

Characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: 17 years of the CANWARD study (2007–23)

Anissa Brahami¹, James A. Karlowsky^{1,2*}, Melissa McCracken³, George R. Golding^{1,3}, Kimberly A. Nichol²,
Melanie R. Baxter¹, Heather J. Adam ^{1,2}, Philippe R. S. Lagacé-Wiens ^{1,2} and George G. Zhanel¹

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Room 543-745 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0J9; ²Clinical Microbiology, Diagnostic Services, Shared Health, MS673-820 Sherbrook Street, Winnipeg, Manitoba, Canada R3A 1R9; ³National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada R3E 3M4

*Corresponding author. E-mail: jkarlowsky@sharedhealthmb.ca

Objectives: This study reviewed the patient demographic parameters, molecular characteristics and *in vitro* antimicrobial susceptibility testing results for MRSA isolates infecting inpatients and outpatients presenting for care to tertiary-care Canadian hospitals between 2007 and 2023.

Methods: DNA sequencing was used to generate *spa* types. Pantone-Valentine leukocidin (PVL) genes were detected by PCR. Broth microdilution antimicrobial susceptibility testing (CLSI M7, 12th edition, 2024) was performed with MICs interpreted by CLSI M100 breakpoints (34th edition, 2024) when available.

Results: In total, 2697 MRSA were identified among 12734 *Staphylococcus aureus* isolates submitted to the CANWARD study between 2007 and 2023. The annual proportion of MRSA decreased significantly from 2007 (26.1%) to 2017 (16.0%) and then increased to 24.2% in 2023 ($P < 0.0001$). From 2007 to 2023, community-associated (CA)-MRSA *spa* types increased from 20.8% to 75.0% ($P < 0.0001$), while hospital-associated (HA)-MRSA decreased from 79.2% to 25.0% ($P < 0.0001$). The predominant MRSA *spa* types identified among all isolates were t002 (36.3%), an HA-MRSA genotype, and t008 (24.5%), a CA-MRSA genotype. PVL was detected in 36.0% of all MRSA isolates (76.3% of CA-MRSA; 2.7% of HA-MRSA). Percent susceptible values for all MRSA isolates were $\geq 99\%$ for ceftobiprole, dalbavancin, daptomycin, linezolid, nitrofurantoin and vancomycin. Notable differences in percent susceptible values were identified for clindamycin (HA-MRSA, 38.9%; CA-MRSA, 86.9%) ($P < 0.0001$) trimethoprim/sulfamethoxazole (HA-MRSA, 91.4%; CA-MRSA, 98.8%) ($P < 0.0001$) and doxycycline (HA-MRSA, 96.6%; CA-MRSA, 98.8%) ($P = 0.0003$).

Conclusions: The changing epidemiology of MRSA in Canadian hospitals warrants continued national surveillance efforts as a resource to support therapeutic guidelines and infection control and prevention programmes.

Introduction

Staphylococcus aureus is a medically important, virulent human pathogen that can cause a multitude of serious infections. Methicillin resistance in *S. aureus* is conferred by penicillin-binding protein-2a (*mecA* gene), a transpeptidase that catalyses cell wall crosslinking in the presence of almost all β -lactam agents with the exception of two newer cephalosporins, ceftobiprole and cef-taroline. Outbreaks of MRSA were initially described in the early 1960s shortly after the introduction of methicillin into clinical use. Since that time, MRSA has spread worldwide and presents infection control and prevention programme challenges and therapeutic issues in both hospital (hospital-associated [HA]-MRSA) and community (community-associated [CA]-MRSA) settings.

HA-MRSA is defined by infection >48 h following hospitalization or within 12 months of exposure to healthcare.¹ HA-MRSA is correlated with severe, invasive infections, multidrug resistance, well-established risk factors, serious morbidity and mortality.¹ CA-MRSA is defined by infection in the absence of healthcare exposure and is most often associated with skin and soft tissue in young, otherwise healthy persons, although invasive disease, including bacteraemia associated with sepsis and necrotizing pneumonia can occur.¹ Many CA-MRSA carry Pantone-Valentine leukocidin (PVL) genes that generate enhanced virulence.² CA-MRSA are generally resistant to fewer non- β -lactam antimicrobial classes than are HA-MRSA.¹

In the current study, we reviewed the patient demographic parameters, molecular characteristics and *in vitro* antimicrobial

susceptibility testing results for MRSA isolates infecting inpatients and outpatients presenting for care to tertiary-care Canadian hospitals over a 17-year period from 2007 to 2023. This study expands upon earlier reports on MRSA from the CANWARD study.^{3,4}

