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The Continued Rise of Extended-Spectrum β-Lactamase-, AmpC β-Lactamase-, and Carbapenemase-Producing *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KPN) in Canadian Hospitals: CANWARD 2007-2013

A.J. DENISUIK[1], H.J. ADAM[1,2], P. LAGACÉ-WIENS[1,2], P.J. SIMNER[2], M.R. MULVEY[1,3], M. BAXTER[1], W. MacDOUGALL[1], M. GILMOUR[1,2], J.A. KARLOWSKY[1,2], D.J. HOBAN[1,2] and G.G. ZHANEL[1]

[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

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ABSTRACT

Objective: To assess the prevalence, patterns of antibiotic resistance, and molecular characteristics of ESBL-, AmpC-, and carbapenemase-producing EC and KPN isolated from Canadian hospitals.

Methods: 6,606 EC and 2,058 KPN were collected from January 2007 to December 2013 as part of the ongoing CANWARD national surveillance study. Antimicrobial susceptibility testing was performed according to CLSI guidelines and putative ESBL-, AmpC-, and carbapenemase-producers were identified. 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.

Results: The prevalence of ESBL-EC [2007: 3.4%, 2013: 9.5%], AmpC-EC [2007: 0.7%, 2013: 3.1%], and ESBL-KPN [2007: 1.5%, 2013: 5.7%] increased significantly during the study period, with all three antibiotic resistant organisms reaching peak incidence in 2013. Antimicrobials demonstrating the greatest activity against ESBL-EC, AmpC-EC, and ESBL-KPN in this study were colistin, amikacin, ertapenem, and meropenem, whereas 78.8%, 34.9%, and 66.7% of ESBL-EC, AmpC-EC, and ESBL-KPN, respectively, were multidrug resistant. The prevalence of the ST131 clone was higher in ESBL-EC (56.9%) compared to AmpC-EC (31.7%; *P*<0.001). CTX-M-15 was the dominant genotype in both ESBL-EC and ESBL-KPN (66.5% and 48.0%, respectively), whereas the dominant genotype in AmpC-EC was CMY-2 (53.2%). KPC-3 represents the dominant genotype among carbapenemase-producers (n=4). In total, 5 isolates demonstrating a meropenem MIC ≥1 μg/ml were collected in 2013, this in comparison to a total of 5 such isolates for the years 2009 to 2012 combined.

Conclusions: The prevalence of ESBL- and AmpC-producing EC and KPN increased significantly between 2007 and 2013. The prevalence of carbapenem-resistant Enterobacteriaceae remains low in Canada.

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 2013, inclusive.

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MATERIALS & METHODS

Bacterial Isolates: A total of 6,606 EC and 2,058 KPN were collected from January 2007 to December 2013, 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: Antimicrobial susceptibility testing was performed using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M07-A9). Minimum inhibitory concentration (MIC) interpretive standards were defined by CLSI M100-S24 breakpoints. US Food and drug administration (FDA) breakpoints were used for colistin (S: ≤2, R: ≥4 μg/ml) and tigecycline (S: ≤2, I: 4, R: ≥8 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-hyperproducers 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]. Isolates negative for all acquired AmpC β-lactamases were analyzed for promoter/attenuator mutations within the chromosomal *ampC* gene [9]. Any EC or KPN with an ertapenem 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 and sequencing [10]. Following genomic extraction and *Xba*I digestion, all isolates were typed by pulsed-field gel electrophoresis (PFGE) using a standardized protocol [7]. Sequence type (ST) 131 was identified with an allele specific PCR for the *pabB* gene as previously described by Clermont *et al.* [11].

Statistical Analysis: Statistical significance was calculated by the chi-squared test, binary logistic regression, or the Fisher exact test using the SPSS statistics (Version 20) program (IBM Corporation).

