ASM Microbe 2022
Poster # 2133

www.can-r.ca

Rates of Extended-Spectrum β-lactamase-Producing (ESBL) *Escherichia coli* and *Klebsiella pneumoniae* Continue to Increase in Canada: CANWARD 2007 – 2020

HJ Adam[1,2], AJ Denisuik[2], MR Mulvey[2,3], M Baxter[2], A Golden[3], PRS Lagacé-Wiens[1,2], JA Karlowsky[1,2], GG Zhanel[2]
and the Canadian Antimicrobial Resistance Alliance (CARA)
[1]Shared Health, [2]University of Manitoba, [3]Public Health Agency of Canada - National Microbiology Laboratory (PHAC-NML), Winnipeg, Canada

Dr. George G. Zhanel
MS673-820 Sherbrook Street
Winnipeg, MB R3A 1R9
CANADA
Email: ggzhanel@pcsinternet.ca

Background

Bacteria that belong to the family Enterobacteriaceae, including *Escherichia coli* and *Klebsiella pneumoniae*, are a common cause of both community-acquired and nosocomial infections (1,2). The oxyiminocephalosporins represent safe and effective therapy for the treatment of these infections. Resistance to oxyiminocephalosporins in the Enterobacteriaceae is largely attributable to the production of extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases, which hydrolyze a variety of β-lactams including the oxyiminocephalosporins and monobactams. Over the last decade, the proportion of *E. coli* and *K. pneumoniae* clinical isolates that harbour an ESBL enzyme has increased in Canada and elsewhere in the world (3-5). ESBL-producing organisms are often multi-drug resistant. Accordingly, treatment options for infections caused by ESBL-producers are limited, and the use of broad-spectrum antimicrobials (e.g. carbapenems) is commonly required (3). The emergence of β-lactamase enzymes with carbapenemase activity presents serious implications for patient care and public health.

The purpose of this study was to assess the prevalence and antimicrobial resistance patterns of ESBL-producing *E. coli* and *K. pneumoniae* in Canada as part of the ongoing CANWARD national surveillance study.

Materials and Methods

Bacterial Isolates:
A total of 10,605 *E. coli* and 3,474 *K. pneumoniae* were collected from January 2007 to December 2020 as part of the CANWARD surveillance study. CANWARD is an ongoing national Public Health Agency of Canada/Canadian Antimicrobial Resistance Alliance (PHAC/CARA) partnered surveillance study evaluating *in vitro* activities of antimicrobial agents against bacterial pathogens isolated by clinical laboratories from patients attending tertiary care hospitals across Canada. Hospitals in 8 of the 10 Canadian provinces submitted clinically relevant isolates from inpatients and outpatients attending hospital clinics, medical and surgical wards, emergency rooms, and intensive care units (ICUs) to the CANWARD coordinating laboratory (Health Sciences Centre, Winnipeg, Canada). The CANWARD study sets annual quotas for respiratory, wound, urine and bloodstream isolates and requires isolates to be collected consecutively, one per patient, per site of infection. Isolates are deemed clinically significant by the submitting sites local testing criteria and the identities of the isolates are confirmed, by colonial appearance, spot testing and/or MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA), upon receipt at the CANWARD coordinating laboratory.

Antimicrobial Susceptibility Testing:
Antimicrobial susceptibility testing was performed using custom-designed in-house prepared 96-well microtiter panels in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (6). The MICs of the antimicrobial agents for the isolates were interpreted utilizing CLSI criteria (7). Putative ESBL-producers were identified as any *E. coli* or *K. pneumoniae* isolate with a ceftriaxone and/or ceftazidime MIC ≥ 1 µg/mL and were phenotypically confirmed by CLSI phenotypic confirmatory disk test (7).

