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A Decade of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Surveillance in Canadian Hospitals Demonstrates Significant Increases in Prevalence: Results of the CANWARD Study 2007 to 2016

A.J. DENISUIK[1], H.J. ADAM[1,2], P. LAGACÉ-WIENS[1,2], M.R. MULVEY[1,3], M. BAXTER[1], J.A. KARLOWSKY[1,2], M. GILMOUR[1,3], D.J. HOBAN[1,2], G.G. ZHANEL[1], and the CANADIAN ANTIMICROBIAL RESISTANCE ALLIANCE (CARA)

[1]University of Manitoba, [2]Diagnostic Services Manitoba, [3]National Microbiology Laboratory, Winnipeg MB, Canada

Andrew Denisuik
Microbiology, Health Sciences Centre
MS673-820 Sherbrook St.
Winnipeg, MB R3A 1R9
Phone: 204-787-4684
Email: adenisuik@mymts.net

www.can-r.ca

UPDATED ABSTRACT

Objective: This study assessed the prevalence, patterns of antibiotic resistance, and molecular characteristics of ESBL-, AmpC-, and carbapenemase-producing *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KPN) isolated from Canadian hospitals over a ten-year period. **Methods:** 8,387 EC and 2,624 KPN were collected from January 2007 to December 2016 as part of the ongoing CANWARD national surveillance study. Antimicrobial susceptibility testing was performed in accordance with CLSI guidelines and putative ESBL-, AmpC-, and carbapenemase-producers were identified by phenotypic and genotypic methods. All putative isolates were characterized by PCR and sequencing to detect resistance genes and by PFGE to assess clonal spread. The EC ST131 clone was identified by an allele-specific PCR for the *pabB* gene. All CTX-M-15-producing EC underwent whole-genome sequencing (WGS) to further characterize this cohort. **Results:** The prevalence of ESBL-EC [2007: 3.4%, 2016: 11.1%] and ESBL-KPN [2007: 1.5%, 2016: 10.3%] increased significantly during the study period (P<0.001 and P<0.001, respectively). Peak incidences were observed in 2015 and 2016, respectively. In comparison to ESBL-producing isolates, the prevalence of AmpC-EC has been variable and does not demonstrate any clear trend. The rate of carbapenem resistance has remained low (<3%) among ESBL-EC, ESBL-KPN, and AmpC-EC. Antimicrobials demonstrating the greatest activity against ESBL-EC, AmpC-EC, and ESBL-KPN in this study were colistin, amikacin, ertapenem, and meropenem, while 77.9%, 36.2%, and 72.2% of ESBL-EC, AmpC-EC, and ESBL-KPN, respectively, were multidrug resistant (resistance to ≥3 antimicrobial classes). The ST131 clone was identified among 56.9% and 31.7% (P<0.001) of ESBL-EC and AmpC-EC, respectively. CTX-M-15 was the dominant genotype in both ESBL-EC and ESBL-KPN, while the dominant genotype in AmpC-EC was CMY-2. KPC-3 represents the dominant genotype among carbapenemase-producers. CTX-M-15-producing EC were largely clonal based on core single nucleotide variant-based phylogeny, which demonstrated a high degree of similarity among CTX-M-15-producing ST131 isolates circulating in Canadian hospitals. **Conclusions:** The prevalence of ESBL-producing EC and KPN increased significantly between 2007 and 2016. The prevalence of AmpC-producing EC remains considerably lower when compared to ESBL-producing EC. The prevalence of carbapenem-resistant Enterobacteriaceae remains low (<3%) among isolates submitted to the CANWARD study.