CONCLUSIONS

1. A significant national increase in the prevalence of ESBL-EC, ESBL-KPN, and AmpC-EC was observed during the study period while the prevalence of carbapenemase-producing isolates remained <1.0%.
   - The national rate of ESBL-EC reached maximum incidence in 2013 with ESBL-EC demonstrating significant increases in prevalence in 2011 (compared to 2007, 2008, 2009, 2010), 2012 (compared to 2007, 2009, 2010), and 2013 (compared to 2007, 2008, 2009, 2010).
2. ESBL-EC are generally polyclonal by PFGE, however ST-131 was identified in 58.9% of isolates.
   - The rate of ST-131 increased significantly among ESBL-EC across the study period and ESBL-EC are significantly more likely to belong to the ST-131 clone as compared to AmpC-EC.
3. The majority of ESBL-EC collected were isolated from female patients over the age of 65 with bloodstream infections located on general medical wards.
   - The frequency of ESBL-EC infections isolated from respiratory specimens was significantly higher as compared to blood and urine sources (*P*=0.006 and *P*<0.001, respectively).
4. CTX-M-type ESBLs represent the dominant family in Canadian hospitals with CTX-M-15 being the most common variant.
5. 60.0% of AmpC-EC produced an acquired AmpC β-lactamase, of which 98.8% produced CMY-2 and 1.2% produced FOX-5.
6. ESBL-EC and ESBL-KPN are frequently MDR (79.2% and 70.1%, respectively) and are significantly more likely to be MDR as compared to AmpC-EC (36.3%), while ESBL-KPN (9.0%) are significantly more likely to be XDR as compared to ESBL-EC and AmpC-EC [3.3% (*P*=0.007) and 1.4% (*P*=0.002), respectively].
7. The majority of ESBL-EC (>95%), AmpC-EC (>97%), and ESBL-KPN (>89%) remained susceptible to colistin, amikacin, 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* (331)							
AMC[b]	8	16	1	>32	54.4	36.8	8.8
Cefazolin	>128	>128	16	>128			100.0
Cefoxitin	8	16	0.5	>32	81.3	10.0	8.7
Ceftriaxone	>64	>64	≤0.25	>64	1.8	1.2	97.0
Ceftazidime	16	>32	≤0.5	>32	33.1	7.5	59.4
Cefepime	8	>32	≤1	>32	58.8	21.9	19.3
TZP[b]	4	16	≤1	>512	93.1	4.8	2.1
Ertapenem	≤0.06	0.25	≤0.06	>32	97.6	0.9	1.5
Meropenem	≤0.12	≤0.12	≤0.12	32	99.7		0.3
Ciprofloxacin	>16	>16	≤0.06	>16	10.6	0.6	88.8
Amikacin	4	8	≤2	>64	96.7	3.0	0.3
Gentamicin	1	>32	≤0.5	>32	55.0	0.6	44.4
Tigecycline	0.5	1	0.12	4	99.7	0.3	
SXT[b]	>8	>8	≤0.12	>8	31.1		68.9
Colistin	0.5	1	≤0.06	4	99.3		0.7

Cohort (n)	MIC (μg/ml)				MIC Interpretation[a]		
Antibiotic	MIC_{50}	MIC_{90}	Min.	Max.	%S	%I	%R
ESBL-*K. pneumoniae* (67)							
AMC[b]	16	32	2	>32	49.2	31.7	19.1
Cefazolin	>128	>128	8	>128			100.0
Cefoxitin	8	>32	2	>32	73.0	11.1	15.9
Ceftriaxone	64	>64	≤0.25	>64	11.9	7.5	80.6
Ceftazidime	32	>32	0.25	>32	28.3	3.3	68.3
Cefepime	8	>32	≤1	>32	64.9	5.3	29.8
TZP[b]	8	512	2	>512	67.2	16.4	16.4
Ertapenem	0.12	0.5	≤0.06	16	95.2	1.6	3.2
Meropenem	≤0.12	≤0.12	≤0.12	2	98.5	1.5	
Ciprofloxacin	4	>16	≤0.06	>16	35.8	7.5	56.7
Amikacin	≤2	16	≤2	>64	92.5	1.5	6.0
Gentamicin	1	>32	≤0.5	>32	55.2		44.8
Tigecycline	1	4	0.5	16	88.1	6.0	6.0
SXT[b]	>8	>8	≤0.12	>8	22.4		77.6
Colistin	0.5	1	0.25	>16	95.2		4.8