Statistical Analysis:
Differences in demographics and susceptibility results of ESBL-producing *E. coli* and *K. pneumoniae* strains were calculated by the chi-squared test (https://www.graphpad.com/quickcalcs). Annual proportions of isolates harbouring ESBL genes were assessed by the Cochrane–Armitage test of trend using JMP version 14 (SAS, Cary, NC, USA). Statistical significance was defined as a *P* value of ≤0.05.

Results

Table 1. Patient demographics associated with ESBL-*E. coli* and ESBL-*K. pneumoniae*

Parameter	Cohort: % (N in cohort / total N collected)	
	ESBL-*E. coli*	ESBL-*K. pneumoniae*
Value	7.8% (832/10605)	5.2% (181/3474)
Gender		
Male	9.6% (414/4308)	5.8% (109/1895)
Female	6.6% (418/6297)	4.6% (72/1579)
Age (years)		
≤17	3.9% (39/998)	4.6% (13/282)
18-64	8.3% (343/4153)	6.0% (84/1411)
≥65	8.3% (450/5454)	4.7% (84/1781)
Specimen Source		
Blood	7.5% (419/5612)	4.8% (88/1827)
Urine	5.8% (208/3614)	5.2% (35/677)
Wound	10.7% (39/365)	7.9% (12/152)
Respiratory	16.4% (166/1014)	5.6% (46/818)
Hospital Location		
Clinic/Office	4.6% (75/1639)	3.3% (14/425)
Emergency Room	5.5% (220/4036)	3.0% (27/890)
Intensive Care Unit	11.5% (123/1070)	6.5% (44/681)
Medical Ward	11.0% (346/3151)	7.0% (82/1171)
Surgical Ward	9.6% (68/708)	4.6% (14/307)
Geographic Region		
West	8.2% (283/3154)	5.4% (58/1080)
Central	8.4% (480/5721)	5.9% (116/1968)
East	4.9% (69/1403)	1.7% (7/419)

ESBL rates were higher in *E. coli* isolates collected from males than females (P<0.0001) and samples collected from the central region compared to the east for *E. coli* and *K. pneumoniae* (P<0.001). There was a significant difference between the proportion of ESBL-producing *E. coli* in the three age groups (P<0.0001) with 3.9% in the <17 age group and 8.3% in both the 18-64 and >65 groups. ESBL rates in *E. coli* were significantly higher in respiratory samples than any other source (P≤0.01).

Significant differences (*P*<0.0001) in the susceptibility rates of ESBL and non-ESBL *E. coli* and *K. pneumoniae* were observed for all reported antimicrobial agents, except colistin.

17 carbapenem-resistant *E. coli* and *K. pneumoniae* were isolated. Isolates were identified in 2009/2012/2013/2014 (N=1 per year), 2015 (N=2), 2018 (N=5), 2019 (N=2), and 2020 (N=3). All isolates were collected from central Canada except for 2, which were collected in the western region. Nine (53%) of isolates were from blood cultures, 5 (29%) were from respiratory samples and 3 (18%) were isolated from wounds. Patients were predominantly male (N=10, 59%) and 94% were isolated from adults (18 – 64 years, N=8; ≥65 years, N=8).

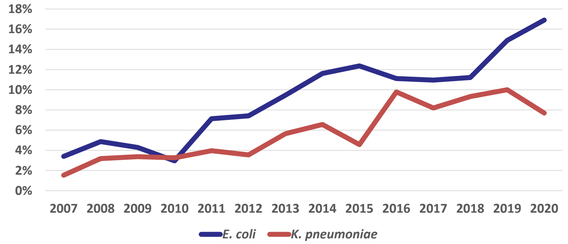

Figure 1. Annual proportion of ESBL-producing *E. coli* and *K. pneumoniae* in isolates collected as part of CANWARD from 2007 to 2020.

Table 2. Antimicrobial susceptibility testing of *E. coli*, ESBL-producing and non-ESBL-producing isolates.

