



Introduction

Antimicrobial-resistant Gram-negative organisms such as extended-spectrumβ-lactamase producing (ESBL) Enterobacterales, carbapenem-resistant Enterobacterales (CRE), and carbapenem-resistant *Pseudomonas aeruginosa* are increasing in prevalence in Canada and around the world (1,2). Available therapeutic options for the treatment of these antimicrobial resistant organisms are limited as these organisms frequently display a multidrug-resistant (MDR) phenotype (1,2).

CANWARD (a collaboration between the Canadian Antimicrobial Resistance Alliance and the National Microbiology Laboratory) is a national ongoing surveillance study which assesses pathogens and their associated antimicrobial susceptibility testing phenotypes for respiratory, bacteremic, urinary, and wound/IV site infection isolates in Canadian hospitalized patients on medical/surgical wards, intensive care units, emergency rooms and outpatient clinics.

Materials and Methods

Participating Sites

From January 2007 to October 2024, tertiary-care medical centres in major population centres in 8 of the 10 provinces in Canada were recruited (1, 2). These sites were geographically distributed in a population-based fashion.

Bacterial Isolates

Tertiary-care medical centres submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. Each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with respiratory, urine, wound, and bloodstream infections. Isolate identification was performed by the submitting site and confirmed at the reference site as required, based on morphological characteristics and antimicrobial susceptibility patterns. Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at -80°C until minimum inhibitory concentration (MIC) testing was carried out. From 2007 to 2024, a total of 7718, 5283, 5373, 4960, 3785, 2802, 3511, 3172, 3206, 3125, 3418, 2949, 3069, 2231, 2331, 2227, 1906, and 3097 Gram-positive and Gram-negative isolates were collected in each study study year (1, 2). The focus of this study was the 35,824 Gram-negative pathogens collected from CANWARD 2007-2024, inclusive.

Antimicrobial Susceptibilities

The *in vitro* activity of selected antimicrobials was determined by broth microdilution in accordance with CLSI guidelines (3). Antimicrobial MIC interpretive standards were defined according to CLSI breakpoints (4). The MICs of the antimicrobial agents were determined using 96-well custom designed microtitre plates. These plates contained doubling antimicrobial dilutions in 100μL/well of cation adjusted Mueller-Hinton broth and inoculated to achieve a final concentration of approximately 5 x 10⁵ CFU/mL then incubated in ambient air for 16-20 hours prior to reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using ATCC QC organisms including *S. pneumoniae* 49619, *S. aureus* 29213, *E. faecalis* 29212, *E. coli* 25922, and *P. aeruginosa* 27853.

Statistical Analysis

Annual proportions of isolates harbouring ESBL genes were assessed by the Cochran–Armitage test of trend using XLSTAT (v.2019). Statistical significance was defined as a *P* value of ≤0.05.

Results

Table 1. The 20 most common organisms isolated from Canadian hospitals

Rank	Organism	N	% of Total
1	<i>Staphylococcus aureus</i>	13,332	20.7
	MSSA	10,478	16.3
	MRSA	2,854	4.4
2	<i>Escherichia coli</i>	12,441	19.4
3	<i>Pseudomonas aeruginosa</i>	6,269	9.8
4	<i>Klebsiella pneumoniae</i>	4,200	6.5
5	<i>Streptococcus pneumoniae</i>	3,600	5.6
6	<i>Haemophilus influenzae</i>	2,438	3.8
7	<i>Enterococcus faecalis</i>	2,321	3.6
8	CNS / <i>Staphylococcus epidermidis</i>	2,228	3.5
9	<i>Enterobacter cloacae</i>	1,755	2.7
10	<i>Stenotrophomonas maltophilia</i>	1,269	2.0
11	<i>Klebsiella oxytoca</i>	1,110	1.7
12	<i>Serratia marcescens</i>	1,054	1.6
13	<i>Proteus mirabilis</i>	1,007	1.6
14	<i>Streptococcus agalactiae</i>	999	1.6
15	Viridans group streptococci	996	1.6
16	<i>Streptococcus pyogenes</i>	911	1.4
17	<i>Enterococcus faecium</i>	843	1.3
18	<i>Moraxella catarrhalis</i>	610	1.0
19	<i>Candida albicans</i>	577	0.9
20	<i>Klebsiella aerogenes</i>	473	0.7
	Other	5,730	8.9
		64,163	