BACKGROUND

The β-lactams (penicillins, cephalosporins, carbapenems, and monobactams) comprise over 60% of the global antibiotic market [1]. Within this class, the oxyimino-cephalosporins and carbapenems represent extremely important agents for the treatment of serious community- and hospital-acquired infections [2]. Though bacterial susceptibility to β-lactam agents can become compromised through a number of mechanisms, β-lactamase production represents the single greatest source of β-lactam resistance among Gram-negative organisms [3]. Members of the Enterobacteriaceae, including *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KPN), are among the top ranked pathogens causing bacterial disease in Canadian hospitals [4]. Within the Enterobacteriaceae, oxyimino-cephalosporin resistance is largely attributable to the production of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases, able to hydrolyze a variety of β-lactams including the oxyimino-cephalosporins and monobactams. In addition, the recent emergence of β-lactamase enzymes with carbapenemase activity (e.g. bla_{KPC}) is of great concern. Such variants have now spread worldwide and threaten the effective use of the carbapenems as last-line agents in many countries. Infections caused by these organisms hold serious implications for both public health and infection control practices. Such infections are often associated with delays in the administration of effective therapy, as β-lactam resistance often undermines empiric regimens [2,5]. Furthermore, the frequent association of such organisms with multidrug resistance (MDR) severely limits available treatment options. As a result, patients are subject to increased length of hospital stay, increased hospital cost, as well as an elevated risk of infection-related mortality [2]. The purpose of this study was to assess the prevalence, patterns of antibiotic resistance, and molecular characteristics of ESBL-, AmpC-, and KPC-producing EC and KPN isolated from Canadian hospitals between January 2007 and December 2016, inclusive.

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MATERIALS & METHODS

Bacterial Isolates: A total of 8,387 EC and 2,624 KPN were collected from January 2007 to December 2016, inclusive, as part of the ongoing CANWARD national surveillance study [4]. Tertiary-care medical centers submitted clinically relevant isolates from in- and outpatients attending hospital clinics, medical and surgical wards, emergency rooms, and intensive care units (ICUs) with blood, urine, wound, and respiratory tract infections. **Antimicrobial Susceptibility Testing (AST):** AST was performed using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M07-A10). Minimum inhibitory concentration (MIC) interpretive standards were defined by CLSI M100-S27 breakpoints. US Food and Drug Administration (FDA) breakpoints were used for colistin (S: ≤2, R: ≥4 μg/ml). MDR is defined as resistance to ≥3 different antimicrobial classes and extreme drug resistance (XDR) is defined as resistance to ≥5 different antimicrobial classes, as described by Magiorakos *et al.* [6]. Putative ESBL-producers were identified as any EC or KPN isolate with a ceftriaxone and/or ceftazidime MIC of ≥1 μg/ml and were phenotypically confirmed by CLSI phenotypic confirmatory disk test. Putative AmpC-producers were identified as any EC with a cefoxitin MIC of ≥32 μg/ml. **Molecular Characterization:** All phenotypically confirmed ESBL-producing isolates were further characterized by PCR and sequencing for the detection of bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and bla_{OXA} genes [7]. All putative AmpC-producing EC were screened for genes encoding the bla_{ENT}, bla_{DHA}, bla_{FOX}, and bla_{CIT} groups of AmpC acquired enzymes using a previously described multiplex PCR [8]. Any EC or KPN with an ertapenem or meropenem MIC of ≥0.5 μg/ml was screened for the production of bla_{KPC}, bla_{IMI}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{GES}, and bla_{OXA-48} by PCR [9]. Sequence type (ST) 131 was identified with an allele specific PCR for the *pabB* gene as previously described by Clermont *et al.* [10]. Some 257 EC found to contain $bla_{CTX-M-15}$ collected between 2007 and 2014 were selected to undergo further characterization by WGS. Following preparation of bacterial DNA, 150-bp paired-end indexed reads were generated on the Illumina MiSeq platform, resulting in an average of 1,529,066 reads and 90-times coverage per genome. Reads were assembled into draft genomes using Spades v3.9 [11] and subsequently processed via the Center for Genomic Epidemiology (CGE) bacterial analysis pipeline in order to characterize resistance genes. Core single nucleotide variant (SNV)-based phylogeny was performed using the SNVPhyl pipeline (v1.0.1b) [12] with reference strain EC JJ1886 (CP006784.1). In total, 3,160,900 of 5,129,938 (62.9%) reference positions were valid and included as part of the core genome, yielding 186,051 high-quality SNVs.