	ESBL (N=832)							Non-ESBL (N=9771)						
	MIC Interpretation[a]			MIC (µg/mL)				MIC Interpretation[a]			MIC (µg/mL)			
Drug	% S	% I	% R	MIC_{50}	MIC_{90}	Min.	Max.	% S	% I	%R	MIC_{50}	MIC_{90}	Min.	Max.
Amikacin	98.0%	1.7%	0.4%	2	8	≤ 1	> 64	99.8%	0.1%	0.1%	2	4	≤ 1	> 64
AMC[b]	49.1%	37.9%	13.0%	16	32	1	> 32	85.8%	9.8%	4.5%	4	16	≤ 0.06	> 32
Cefazolin		0.1%	99.9%	> 128	> 128	4	> 128	74.8%	12.7%	12.5%	2	8	≤ 0.5	> 128
Cefepime	24.3%	29.1%	46.6%	8	> 64	≤ 0.25	> 64	99.6%	0.2%	0.2%	≤ 0.25	≤ 1	≤ 0.25	> 64
Cefoxitin	77.6%	13.7%	8.7%	8	16	0.5	> 32	92.6%	3.9%	3.5%	4	8	≤ 0.06	> 32
Ceftazidime	27.9%	10.3%	61.9%	16	> 32	≤ 0.5	> 32	97.8%	0.4%	1.9%	≤ 0.25	0.5	≤ 0.25	> 32
Ceftriaxone	1.4%	1.1%	97.5%	> 64	> 64	≤ 0.25	> 64	97.7%	0.3%	2.1%	≤ 0.25	≤ 1	≤ 0.25	> 256
Ciprofloxacin	11.8%	1.0%	87.3%	> 16	> 16	≤ 0.06	> 16	79.4%	1.6%	19.0%	≤ 0.06	> 16	≤ 0.06	> 16
Colistin		99.6%	0.4%	0.5	1	≤ 0.06	8		99.7%	0.3%	0.25	0.5	≤ 0.06	> 16
Ertapenem	97.6%	1.1%	1.3%	≤ 0.03	0.12	≤ 0.03	> 32	99.9%	0.1%	0.1%	≤ 0.03	≤ 0.06	≤ 0.03	> 32
Gentamicin	64.1%	1.2%	34.7%	1	> 32	≤ 0.5	> 32	92.9%	0.4%	6.8%	≤ 0.5	1	≤ 0.5	> 32
Meropenem	99.6%		0.4%	≤ 0.03	0.06	≤ 0.03	> 32	100%		0%	≤ 0.03	≤ 0.12	≤ 0.03	> 32
TZP[b]	92.2%	4.6%	3.2%	4	16	≤ 1	> 512	97.7%	1.0%	1.3%	2	4	≤ 1	> 512
Tigecycline	99.9%	0.1%		0.5	1	0.12	4	100%	0%		0.25	0.5	≤ 0.03	4
SXT[b]	32.5%		67.5%	> 8	> 8	≤ 0.12	> 8	76.2%		23.8%	≤ 0.12	> 8	≤ 0.12	> 8

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

Table 3. Antimicrobial susceptibility testing of *K. pneumoniae*, ESBL-producing and non-ESBL-producing isolates.

