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Surveillance and Molecular Characterization of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Canadian Hospitals: Results of the CANWARD Study 2007-2017

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Introduction

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 2017, inclusive.

Materials and Methods

Bacterial Isolates: A total of 9,037 EC and 2,868 KPN were collected from January 2007 to December 2017, 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.

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* (611)							
AMC[b]	8	32	1	>32	50.7	38.5	10.8
Cefazolin	>128	>128	4	>128		0.2	99.8
Cefoxitin	8	16	0.5	>32	80.5	11.5	8.0
Ceftriaxone	>64	>64	≤0.25	>64	2.0	1.5	96.6
Ceftazidime	16	>32	≤0.5	>32	32.6	10.2	57.2
Cefepime	8	>32	≤0.25	>32	28.9	32.0	40.1
TZP[b]	4	16	≤1	>512	92.8	4.3	3.0
Ertapenem	≤0.06	0.25	≤0.06	>32	97.9	1.0	1.1
Meropenem	≤0.12	≤0.12	≤0.12	32	99.8		0.2
Ciprofloxacin	>16	>16	≤0.06	>16	11.5	0.3	88.2
Amikacin	≤2	8	≤2	>64	97.6	2.1	0.3
Gentamicin	1	>32	≤0.5	>32	60.7	1.2	38.1
Tigecycline	0.5	1	0.12	4	99.8	0.2	
SXT[b]	>8	>8	≤0.12	>8	31.9		68.1
Colistin	0.25	1	≤0.06	4	99.7		0.3
ESBL-*K. pneumoniae* (127)							
AMC[b]	16	32	2	>32	35.0	38.2	26.8
Cefazolin	>128	>128	8	>128			100.0
Cefoxitin	8	>32	2	>32	64.2	15.5	20.3
Ceftriaxone	>64	>64	≤0.25	>64	8.7	3.9	87.4
Ceftazidime	>32	>32	0.25	>32	23.3	4.2	72.5
Cefepime	8	>32	≤0.25	>32	27.4	27.4	45.3
TZP[b]	16	>512	2	>512	64.6	15.8	19.7
Ertapenem	0.12	1	≤0.06	>32	88.6	4.1	7.3
Meropenem	≤0.12	≤0.12	≤0.12	16	96.1	1.6	2.4
Ciprofloxacin	4	>16	≤0.06	>16	27.6	10.2	62.2
Amikacin	≤2	8	≤2	>64	96.1	0.8	3.2
Gentamicin	2	>32	≤0.5	>32	51.2		48.8
Tigecycline	1	4	0.5	16	89.0	7.9	3.1
SXT[b]	>8	>8	≤0.12	>8	15.8		84.3
Colistin	0.5	1	0.25	>16	95.1		4.9
AmpC-*E. coli* (187)							
AMC[b]	32	>32	1	>32	19.6	16.2	64.3
Cefazolin	>128	>128	0.5	>128	1.1	3.4	95.5
Cefoxitin	>32	>32	32	>32			100.0
Ceftriaxone	8	32	≤0.25	>64	40.8	3.9	55.3
Ceftazidime	16	>32	≤0.25	>32	39.4	6.3	54.3
Cefepime	≤0.25	1	≤0.25	>32	94.1	3.3	2.6
TZP[b]	4	32	≤1	>512	89.4	6.7	3.9
Ertapenem	≤0.06	0.25	≤0.06	2	96.6	2.8	0.6
Meropenem	≤0.06	≤0.06	≤0.06	0.25	100.0		
Ciprofloxacin	≤0.06	>16	≤0.06	>16	64.8	0.6	36.6
Amikacin	≤2	4	≤2	>64	98.3	0.6	1.1
Gentamicin	≤0.5	32	≤0.5	>32	86.0		14.0
Tigecycline	0.5	1	0.12	2	100.0		
SXT[b]	0.25	>8	≤0.12	>8	67.0		33.0
Colistin	0.25	0.5	0.12	4	99.4		0.6

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

Table 2. The national prevalence of ESBL-*E. coli*, ESBL-*K. pneumoniae*, and AmpC-*E. coli* from 2007 to 2017

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	2017	2007-2017	
ESBL-*E. coli* (611)	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)	11.1 (72/650)	6.8 (611/9037)	<0.001
ESBL-*K. pneumoniae* (127)	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)	8.2 (20/244)	4.4 (127/2868)	<0.001
AmpC-*E. coli* (187)	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)	2.6 (17/650)	2.3 (187/8037)	0.019↘

[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 2007-2017; [c]Statistical significance defined as *P*<0.05.