Table 2. Demographics of patients with Gram-negative isolates from CANWARD (n=35,824)

Demographic Variable	N	% of Total
Biological Sex ^a		
Female	17,227	48.1
Male	18,594	51.9
Age Group		
≤ 17 years	3,543	9.9
18 - 64 years	14,537	40.6
≥ 65 years	17,744	49.5
Specimen Source		
Blood	13,226	36.9
Respiratory	14,192	39.6
Urine	6,301	17.6
Wound	2,105	5.9
Ward Type ^b		
Clinic	6,640	18.5
ICU	8,529	23.8
ER	6,784	18.9
Medical	11,198	31.3
Surgical	2,670	7.5

^a Biological sex unknown for 3 isolates

^b Patient location unknown for 3 isolates

Table 3. Antimicrobial activity against the most common Gram-negative bacilli isolated from Canadian hospitals

Organism (no. tested) / Antimicrobial Agent [†]	% S	% I	% R	MIC ₅₀	MIC ₉₀	Range Min	Range Max
<i>Escherichia coli</i> (12,441)							
Amox-Clav	79.1	13.5	7.4	8	16	≤ 0.06	> 32
Cefazolin	67.3	11.9	20.8	2	128	≤ 0.5	> 128
Cefepime	92.1	2.7*	5.2	≤ 1	≤ 1	≤ 1	> 32
Cefoxitin	90.8	5.1	4.1	4	8	≤ 0.06	> 32
Ceftazidime	90.6	1.6	7.8	≤ 0.5	4	≤ 0.5	> 32
Ceftaz-Avi	99.8	0.2	0.12	0.25	≤ 0.06	≤ 0.06	> 16
Ceftobiprole [‡]	90.7	0.2	9.1	≤ 1	≤ 1	≤ 1	> 16
Ceftol-Tazo	98.9	0.3	0.8	0.25	0.5	≤ 0.12	> 64
Ceftriaxone	89.1	0.3	10.6	≤ 1	8	≤ 1	> 64
Ciprofloxacin	73.9	1.8	24.3	≤ 0.06	> 16	≤ 0.06	> 16
Colistin	99.6	0.4	0.25	0.5	≤ 0.06	≤ 0.06	> 16
Doxycycline	72.6	6.5	20.9	2	32	≤ 0.12	> 32
Ertapenem	99.6	0.2	0.2	≤ 0.06	≤ 0.06	≤ 0.06	> 32
Gentamicin	89.8	0.5	9.7	≤ 0.5	4	≤ 0.5	> 32
Imipenem	99.8	0.1	0.1	0.25	0.25	≤ 0.03	> 32
Imi-Rele	99.9	0.1	0.25	0.25	≤ 0.03	≤ 0.03	> 32
Meropenem	99.9	0.1	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	> 32
Mero-Vabor	99.9	0.1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	> 32
Moxifloxacin						> 16	≤ 0.06
Nitrofurantoin	97.1	1.8	1.1		32	≤ 1	> 256
Pip-Tazo	95.5	1.7*	2.8	2	4	≤ 1	> 512
Tigecycline [§]	99.9	0.1		0.25	0.5	≤ 0.03	4
TMP-SMX	72.7		27.3	≤ 0.12	> 8	≤ 0.12	> 8
ESBL <i>E. coli</i> (1,091)							
Amox-Clav	41.4	38.6	20.0	16	32	1	> 32
Cefazolin	0.1	0.1	99.8	> 128	> 128	2	> 128
Cefepime	24.3	28.8	46.9	16	> 32	≤ 1	> 32
Cefoxitin	77.1	14.4	8.5	8	16	0.5	> 32
Ceftazidime	26.1	14.1	59.8	16	> 32	≤ 0.5	> 32
Ceftaz-Avi	99.5	1.3	0.5	0.25	0.5	≤ 0.06	> 16
Ceftobiprole	2.0	1.3	96.7	> 16	> 16	≤ 1	> 16
Ceftol-Tazo	96.2	2.6	0.5	0.5	1	≤ 0.12	> 64
Ceftriaxone	1.4	1.2	97.4	> 64	> 64	≤ 1	> 64
Ciprofloxacin	11.7	0.9	87.4	> 16	> 16	≤ 0.06	> 16
Colistin	99.3	0.7	0.5	1	≤ 0.06	8	> 32
Doxycycline	34.6	19.1	46.3	16	> 32	0.25	> 32
Ertapenem	97.9	12.1	0.9	≤ 0.06	0.12	≤ 0.06	> 32
Gentamicin	96.2	0.1	31.3	> 32	> 32	≤ 0.5	> 32
Imipenem	99.7	0.3	0.25	0.25	0.25	≤ 0.03	16