	ESBL (N=181)							Non-ESBL (N=3291)						
	MIC Interpretation[a]			MIC (µg/mL)				MIC Interpretation[a]			MIC (µg/mL)			
Drug	% Sus	% Int	% Res	MIC_{50}	MIC_{90}	Min.	Max.	% Sus	% Int	% Res	MIC_{50}	MIC_{90}	Min.	Max.
Amikacin	96.7%	1.1%	2.2%	2	8	≤ 1	> 64	99.9%	0%	0.1%	≤ 1	2	≤ 1	> 64
AMC[b]	31.7%	34.8%	33.5%	16	> 32	2	> 32	95.7%	2.3%	2.0%	2	8	≤ 0.06	> 32
Cefazolin			100%	> 128	> 128	8	> 128	86.6%	6.3%	7.1%	1	4	≤ 0.5	> 128
Cefepime	19.9%	21.1%	59.1%	32	> 64	≤ 0.25	128	99.2%	0.3%	0.5%	≤ 0.25	≤ 1	≤ 0.25	> 64
Cefoxitin	66.7%	12.4%	20.9%	8	> 32	2	> 32	92.2%	3.5%	4.2%	4	8	0.12	> 32
Ceftazidime	19.5%	4.6%	75.9%	> 32	> 32	0.25	> 32	98.7%	0.2%	1.0%	≤ 0.25	0.5	≤ 0.25	> 32
Ceftriaxone	6.1%	2.8%	91.2%	> 64	> 64	≤ 0.25	> 256	98.2%	0.2%	1.6%	≤ 0.25	0.5	≤ 0.25	256
Ciprofloxacin	14.9%	10.5%	74.6%	4	> 16	≤ 0.06	> 16	90.9%	3.1%	6.0%	≤ 0.06	0.25	≤ 0.06	> 16
Colistin		96.0%	4.0%	0.5	1	0.25	> 16		98.0%	2.0%	0.5	1	≤ 0.06	> 16
Ertapenem	88.7%	2.8%	8.5%	0.12	1	≤ 0.03	> 32	99.6%	0.2%	0.2%	≤ 0.03	≤ 0.06	≤ 0.03	> 32
Gentamicin	55.2%	1.1%	43.6%	≤ 0.5	> 32	≤ 0.5	> 32	98.8%	0.2%	1.1%	≤ 0.5	≤ 0.5	≤ 0.5	> 32
Meropenem	94.5%	1.1%	4.4%	≤ 0.06	0.12	≤ 0.03	> 32	99.8%		0.2%	≤ 0.03	0.06	≤ 0.03	32
TZP[b]	65.2%	14.9%	19.9%	16	> 512	2	> 512	97.8%	0.7%	1.5%	2	8	≤ 1	> 512
Tigecycline	86.7%	10.5%	2.8%	1	4	0.5	16	96.0%	3.3%	0.7%	1	1	0.06	> 16
SXT[b]	16.6%		83.4%	> 8	> 8	≤ 0.12	> 8	93.1%		6.9%	≤ 0.12	1	≤ 0.12	> 8

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

Table 4. Antimicrobial susceptibility testing of carbapenem-resistant *E. coli* and *K. pneumoniae*.

	E. coli (N=4)					*K. pneumoniae* (N=13)				
	MIC Interpretation[a]			MIC (µg/mL)		MIC Interpretation[a]			MIC (µg/mL)	
Drug	% Sus	% Int	% Res	Min.	Max.	% Sus	% Int	% Res	Min.	Max.
Amikacin	75.0%		25.0%	2	> 64	76.9%	15.4%	7.7%	≤ 1	> 64
AMC[b]			100%	> 32	> 32			100%	32	> 32
Cefazolin			100%	> 128	> 128			100%	> 128	> 128
Cefepime			100%	> 64	> 64		7.7%	92.3%	4	> 64
Cefoxitin			100%	> 32	> 32	7.7%		92.3%	8	> 32
Ceftazidime			100%	> 32	> 32			100%	32	> 32
Ceftriaxone			100%	> 64	> 64			100%	8	> 64
Ciprofloxacin			100%	> 16	> 16	7.7%		92.3%	≤ 0.06	> 16
Colistin		100%		0.25	0.5		84.6%	15.4%	0.25	16
Ertapenem			100%	> 32	> 32			100%	4	> 32
Gentamicin	25.0%		75.0%	1	> 32	61.5%	7.7%	30.8%	≤ 0.5	> 32
Meropenem			100%	8	> 32			100%	4	> 32
TZP[b]			100%	512	> 512		7.7%	92.3%	64	> 512
Tigecycline	100%			0.12	0.5	69.2%	30.8%		0.5	4
SXT[b]			100%	> 8	> 8	15.4%		84.6%	≤ 0.12	> 8

