



Introduction

Whether in Canada, the US or globally, antimicrobial-resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA, community associated [CA] and healthcare associated [HA]), vancomycin-resistant *Enterococcus* species (VRE), and penicillin-resistant *Streptococcus pneumoniae* are increasing in prevalence (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 from 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, 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 year (1, 2).The focus of this study was the of 27,293 Gram-positive pathogens collected over the years.

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 MRSA isolates 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	<i>CNS / 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	<i>Viridans group streptococci</i>	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-positive isolates from CANWARD (n=27,293)

Demographic Variable	N	% of Total
Biological Sex ^a		
Female	11,221	41.1
Male	16,065	58.9
Age Group		
≤ 17 years	3,604	13.2
18 - 64 years	13,256	48.6
≥ 65 years	10,433	38.2
Specimen Source		
Blood	13,172	48.3
Respiratory	8,252	30.2
Urine	1,496	5.5
Wound	4,373	16.0
Ward Type ^b		
Clinic	4,652	17.0
ER	7,093	26.0
ICU	5,040	18.5
Medical	8,340	30.6
Surgical	2,164	7.9

^a Biological sex unknown for 7 isolates
^b Patient location unknown for 4 isolates

Table 3. Antimicrobial activity against the most common Gram-positive cocci isolated from Canadian hospitals

Organism (no. tested) / Antimicrobial Agent [†]	% S	% I	% R	MIC (µg/mL)			
				MIC ₅₀	MIC ₉₀	Range Min	Range Max
Staphylococcus aureus - MSSA (10,478)							
Cefoxitin	100			4	4	0.12	4
Ceftobiprole ^a	100			≤ 1	≤ 1	≤ 1	2
Ceftriaxone				4	4	≤ 1	> 64
Ciprofloxacin	87.6	3.0	9.4	0.5	2	≤ 0.06	> 16
Clarithromycin	75.5	0.7	23.8	≤ 0.25	> 16	≤ 0.25	> 16
Clindamycin	94.1	0.4	5.5	≤ 0.25	≤ 0.25	≤ 0.25	> 8
Dalbavancin	100			0.06	0.06	≤ 0.03	0.25
Daptomycin	99.9			0.25	0.5	≤ 0.06	2
Doxycycline	98.8	0.7	0.4	≤ 0.25	≤ 0.25	≤ 0.25	> 16
Gentamicin	98.3	0.1	1.6	≤ 0.5	≤ 0.5	≤ 0.5	> 32
Lefamulin	98.7		1.3	0.12	0.12	0.03	> 8
Levofloxacin	90.1	0.3	9.6	0.25	1	≤ 0.06	> 32
Linezolid	99.9			2	4	≤ 0.12	8
Nitrofurantoin	99.8	0.1	0.1	16	16	≤ 1	> 512
Tigecycline ^b	99.0		1.0	0.25	0.25	≤ 0.03	2
Trimethoprim Sulfate	99.4		0.6	≤ 0.12	≤ 0.12	≤ 0.12	> 8
Vancomycin	100			1	1	≤ 0.25	2
Staphylococcus aureus - MRSA (2,854)							
Cefoxitin			100	> 32	> 32	8	> 32
Ceftobiprole ^a	100			≤ 1	2	≤ 1	4
Ceftriaxone				> 64	> 64	2	> 64
Ciprofloxacin	23.5	0.7	75.8	> 16	> 16	≤ 0.06	> 16
Clarithromycin	20.0	0.7	79.3	> 16	> 16	≤ 0.25	> 16
Clindamycin	62.0	0.1	37.9	≤ 0.25	> 8	≤ 0.25	> 8
Dalbavancin	100			0.06	0.06	≤ 0.03	0.25
Daptomycin	99.8		0.2	0.25	0.5	≤ 0.06	4
Doxycycline	97.6	1.0	1.4	≤ 0.25	1	≤ 0.25	16
Gentamicin	93.2	0.4	6.4	≤ 0.5	1	≤ 0.5	> 32
Lefamulin	98.9		1.1	0.12	0.25	0.03	0.5
Levofloxacin	14.1		85.9	> 32	> 32	0.12	> 32
Linezolid	99.9		0.1	2	4	≤ 0.12	> 16
Nitrofurantoin	99.6	0.1	0.3	16	16	8	256
Tigecycline ^b	98.1		1.9	0.25	0.5	≤ 0.03	4
Trimethoprim Sulfate	94.5		5.5	≤ 0.12	0.25	≤ 0.12	> 8
Vancomycin	99.9	0.1		1	1	≤ 0.25	4
Staphylococcus epidermidis (1,390)							
Amox-Clav				1	8	≤ 0.06	> 32
Cefazolin				1	32	≤ 0.5	> 128
Cefepime				4	> 32	≤ 1	> 32
Cefoxitin				8	> 32	≤ 0.06	> 32
Ceftobiprole				≤ 1	≤ 1	≤ 1	8
Ceftriaxone				8	> 64	≤ 1	> 64
Ciprofloxacin	49.1	1.8	49.1	2	> 16	≤ 0.06	> 16
Clarithromycin	35.2	1.8	63.0	> 16	> 16	≤ 0.25	> 16
Clindamycin	60.4	1.4	38.2	≤ 0.25	> 8	≤ 0.25	> 8
Dalbavancin				≤ 0.03	0.06	≤ 0.03	1
Daptomycin	99.8		0.2	0.25	0.25	≤ 0.06	2
Doxycycline	94.4	3.0	2.6	0.5	2	≤ 0.25	> 16
Ertapenem				2	> 32	≤ 0.06	> 32
Gentamicin	63.6	5.2	31.2	≤ 0.5	> 32	≤ 0.5	> 32
Levofloxacin	44.2	1.6	54.2	4	> 32	0.12	> 32
Linezolid	100			1	1	≤ 0.12	4
Meropenem				1	16	≤ 0.12	> 32
Pip-Tazo				≤ 1	16	≤ 1	256
Tigecycline				0.12	0.5	≤ 0.03	2
Trimethoprim Sulfate	63.2		36.8	0.25	8	≤ 0.12	> 8
Vancomycin	100			1	2	≤ 0.25	4
Streptococcus pneumoniae (3,600)							
Amox-Clav	97.7	1.3	0.1	≤ 0.06	0.12	≤ 0.06	16
Ceftobiprole ^a	99.9		1.0	≤ 1	≤ 1	≤ 1	≤ 1
Ceftriaxone	99.3	0.5	0.2	≤ 1	≤ 1	≤ 1	> 8
Cefuroxime	92.0	1.2	6.8	≤ 0.25	≤ 0.25	≤ 0.25	> 16
Chloramphenicol	97.7		2.3	2	4	≤ 0.12	32
Ciprofloxacin				1	2	≤ 0.06	> 16
Clarithromycin	76.7	2.9	20.4	≤ 0.25	4	≤ 0.25	> 16
Clindamycin	92.0	0.4	7.6	≤ 0.25	≤ 0.25	≤ 0.25	> 8
Dalbavancin				≤ 0.03	≤ 0.03	≤ 0.03	0.12
Daptomycin				≤ 0.06	0.12	≤ 0.06	0.5
Doxycycline	85.3	1.1	13.6	≤ 0.25	2	≤ 0.25	> 16
Ertapenem	98.9	1.0	0.1	≤ 0.06	0.12	≤ 0.06	4
Imipenem	92.7	5.8	1.5	≤ 0.06	≤ 0.06	≤ 0.06	1
Lefamulin	100			0.12	0.12	≤ 0.015	0.25
Levofloxacin	99.2	0.1	0.7	1	1	≤ 0.06	32
Linezolid	100			1	1	≤ 0.12	2
Meropenem	94.2	3.9	1.9	≤ 0.12	≤ 0.12	≤ 0.12	2
Penicillin	81.7	13.7	4.6	≤ 0.03	0.25	≤ 0.03	> 8
Pip-Tazo				≤ 1	≤ 1	≤ 1	16
Tigecycline ^b	99.7		0.3	≤ 0.03	0.06	≤ 0.03	0.25
Trimethoprim Sulfate	84.2	6.0	9.8	≤ 0.12	2	≤ 0.12	> 8
Vancomycin	100			≤ 0.25	≤ 0.25	≤ 0.25	1