Cohort (n)	MIC (μg/ml)				MIC Interpretation[a]		
Antibiotic	MIC_{50}	MIC_{90}	Min.	Max.	%S	%I	%R
AmpC-*E. coli* (146)							
AMC[b]	32	>32	1	>32	23.3	18.5	58.2
Cefazolin	>128	>128	0.5	>128	0.7	2.7	96.6
Cefoxitin	>32	>32	32	>32			100.0
Ceftriaxone	8	32	≤0.25	>64	39.7	2.7	57.5
Ceftazidime	16	>32	1	>32	40.9	5.6	53.5
Cefepime	≤0.25	1	≤0.25	>32	97.5	0.8	1.7
TZP[b]	4	32	≤1	>512	89.7	6.9	3.4
Ertapenem	≤0.06	0.25	≤0.06	1	97.3	2.7	
Meropenem	≤0.06	≤0.06	≤0.06	0.12	100.0		
Ciprofloxacin	0.12	>16	≤0.06	>16	61.6	0.7	37.7
Amikacin	2	4	≤2	>64	98.6		1.4
Gentamicin	≤0.5	32	≤0.5	>32	82.9		17.1
Tigecycline	0.5	1	0.12	2	100.0		
SXT[b]	0.25	>8	≤0.12	>8	65.8		34.2
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.

TABLE 2. Patient demographics associated with ESBL-*E. coli*, ESBL-*K. pneumoniae*, and AmpC-*E. coli* infections.

Parameter	Cohort: % (no. in cohort/total no. collected)		
Value	ESBL-*E. coli* (n=331)	AmpC-*E. coli* (n=146)	ESBL-*K. pneumo.* (n=67)
Gender			
Male	5.9 (154/2594)	2.9 (64/2211)	3.9 (43/1110)
Female	4.4 (177/4012)	2.4 (82/3393)	2.5 (24/948)
Age (years)			
≤17	1.2 (8/685)	2.3 (13/555)	3.6 (7/196)
18-64	5.2 (139/2697)	2.6 (60/2274)	4.4 (37/838)
≥65	5.7 (184/3224)	2.6 (73/2775)	2.2 (23/1024)
Hospital Location			
Clinic/Office	3.2 (36/1126)	1.5 (14/938)	1.7 (4/232)
Emergency Room	3.6 (91/2508)	1.9 (41/2143)	1.8 (10/542)
Intensive Care Unit	8.2 (53/649)	5.3 (30/563)	4.4 (18/407)
Medical Ward	6.9 (130/1891)	3.0 (49/1616)	4.6 (31/680)
Surgical Ward	4.9 (21/432)	3.5 (12/344)	2.0 (4/197)
Specimen Source			
Blood	5.2 (176/3353)	2.3 (71/3033)	3.2 (35/1098)
Urine	3.8 (95/2496)	2.3 (45/1934)	3.6 (16/442)
Wound	4.7 (11/236)	4.5 (9/201)	3.4 (3/88)
Respiratory	9.4 (49/521)	4.8 (21/436)	3.0 (13/430)
Multi-Drug Resistance			
MDR	79.2 (262/331)	36.3 (53/146)	70.1 (47/67)
XDR	3.3 (11/331)	1.4 (2/146)	9.0 (8/67)
***E. coli* ST-131**	58.9 (195/331)	30.1 (44/146)	Not Applicable

TABLE 3. Resistance profile and patient demographics associated with KPC-*E. coli* and KPC-*K. pneumoniae.*