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

Conclusions

1. A significant increase in the proportion of *E. coli* and *K. pneumoniae*-producing ESBLs was observed in isolates collected as part of CANWARD from 2007 to 2020
   a. The proportion of ESBL-producing *E. coli* increased from 3.4% in 2007 to 16.9% in 2020 (P<0.0001).
   b. The proportion of ESBL-producing *K. pneumoniae* increased from 1.5% in 2007 to 7.7% in 2020 (P<0.0001).
   c. Antimicrobial susceptibility of ESBL-producing *E. coli* and *K. pneumoniae* was significantly reduced compared to non-ESBL-producing isolates for most agents tested, most notably ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole.
2. Carbapenem-resistance in *E. coli* and *K. pneumoniae* remains low in Canada.
   a. Overall, only 0.04% of *E. coli* (4/10,605) and 0.4% (13/3,474) of *K. pneumoniae* demonstrated carbapenem resistance.
   b. Amikacin, tigecycline, and gentamicin retained activity against many of the carbapenem-resistant isolates.

Acknowledgements

The CANWARD study was supported in part by the University of Manitoba, Shared Health Manitoba, Public Health Agency of Canada – National Microbiology Laboratory, Astellas, Avir/Basilea, Iterum, Merck, Paladin Labs, Paratek, Pfizer, Sunovion, Tetraphase and Verity. CARA acknowledges the continued efforts of the CANWARD-supporting laboratories: Vancouver Hospital, Vancouver, BC; University of Alberta Hospital, Edmonton, AB; Royal University Hospital, Saskatoon, SK; Health Sciences Centre, Winnipeg, MB; London Health Sciences Centre, London, ON; Mount Sinai Hospital/University Health Network, Toronto, ON; The Ottawa Hospital, Ottawa, ON; St. Michael's Hospital, Toronto, ON; St. Joseph's Hospital, Hamilton, ON; Children's Hospital of Eastern Ontario, Ottawa, ON; Hôpital Maisonneuve-Rosemont, Montreal, QC; Montreal General Hospital, Montreal, QC; Royal Victoria Hospital, Montreal, QC; CHRTR Pavillon Ste. Marie, Trois-Rivières, QC; Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC; Cité de la Santé, Laval, QC; L'Hôtel-Dieu de Quebec, Quebec City, QC; South East Health Care Corp., Moncton, NB; and Queen Elizabeth II Health Sciences Centre, Halifax, NS.

References

1. Zhanel GG, Adam HJ, Baxter MR et al. 42,936 pathogens from Canadian hospitals: 10 years of results (2007-2016) from the CANWARD surveillance study. J Antimicrob Chemother 2019; 74 Suppl 4: 5-21.
2. Nelson GE, Greene MH. Enterobacteriaceae. In: Bennett JE, Dolin R, Blaser MJ, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elsevier, Inc., 2020: 2669–85.
3. Peirano G, Pitout JDD. Extended-spectrum β-lactamase-producing Enterobacteriaceae: update on molecular epidemiology and treatment options. Drugs 2019; 79: 1529–41.
4. Denisuik AJ, Karlowsky JA, Adam HJ, Baxter MR, Lagacé-Wiens PRS, Mulvey MR, Hoban DJ, Zhanel GG; Canadian Antimicrobial Resistance Alliance (CARA) and CANWARD. Dramatic rise in the proportion of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among clinical isolates identified in Canadian hospital laboratories from 2007 to 2016. J Antimicrob Chemother. 2019;74:iv64-iv71.
5. McDanel J, Schweizer M, Crabb V et al. Incidence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella infections* in the United States: a systematic literature review. Infect Control Hosp Epidemiol 2017; 38: 1209–15.
6. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically - eleventh Edition:M07. 2018.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing - Thirtieth Edition:M100. 2020.