	**% I**	**% R**	**MIC_{50}**	**MIC_{90}**	**Range**	**% S**	**% I**	**% R**	**P***
Amox/Clav†	8	16	2–32	60.4	37.7	1.9	4	8	0.5–32	93.2	5.6	1.2	NS
Cefazolin	128	>128	64–>128			100	2	8	≤0.5–>128	92.2	3.5	4.3	0.0001
Ceftriaxone	>64	>64	2–>64	3.8	15.1	81.1	≤1	≤1	≤1–>64	98.2	1.0	0.8	0.0001
Cefepime	16	>32	≤1–>32	45.3	30.2	24.5	≤1	≤1	≤1–>32	99.6	0.3	0.1	0.0001
TZP	4	16	≤1–>512	92.4	5.7	1.9	≤1	4	≤1–>512	98.0	0.8	1.2	NS
Cefoxitin†	8	8	0.5–>32	92.4	5.7	1.9	4	8	≤0.06–>32	95.4	2.4	2.2	NS
Ertapenem†	≤0.06	0.12	≤0.06–0.25	100			≤0.06	≤0.06	≤0.06	100			NS
Meropenem	≤0.12	≤0.12	≤0.12	100			≤0.12	≤0.12	≤0.12–0.5	100			NS
Ciprofloxacin	>16	>16	≤0.06–>16	7.5		92.5	≤0.06	>16	≤0.06–>16	81.7	0.3	18.0	0.0001
Levofloxacin	16	32	≤0.06–>32	7.5		92.5	≤0.06	16	≤0.06–>32	82.2	0.8	17.0	0.0001
Amikacin	4	8	≤2–>64	94.3	3.8	1.9	≤2	4	≤2–>64	99.9		0.1	NS
Gentamicin	32	>32	≤0.5–>32	41.5		58.5	≤0.5	1	≤0.5–>32	93.1	0.5	6.4	0.0001
Doxycycline	32	>256	2–>256	22.6	1.9	75.5	–	–	–	–	–	–	N/A
Tigecycline	0.5	1	0.25–2	100			0.25	0.5	0.06–4	99.9	0.1		NS
TMP/SMX	>8	>8	≤0.12–>8	32.1		67.9	≤0.12	>8	≤0.12–>8	77.2		22.8	0.0001
Nitrofurantoin†	16	32	1–64	96.2	3.8		16	32	≤0.5–128	96.6	2.6	0.8	NS
Colistin†	1	1	≤0.06–4	98.1		1.9	0.5	1	≤0.06–>16	99.0		1.0	NS
	ESBL-producing *K pneumoniae* (n=7)						**Non-ESBL-producing *K pneumoniae* (n=435, †n=185)**						
Antibiotic	**MIC_{50}**	**MIC_{90}**	**Range**	**% S**	**% I**	**% R**	**MIC_{50}**	**MIC_{90}**	**Range**	**% S**	**% I**	**% R**	**P***
Amox/Clav†	8	16	8–16	71.4	28.6		2	4	1–16	96.8	3.2		NS
Cefazolin	128	>128	16–>128		14.3	85.7	2	4	≤0.5–>128	96.1	1.4	2.5	0.0001
Ceftriaxone	>64	>64	2–>64	28.6		71.4	1	1	≤1–32	99.8	0.2		0.0001
Cefepime	32	128	≤1–128	42.9		57.1	1	1	≤1–>32	99.8	0.2		0.0001
TZP	16	>512	4–>512	57.1	14.3	28.6	2	8	≤1–512	98.4	0.7	0.9	0.0031
Cefoxitin†	8	>256	2–>256	71.4		28.6	4	8	1–>32	93.0	4.3	2.7	0.0011
Ertapenem	≤0.06	≤0.06	≤0.06–0.12	100			≤0.06	≤0.06	≤0.06–0.25	100			NS
Meropenem	≤0.12	≤0.12	≤0.12	100			≤0.12	≤0.12	≤0.12–0.25	100			NS
Ciprofloxacin	>16	>16	≤0.06–>16	28.6		71.4	≤0.06	0.5	≤0.06–>16	95.9	0.7	3.4	0.0001
Levofloxacin	4	>16	≤0.06–>16	28.6		71.4	≤0.06	0.5	≤0.06–32	97.0	0.9	2.1	0.0001
Amikacin	8	>64	≤2–>64	85.7		14.3	≤2	≤2	≤2–16	100			0.0158
Gentamicin	32	>32	≤0.5–>32	42.9		57.1	≤0.5	≤0.5	≤0.5–>32	99.0	0.5	0.5	0.0001
Doxycycline	32	128	2–128	14.3	14.3	71.4	–	–	–	–	–	–	N/A
Tigecycline	1	8	0.5–8	85.7		14.3	1	2	0.25–8	95.2	3.7	1.1	NS
TMP/SMX	>8	>8	≤0.12–>8	42.9		57.1	≤0.12	0.5	≤0.12–>8	94.7		5.3	0.0003
Nitrofurantoin†	128	128	16–128	14.3	14.3	71.4	64	128	8–256	37.3	34.6	28.1	0.0252
Colistin†	0.5	0.5	0.5–>16	85.7		14.3	0.5	1	0.12–>16	97.8		2.2	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); $MIC_{50/90}$ 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*