CONCLUSIONS

1. A significant national increase in the prevalence of ESBL-EC and ESBL-KPN was observed during the study period; the prevalence of carbapenemase-producing isolates remained <1.0%.
   - The national rate of ESBL-EC reached maximum incidence in 2015; From 2007 to 2010 3.9% (185/4798) of EC collected were found to produce an ESBL in comparison to 9.9% (354/3589) of EC collected from 2011 to 2016 (P<0.001).
2. Overall, ESBL-EC were most commonly isolated from female patients over the age of 65 with bloodstream infections located on general medical wards.
3. CTX-M-type ESBLs represent the dominant family in Canadian hospitals with CTX-M-15 being the most common variant. CTX-M-15 comprised 64.4% of all ESBL-EC isolates collected.
4. According to core SNV-based phylogeny, CTX-M-15-producing EC cluster largely according to ST.
   - Here, the 5 most common STs made up 87.9% of all isolates. These included: ST-131 (72.8%), ST-405 (6.6%), ST-648 (4.7%), ST-38 (1.9%), and ST-410 (1.9%).
5. CTX-M-15-producing EC contained a large variety of resistance genes. The most commonly identified genes included *aac(3)-II* variants (44.4%) conferring resistance to the aminoglycosides, *aac(6')-Ib-cr* (68.5%) conferring reduced susceptibility or resistance to ciprofloxacin, the tetracycline resistance gene *tetA* (59.1%), *dfr17* (61.1%) conferring resistance to trimethoprim-sulfamethoxazole, as well as a variety of other β-lactamase genes.
6. 55.9% of AmpC-EC produced an acquired AmpC β-lactamase, of which 98.8% produced CMY-2
7. ESBL-EC and ESBL-KPN are frequently MDR (77.9% and 72.2%, respectively) and are significantly more likely to be MDR as compared to AmpC-EC (36.2%),
8. The majority of ESBL-EC (>98%), AmpC-EC (>98%), and ESBL-KPN (>89%) remained susceptible to colistin, tigecycline, ertapenem, and meropenem.

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RESULTS

TABLE 1. Antimicrobial susceptibility testing of ESBL-*E. coli*, ESBL-*K. pneumoniae* and AmpC-*E. coli*.