Parameter	KPC-*E. coli* (N=2)		KPC-*K. pneumoniae* (N=1)	
	MIC (μg/ml)	S / I /R	MIC (μg/ml)	S / I / R
Susceptibility				
AMC	>32, >32	R, R	>32	R
Cefazolin	>128, >128	R, R	>128	R
Cefoxitin	16, 16	I, I	>32	R
Ceftriaxone	32, 64	R, R	>64	R
Ceftazidime	>32, >32	R, R	>32	R
TZP	128, 256	R, R	512	R
Ertapenem	2, 8	R, R	16	R
Meropenem	1, 1	S, S	4	R
Ciprofloxacin	>16, >16	R, R	>16	R
Amikacin	32, 8	I, S	32	I
Gentamicin	>32, 2	R, S	8	S
Tigecycline	0.5, 0.25	S, S	2	S
SXT	>8, >8	R, R	>8	R
Colistin	0.5, 0.25	S, S	0.25	S
Demographics				
Year	2010, 2011		2009	
Region	Quebec, Quebec		Ontario	
Gender	Male, Male		Female	
Age	77, 74		67	
Source	Resp., Resp.		Blood	
Location	ICU, ICU		Med. Ward	
***E. coli* ST-131**	POS, POS		Not Applicable	

TABLE 4. Genotypic characterization of ESBL-*E. coli*, ESBL-*K. pneumoniae, and* AmpC-*E. coli.*

Cohort (n)	Genotype	No. of Isolates (%)
ESBL-*E. coli* (331)	CTX-M-3	2 (0.6)
	CTX-M-14	56 (16.9)
	CTX-M-15	222 (67.1)
	CTX-M-24	2 (0.6)
	CTX-M-27	26 (7.9)
	CTX-M-65	1 (0.3)
	SHV-2a	3 (0.9)
	SHV-12	6 (1.8)
	TEM-12	1 (0.3)
	Unknown	12 (3.6)
	[TEM-1[a]	114 (34.4)]
ESBL-*K. pneumo.* (67)	CTX-M-2	1 (1.5)
	CTX-M-3	1 (1.5)
	CTX-M-14	8 (11.9)
	CTX-M-15	32 (47.8)
	CTX-M-27	3 (4.5)
	SHV-2	1 (1.5)
	SHV-2a	6 (9.0)
	SHV-5	1 (1.5)
	SHV-11	20 (29.9)
	SHV-12	9 (13.4)
	SHV-28	4 (6.0)
	SHV-31	1 (1.5)
	SHV-108	1 (1.5)
	SHV-168	1 (1.5)
	Unknown	6 (9.0)
	[SHV-1[a]	19 (28.4)]
	[TEM-1[a]	32 (47.8)]
AmpC-*E. coli* (146)	Chromosomal AmpC hyperproduction	65 (44.5)
	CMY-2	80 (54.8)
	FOX-5	1 (0.7)

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

TABLE 5. The national prevalence of ESBL-*E. coli*, ESBL-*K. pneumoniae*, and AmpC-*E. coli from* 2007 to 2013.

Cohort (n)	CANWARD Study Year: % (no. in cohort/total no. of species collected)								*P*-value[b,c]
	2007	2008	2009	2010	2011	2012	2013	2007-2013	
ESBL-*E. coli* (331)	3.4 (53/1560)	4.9 (55/1131)	4.3 (47/1097)	2.9 (30/1017)	7.1 (46/646)	7.6 (38/500)	9.5 (62/655)	5.0 (331/6606)	<0.001
ESBL-*K. pneumoniae* (67)	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)	3.3 (67/2058)	0.004
AmpC-*E. coli* (146)	0.7 (4/558[a])	3.1 (35/1131)	2.7 (30/1097)	2.7 (27/1017)	2.9 (19/646)	2.2 (11/500)	3.1 (20/655)	2.6 (146/5604)	0.003

[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 2007-2013; [c]Statistical significance defined as *P*<0.05.