Figure 1. Minimum Spanning Tree Constructed from the Multilocus Sequence Typing Profiles of ESBL-*E. coli*

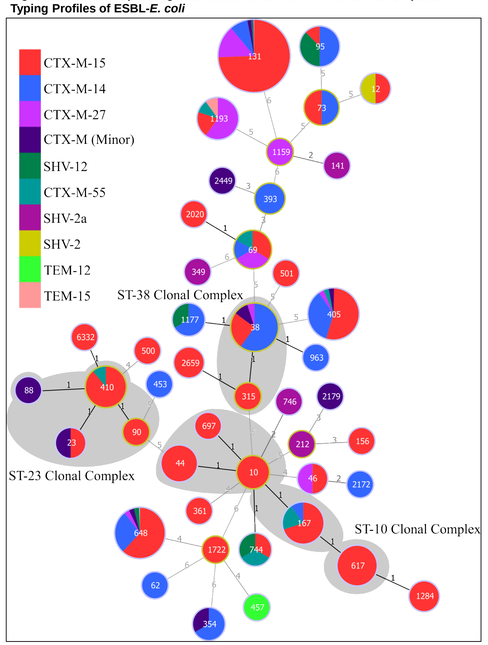

Figure 2. (A) Core SNV-based phylogeny of CTX-M-15-*E. coli*. (B) Frequency of resistance genes by sequence type in CTX-M-15-*E. coli*

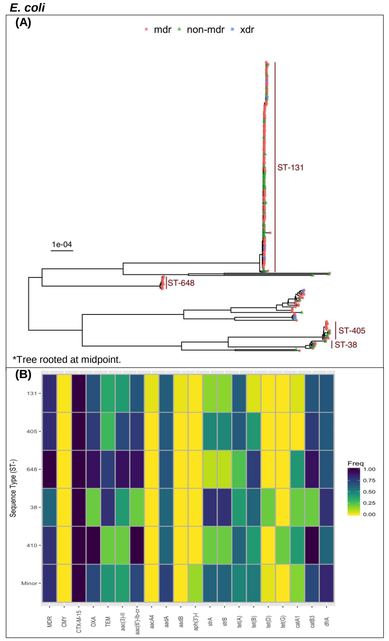

Table 3. Genotypic characterization of ESBL-*E. coli* and ESBL-*K. pneumoniae*

Cohort (n)	Genotype	2017: No. of Isolates (%)	2007-2017: No. of Isolates (%)
ESBL-*E. coli* (2017: 72) (2007-17: 611)	CTX-M-2		1 (0.2)
	CTX-M-3		3 (0.5)
	CTX-M-14	12 (16.7)	99 (16.2)
	CTX-M-15	47 (65.3)	394 (64.5)
	CTX-M-24		3 (0.5)
	CTX-M-27	10 (13.9)	65 (10.6)
	CTX-M-55		1 (0.2
	CTX-M-65		1 (0.2
	SHV-2a		11 (1.8)
	SHV-12		10 (1.6)
	TEM-12	1 (1.4)	5 (0.8)
	Unknown	2 (2.8)	21 (3.4)
	[TEM-1[a]	14 (19.4)	181 (29.6)]
ESBL-*K. pneumoniae* (2017: 20) (2007-17: 127)	CTX-M-2		1 (0.8)
	CTX-M-3		2 (1.6)
	CTX-M-14	3 (15.0)	15 (11.8)
	CTX-M-15	16 (80.0)	71 (55.9)
	CTX-M-27		3 (2.4)
	SHV-2		1 (0.8)
	SHV-2a	1 (5.0)	10 (7.9)
	SHV-5		1 (0.8)
	SHV-11	4 (20.0)	38 (29.9)
	SHV-12	3 (15.0)	21 (16.5)
	SHV-28		5 (3.9)
	SHV-31		1 (0.8)
	SHV-108		1 (0.8)
	SHV-168		1 (0.8)
	Unknown		7 (5.5)
	[SHV-1[a]	12 (60.0)	42 (33.1)
	[TEM-1[a]	11 (55.0)	69 (54.3)]

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

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 prevalence in 2015; From 2007 to 2010 3.9% (185/4798) of EC collected were found to produce an ESBL in comparison to 10.0% (426/4239) of EC collected from 2011 to 2017 (*P*<0.001).

Overall, ESBL-EC were most commonly isolated from female patients over the age of 65 with bloodstream infections located on general medical wards.

2. CTX-M-type ESBLs represent the dominant family in Canadian hospitals with CTX-M-15 being the most common variant. CTX-M-15 comprised 64.5% of all ESBL-EC isolates collected.
3. According to core SNV-based phylogeny, CTX-M-15-producing EC clustered 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%).
4. 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.
5. 56.0% of AmpC-EC produced an acquired AmpC β-lactamase, of which 98.8% produced CMY-2
6. ESBL-EC and ESBL-KPN are frequently MDR (77.2% and 72.9%, respectively) and are significantly more likely to be MDR as compared to AmpC-EC (43.5%),
7. The majority of ESBL-EC (>97%), AmpC-EC (>96%), and ESBL-KPN (>88%) remained susceptible to colistin, tigecycline, ertapenem, and meropenem.

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