Cohort (n)	MIC (μg/ml)				MIC Interpretation[a]		
Antibiotic	MIC_{50}	MIC_{90}	Min.	Max.	%S	%I	%R
ESBL-*E. coli* (539)							
AMC[b]	8	32	1	>32	50.7	39.3	10.0
Cefazolin	>128	>128	4	>128		0.2	99.8
Cefoxitin	8	16	0.5	>32	81.8	9.8	8.4
Ceftriaxone	>64	>64	≤0.25	>64	2.2	1.5	96.3
Ceftazidime	16	>32	≤0.5	>32	35.4	9.1	55.6
Cefepime	8	32	≤0.25	>32	31.8	31.8	36.4
TZP[b]	4	16	≤1	>512	93.5	3.9	2.6
Ertapenem	≤0.06	0.25	≤0.06	>32	98.0	1.0	1.1
Meropenem	≤0.12	≤0.12	≤0.12	32	99.8		0.2
Ciprofloxacin	>16	>16	≤0.06	>16	11.7	0.4	87.9
Amikacin	2	8	≤2	>64	97.6	2.0	0.4
Gentamicin	1	>32	≤0.5	>32	59.2	1.1	39.7
Tigecycline	0.5	1	0.12	4	99.8	0.2	
SXT[b]	>8	>8	≤0.12	>8	31.2		68.8
Colistin	0.5	1	≤0.06	2	100.0		
ESBL-*K. pneumoniae* (107)							
AMC[b]	16	32	2	>32	35.9	37.9	26.2
Cefazolin	>128	>128	8	>128			100.0
Cefoxitin	8	>32	2	>32	66.0	12.6	21.4
Ceftriaxone	>64	>64	≤0.25	>64	10.3	4.7	85.1
Ceftazidime	32	>32	0.25	>32	25.0	5.0	70.0
Cefepime	8	64	≤0.25	>128	32.0	29.9	38.1
TZP[b]	16	>512	2	>512	63.6	15.9	20.6
Ertapenem	0.12	1	≤0.06	>32	89.3	3.9	6.8
Meropenem	≤0.12	≤0.12	≤0.12	16	95.3	1.9	2.8
Ciprofloxacin	4	>16	≤0.06	>16	28.0	11.2	60.8
Amikacin	≤2	16	≤2	>64	95.3	0.9	3.7
Gentamicin	2	>32	≤0.5	>32	51.4		48.6
Tigecycline	1	2	0.5	16	90.7	5.6	3.7
SXT[b]	>8	>8	≤0.12	>8	17.8		82.2
Colistin	0.5	1	0.25	>16	94.2		5.8
AmpC-*E. coli* (170)							
AMC[b]	32	>32	1	>32	21.4	17.3	61.3
Cefazolin	>128	>128	0.5	>128	1.2	3.0	95.8
Cefoxitin	>32	>32	32	>32			100.0
Ceftriaxone	8	32	≤0.25	>64	39.9	3.0	57.1
Ceftazidime	16	>32	≤0.25	>32	39.0	6.7	54.3
Cefepime	≤0.25	1	≤0.25	>32	93.6	3.6	2.8
TZP[b]	4	32	≤1	>512	89.3	7.1	3.6
Ertapenem	≤0.06	0.25	≤0.06	1	97.0	3.0	
Meropenem	≤0.06	≤0.06	≤0.06	0.12	100.0		
Ciprofloxacin	0.12	>16	≤0.06	>16	63.1	0.6	36.3
Amikacin	2	4	≤2	>64	98.2	0.6	1.2
Gentamicin	≤0.5	32	≤0.5	>32	83.9		16.1
Tigecycline	0.5	1	0.12	2	100.0		
SXT[b]	0.25	>8	≤0.12	>8	66.1		33.9
Colistin	0.25	0.5	0.12	2	100.0		

[a]%S: % susceptible, %I: % intermediate, %R: % resistant; [b]AMC: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; SXT: trimethoprim-sulfamethoxazole.

FIGURE 1. Frequency of resistance genes by sequence type in CTX-M-15-producing *E. coli*.

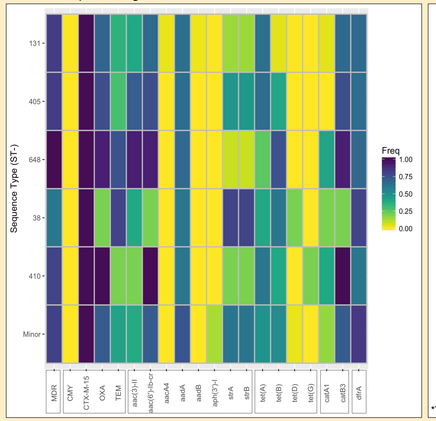
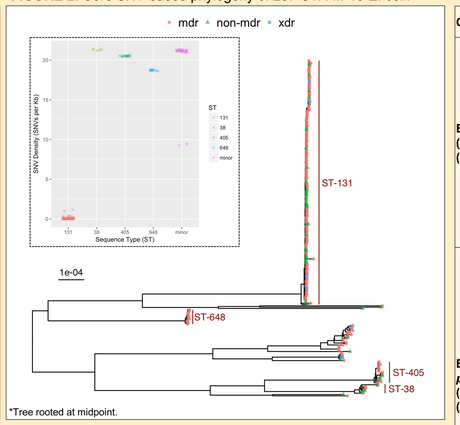


FIGURE 2. Core SNV-based phylogeny of 257 CTX-M-15-*E. coli*.