Amox-Clav, Amoxicillin-Clavulanate; Pip-Tazo, Piperacillin-Tazobactam; [†] Not all isolates were tested against each antimicrobial agent ^a For ceftobiprole, Health Canada breakpoints applied. ^b For tigecycline, FDA breakpoints applied.
^c susceptible dose-dependent breakpoint.

Conclusions

- Of the 27,293 Gram-positive pathogens obtained, the most common were *S. aureus* (20.7% of all isolates collected [MSSA 16.3% / MRSA 4.4%]), *S. pneumoniae* (5.6%), and *Enterococcus* spp. (*E. faecalis* and *E. faecium*) (4.9%).
- Gram-positive isolates were obtained from blood (48.3%), respiratory (30.2%), urine (5.5%) and wound specimens (16.0%) from patients on wards 38.5% (30.6% medical/7.9% surgical), ER 26.0%, ICU 18.5%, and clinics 17.0%.
- MRSA rates decreased significantly during 2007-2015 from 26.0% to 19.3% (*P*<0.0001), however, it increased significantly through study years 2016-2024 (16.9% to 26.3% *P*<0.0001) [data not shown].
- VRE rates ranged from 1.8% to 20.2%, with marked fluctuation year-to-year (data not shown).
- Susceptibility rates for MRSA were: 100% ceftobiprole and dalbavancin, 99.9% linezolid and vancomycin, 99.8% daptomycin, 98.9% lefamulin, 98.1% tigecycline, 94.5% trimethoprim-sulfamethoxazole, 62.0% clindamycin, 23.5% ciprofloxacin and 20.0% for clarithromycin.

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