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 4.5% 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 3
Distribution of beta-lactamase genes among extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (n=53) and *Klebsiella pneumoniae* (n=7) isolates

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*

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 antimicrobials 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 producers.

Distribution of beta-lactamase genes

All isolates phenotypically identified as ESBL producers by the CLSI disk diffusion assay were subjected to PCR and sequencing to detect ESBL genes. All bla_{TEM}, bla_{SHV}, bla_{CTX-M} and $bla_{OXA-1, -2, -10}$ groups were detected in 49%, 1.9%, 96.2% and 47.2% of ESBL-producing *E coli*, respectively, and 14.3%, 100%, 57.1% and 42.9% of ESBL-producing *K pneumoniae*, respectively (Table 3). Of the 53 ESBL-producing *E coli*, 51 (96.2%) carried a CTX-M gene with 28 (52.8%) $bla_{CTX-M-15}$, 17 (32.1%) $bla_{CTX-M-14}$, two (3.8%) $bla_{CTX-M-27}$, and one (1.9%) each of $bla_{CTX-M-3}$, $bla_{CTX-M-24}$, $bla_{CTX-M-65}$, bla_{SHV2a} and an unknown. Of the seven ESBL-producing *K pneumoniae*, three (42.9%) carried $bla_{CTX-M-15}$, one (14.3) carried $bla_{CTX-M-2}$ and three were unknown. Forty-nine of 60 (81.7%) ESBL producers carried several beta-lactamase genes (one to four beta-lactamase genes). bla_{TEM-1} or a narrow-spectrum bla_{SHV} gene were also carried among 26 (49.1%) and zero of the ESBL-producing *E coli* and one (14.3%) and five (71.4%) of the ESBL-producing *K pneumoniae*, respectively. The promoter regions of the bla_{TEM-1} and bla_{SHV-1} genes of the one *E coli* and three *K pneumoniae* with unidentified ESBL genes were investigated for mutations leading to hyperproduction causing the ESBL phenotype. However, no mutations in the promoter region were observed. Twenty-four of 28 (85.7%) CTX-M-15 and the one CTX-M-3 ESBL-producing *E coli* as well as all three CTX-M-15-producing *K pneumoniae* also carried bla_{OXA-1}. The distribution of beta-lactamase genes among ESBL-producing *E coli* and *K pneumoniae* are summarized in Table 3.