Imi-Rele	99.8	0.2	0.25	0.25	0.25	≤ 0.03	16
Meropenem	99.9	0.1	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	> 32
Mero-Vabor	100		≤ 0.06	≤ 0.06	≤ 0.06	0.5	> 16
Moxifloxacin						> 16	≤ 0.06
Nitrofurantoin	93.5	4.2	2.3	16	> 32	≤ 1	> 256
Pip-Tazo	83.0	9.4	7.6	4	16	≤ 1	> 512
Tigecycline	99.9	0.1	0.5	1	0.12	4	> 32
TMP-SMX	35.5		64.3	> 8	> 8	≤ 0.12	> 8
<i>Klebsiella pneumoniae</i> (4,200)							
Amox-Clav	90.4	4.8	4.8	2	8	≤ 0.06	> 32
Cefazolin	81.0	6.2	12.8	1	8	≤ 0.5	> 128
Cefepime	93.7	1.4	4.9	≤ 1	≤ 1	≤ 1	> 32
Cefoxitin	90.3	4.1	5.6	4	8	0.12	> 32
Ceftazidime	92.9	0.5	6.6	≤ 0.5	1	≤ 0.5	> 32
Ceftaz-Avi	99.7	0.3	0.3	0.25	0.5	≤ 0.06	> 16
Ceftobiprole	92.6	0.4	7.0	≤ 1	≤ 1	≤ 1	> 16
Ceftol-Tazo	97.3	0.4	2.3	0.25	1	≤ 0.12	> 64
Ceftriaxone	92.3	0.4	7.3	≤ 1	≤ 1	≤ 1	> 64
Ciprofloxacin	86.8	3.7	9.5	≤ 0.06	0.5	≤ 0.06	> 16
Colistin	97.9	2.1	0.5	1	≤ 0.06	> 16	> 16
Doxycycline	80.4	4.2	15.4	2	16	≤ 0.12	> 32
Ertapenem	98.8	0.4	0.8	≤ 0.06	≤ 0.06	≤ 0.06	> 32
Gentamicin	96.0	0.2	3.8	≤ 0.5	≤ 0.5	≤ 0.5	> 32
Imipenem	98.9	0.4	0.7	0.25	0.5	0.06	> 32
Imi-Rele	99.1	0.6	0.3	0.25	0.5	≤ 0.03	> 32
Meropenem	99.5	0.1	0.4	≤ 0.12	≤ 0.12	≤ 0.12	> 32
Mero-Vabor	99.5	0.5	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	> 32
Moxifloxacin						0.12	≤ 0.06
Nitrofurantoin	39.3	36.8	23.9	64	128	≤ 1	> 256
Pip-Tazo	91.7	4.3	4.0	2	8	≤ 1	> 512
Tigecycline	95.7	3.5	0.8	1	2	0.06	> 16
TMP-SMX	88.3		11.7	≤ 0.12	> 8	≤ 0.12	> 8
<i>Stenotrophomonas maltophilia</i> (1,269)							
Cefepime				> 32	> 32	≤ 1	> 32
Ceftazidime				> 32	> 32	≤ 0.5	> 32
Ceftaz-Avi				> 16	> 16	0.25	> 16
Ceftobiprole				> 16	> 16	≤ 1	> 16
Ceftol-Tazo				32	> 64	≤ 0.12	> 64
Ciprofloxacin				4	16	≤ 0.06	> 16
Colistin				8	> 16	0.12	> 16
Doxycycline				2	8	0.25	> 32
Imi-Rele				> 32	> 32	0.5	> 32
Moxifloxacin				1	4	≤ 0.06	> 16
Nitrofurantoin				> 256	> 256	32	> 256
Tigecycline				2	4	0.12	> 16
TMP-SMX	98.0		2.0	0.25	1	≤ 0.12	> 8
<i>Acinetobacter baumannii</i> (956)							
Amikacin	29.6			≤ 2	4	≤ 2	> 64
Cefepime	84.5	5.7	9.8	4	16	≤ 1	> 32
Ceftazidime	77.6	11.2	11.2	8	32	≤ 0.5	> 32
Ceftaz-Avi				8	> 16	0.12	> 16
Ceftobiprole				≤ 1	4	≤ 1	> 16
Ceftol-Tazo				0.5	8	≤ 0.12	> 64
Ceftriaxone	41.4	48.3	10.3	16	64	≤ 1	> 64
Ciprofloxacin	92.8	1.4	5.8	0.25	1	≤ 0.06	> 16
Gentamicin	92.5	1.7	5.8	≤ 0.5	2	≤ 0.5	> 32
Imi-Rele	95.2	4.8	0.25	0.5	0.06	≤ 0.06	> 32
Meropenem	94.2	1.7	4.1	0.5	2	≤ 0.12	> 32
Moxifloxacin				≤ 0.06	0.5	≤ 0.06	> 16
Nitrofurantoin				> 256	> 256	64	> 256
Pip-Tazo	80.1	9.6	10.3	4	128	≤ 1	> 512
Tigecycline				0.5	1	0.12	> 16
TMP-SMX	91.4		8.6	≤ 0.12	2	≤ 0.12	> 8