*Tree rooted at midpoint.

TABLE 2. The national prevalence of ESBL-*E. coli*, ESBL-*K. pneumoniae*, and AmpC-*E. coli from* 2007 to 2016.

Cohort (n)	CANWARD Study Year: % (no. in cohort/total no. of species collected)											*P*-value[b,c]
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2007-2016	
ESBL-*E. coli* (539)	3.4 (53/1558)	4.9 (55/1130)	4.3 (47/1097)	3.0 (30/1013)	7.1 (46/645)	7.4 (37/499)	9.5 (62/655)	11.6 (72/620)	12.4 (69/558)	11.1 (68/612)	6.4 (539/8387)	<0.001
ESBL-*K. pneumo.* (107)	1.5 (7/455)	3.2 (10/314)	3.4 (12/356)	3.3 (10/307)	4.0 (9/227)	3.6 (6/169)	5.7 (13/230)	6.5 (12/184)	4.6 (9/197)	10.3 (19/185)	4.1 (107/2624)	<0.001
AmpC-*E. coli* (170)	0.7 (4/558[a])	3.1 (35/1130)	2.7 (30/1097)	2.7 (27/1013)	2.9 (19/645)	2.2 (11/499)	3.1 (20/655)	1.0 (6/620)	1.3 (7/558)	1.8 (11/612)	2.3 (170/7387)	0.009

[a]Cefoxitin was tested against 558 E. coli during CANWARD 2007; [b]*P*-value comparing the rate of ESBL-*E. coli*, ESBL-*K. pneumoniae*, and AmpC-*E. coli* from 2007-2014; [c]Statistical significance defined as P<0.05.

TABLE 3. Genotypic characterization of ESBL-*E. coli* and ESBL-*K. pneumoniae*.

Cohort (n)	Genotype	2016: No. of Isolates (%)	2007-2016: No. of Isolates (%)
ESBL-*E. coli* (2016: 68) (2007-16: 539)	CTX-M-2	1 (1.5)	1 (0.2)
	CTX-M-3		3 (0.6)
	CTX-M-14	12 (17.6)	87 (16.1)
	CTX-M-15	44 (64.7)	347 (64.4)
	CTX-M-24		3 (0.6)
	CTX-M-27	10 (14.7)	55 (10.2)
	CTX-M-55		1 (0.2)
	CTX-M-65		1 (0.2)
	SHV-2a	1 (1.5)	11 (2.0)
	SHV-12		10 (1.9)
	TEM-12		4 (0.7)
	Unknown		19 (3.5)
	[TEM-1[a]	17 (25.0)	167 (31.0)]
ESBL-*K. pneumo.* (2016: 19) (2007-16: 107)	CTX-M-2		1 (0.9)
	CTX-M-3	1 (5.3)	2 (1.9)
	CTX-M-14	1 (5.3)	12 (11.2)
	CTX-M-15	12 (63.2)	55 (51.4)
	CTX-M-27		3 (2.8)
	SHV-2		1 (0.9)
	SHV-2a	1 (5.3)	9 (8.4)
	SHV-5		1 (0.9)
	SHV-11	6 (31.6)	34 (31.8)
	SHV-12	4 (21.1)	18 (16.8)
	SHV-28		5 (4.7)
	SHV-31		1 (0.9)
	SHV-108		1 (0.9)
	SHV-168		1 (0.9)
	Unknown		7 (6.5)
	[SHV-1[a]	6 (31.6)	30 (28.0)]
	[TEM-1[a]	16 (84.2)	58 (54.2)]

[a]bla_{TEM-1} and bla_{SHV-1} are not ESBLs, however they have been included due to frequent co-expression.

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