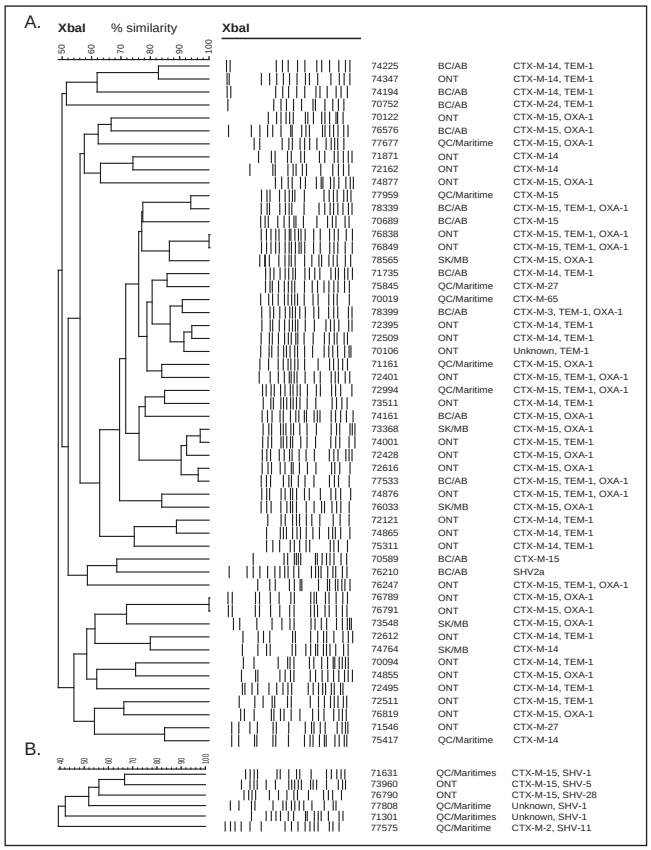

Figure 1) *Pulsed-field gel electrophoresis dendrogram depicting the genetic relationships among extended-spectrum beta-lactamase (ESBL) producers*. **A** *ESBL-producing Escherichia coli;* **B** *ESBL-producing Klebsiella pneumoniae*

Genetic relationships among ESBL-producing *E coli* and *K pneumoniae*

Molecular typing using pulsed-field gel electrophoresis was conducted to study clonal relationships among ESBL-producing *E coli* 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 genes from different CTX-M groups (CTX-M-1 and CTX-M-9 groups) 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.

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 CTX-M 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 $bla_{\text{TEM-1}}$ gene, whereas ESBL-producing *K pneumoniae* more commonly carried a narrow-spectrum bla_{SHV} 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.

Therapeutic options for ESBL producers are severely limited because they are often MDR. Both ESBL-producing *E coli* and *K pneumoniae* displayed high coresistance 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*. Coresistance 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.

ACKNOWLEDGEMENTS: Patricia Baudry is supported by the Winnipeg Health Sciences Centre Department of Research, the Winnipeg Health Sciences Centre Foundation and the Manitoba Health Research Council. Funding for the CANWARD 2007 study was provided in part by the University of Manitoba, Health Sciences Centre in Winnipeg, National Microbiology Laboratory-Health Canada, Affinium Inc, Astellas, Bayer, Janssen Ortho Inc, Oryx, Pfizer Canada, TaiGen, Targanta and Wyeth Inc. Special thanks to Nancy Laing, Barb Weshnoweski, Ravi Vashisht, Lisa Bittner and Haley Butcher for technological assistance. The authors wish to thank M Tarka for expert secretarial assistance. The authors also thank the investigators and laboratory site staff at each medical centre that participated in the CANWARD 2007 study. CANWARD data are also displayed at www.can-r.ca, the official Web site of the Canadian Antimicrobial Resistance Alliance (CARA).

REFERENCES

1. Ko KS, Lee MY, Song JH, et al. Prevalence and characterization of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated in Korean hospitals. Diag Microbiol Infect Dis 2008;61:453-9.
2. Rodriguez-Bano J, Navarro MD, Romero L, et al. Bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli* in the CTX-M era: A new clinical challenge. Clin Infect Dis 2006;43:1407-14.
3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
4. Canton R, Coque TM. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol 2006;9:466-75.
5. Coque TM, Novais A, Carattoli A, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis 2008;14:195-200.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement. CLSI document M100-S18. CLSI, Wayne, PA, USA, 2008.
7. Mulvey MR, Bryce E, Boyd D, et al. Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* spp. in Canadian hospitals. Antimicrob Agents Chemother 2004;48:1204-14.
8. Boyd DA, Tyler S, Christianson S, et al. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 2004;48:3758-64.
9. Baudry PJ, Nichol K, DeCorby M, et al. Comparison of antimicrobial resistance profiles among extended-spectrum-beta-lactamase-producing

and acquired AmpC beta-lactamase-producing *Escherichia coli* isolates from Canadian intensive care units. Antimicrob Agents Chemother 2008;52:1846-9.
10. Pitout JD, Church DL, Gregson DB, et al. Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: The emergence of CTX-M-15-producing isolates. Antimicrob Agents Chemother 2007;51:1281-86.
11. Melano RG, Davidson RJ, Musgrave HL, Forward KR. Cephalosporin resistance in *Klebsiella pneumonia*e from Nova Scotia, Canada. Diag Microbiol Infect Dis 2006;56:197-205.
12. Zhanel GG, DeCorby M, Laing N, et al. Antimicrobial-resistant pathogens in intensive care units in Canada: Results of the Canadian National Intensive Care Unit (CAN-ICU) study, 2005-2006. Antimicrob Agents Chemother 2008;52:1430-7.
13. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother 2005;56:52-9.
14. Doi Y, Adams-Haduch JM, Paterson DL. *Escherichia coli* isolate coproducing 16S rRNA methylase and CTX-M-type extended-spectrum beta-lactamase isolated from an outpatient in the United States. Antimicrob Agents Chemother 2008;52:1204-5.