Amox-Clav, Amoxicillin-Clavulanate; Ceftaz-Avi, Ceftazidime-Avibactam; Imi-Rele, Imipenem-relebactam; Mero-Vabor, Meropenem-vaborbactam; Pip-Tazo, Piperacillin-Tazobactam; TMP-SMX, Trimethoprim-sulfamethoxazole. [†] Not all isolates were tested against each antimicrobial agent. [‡] SDD breakpoint applied for Enterobacterales. [§] For ceftobiprole, Health Canada breakpoints applied. [¶] For tigecycline, FDA breakpoints applied. ^{||} For amikacin, breakpoints applied to isolates from all specimen sources. ^{||} Susceptible breakpoint for meropenem-vaborbactam MIC ≤8 μg/mL.

Conclusions

- Of the 35,824 Gram-negative pathogens obtained, the most common were *E. coli* (19.4% of all isolates collected), *P. aeruginosa* (9.8%), *K. pneumoniae* (6.5%), and *H. influenzae* (3.8%).
- Gram-negative isolates were obtained from blood (36.9%), respiratory (39.6%), urine (17.6%) and wound specimens (5.9%) from patients on wards 38.8% (31.3% medical / 7.5% surgical), ER 23.8%, ICU 18.9%, and clinics 18.5%.
- ESBL-producing *E. coli* (3.4%-16.5%; *P* <0.0001) and *K. pneumoniae* (1.5%-16.3%; *P* <0.0001) increased significantly over the study period.
- Overall, carbapenem-resistant *E. coli* and *K. pneumoniae* remained at less than 1% for the entire study period. Annual rates of carbapenem-resistance for *E. coli* and *K. pneumoniae* ranged from 0-0.54% and 0-2.6%, respectively (data not shown).
- Susceptibility rates for *E. coli* were: 99.9% imipenem-relebactam, meropenem, meropenem-vaborbactam and tigecycline; 99.8% ceftazidime-avibactam; 99.6% ertapenem; 98.9% ceftolozane-tazobactam; 95.5% piperacillin-tazobactam; 90.7% ceftobiprole; 89.8% gentamicin; 89.1% ceftriaxone; 79.1% amoxicillin-clavulanate; 73.9% ciprofloxacin; and 72.7% trimethoprim-sulfamethoxazole.