Materials and methods

Bacterial isolates

Between 2007 and 2023, 12 734 *S. aureus* isolates were submitted to the CANWARD study, an annual, ongoing national surveillance study assessing bacterial pathogen prevalence and antimicrobial resistance in Canadian hospitals. CANWARD is comprised of tertiary-care medical centres that are geographically distributed in a population-based fashion including eight of the 10 Canadian provinces. Each centre submitted isolates from inpatients and outpatients attending hospital clinics, emergency rooms, medical/surgical wards or intensive care units. Centres were requested to submit defined numbers of clinically significant isolates (consecutive, one per patient per infection site per year) as defined by their local hospital criteria, from respiratory, urine, wound and blood-stream infections. Centre participation varied slightly from year-to-year as did the numbers of isolates requested by infection source.⁵

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

The *in vitro* activity of ceftaxime and other relevant antimicrobial agents was determined by broth microdilution following CLSI guidelines.⁶ MICs were interpreted as susceptible, intermediate or resistant using breakpoints established by the CLSI⁷ when available. For tigecycline, MIC interpretation was based on the US FDA package insert label (susceptible breakpoint ≤ 0.5 mg/L; <https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>). Ceftobiprole MICs were interpreted using EUCAST breakpoints (susceptible, ≤ 2 mg/L; resistant, >2 mg/L).⁸

MRSA and MSSA isolates were submitted by centres to CANWARD with their methicillin phenotype indicated. Following broth microdilution testing with ceftaxime,⁶ discrepant MRSA and MSSA isolates were sometimes identified. These rare discrepant isolates were re-tested by broth microdilution. In addition, CLSI reference ceftaxime disk diffusion testing⁹ was performed and isolates were assessed for growth on MRSA Select chromogenic media (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) to resolve phenotype discrepancies. Ceftobiprole-resistant isolates and isolates with not susceptible phenotypes for dalbavancin, daptomycin and vancomycin were confirmed by broth microdilution re-testing given their accepted infrequency.

Molecular typing

From 2007 to 2021, all isolates with an MRSA phenotype were confirmed as MRSA by real-time PCR for *mecA* and species-specific *nuc* genes.¹⁰ Simultaneous detection of the PVL-encoding *lukF-PV* and *lukS-PV* genes was also performed as part of this triplex assay.¹⁰ MRSA isolates were then characterized by sequence-based typing of the repeat region of the staphylococcal protein A (*spa*) gene.¹¹ Sequences were analysed and assigned *spa* types according to the Ridom *spa* server (spaServer.ridom.de; Ridom GmbH).¹² In 2022 and 2023, WGS was used to obtain the aforementioned genetic data. Genomic DNA was extracted using Qiagen DNeasy 96 Blood and Tissue Kits (Hilden, Germany). Short-read libraries were created with TruSeq Nano DNA HT sample preparation kits (Illumina, San Diego, CA, USA). Isolates were sequenced using an Illumina NextSeq™ platform. Paired-end, 151 bp indexed reads were generated using the Illumina NextSeq™ platform. FastQC v0.11.9 (<https://github.com/s-andrews/FastQC>) was used to

assess quality control metrics for Illumina reads. Contigs were assembled using Shovill v1.1.0 on an internal Galaxy platform (<https://github.com/tseemann/shovill>). StarAMR v0.9.1¹³ was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24.¹⁴ ABRicate v1.0.1¹⁵ was used to detect PVL using the virulence factor database (VFDB).¹⁶ WGS data can be found on NCBI under BioProject PRJNA1225611.

A high degree of concordance between *spa* types and established Canadian (and USA) epidemic clones has been documented.¹² For the purpose of this study, CA-MRSA and HA-MRSA were defined genotypically based upon their *spa* type and the assignment of that *spa* type to an established Canadian (and USA) epidemic clone.¹² Isolates with *spa* types assigned to PFGE types CMRSA1 (USA600), CMRSA2 (USA100/800), CMRSA3/6, CMRSA4 (USA200), CMRSA5 (USA500), CMRSA8 or CMRSA9 were categorized as HA-MRSA.¹⁷ Isolates with *spa* types identified as PFGE types CMRSA7 (USA400) or CMRSA10 (USA300) were classified as CA-MRSA.¹⁷ All other isolates, such as those with infrequent *spa* types or isolates without an equivalent Canadian epidemic type (such as the USA700, USA1000 and USA1100 strains) were also considered CA-MRSA.

Statistical analysis

Fisher's exact two-tailed test was used to identify statistically significant differences in demographic characteristics, antimicrobial susceptibilities and changes in antimicrobial susceptibility over time for CA-MRSA and HA-MRSA. The Cochran–Armitage test was used to identify statistically significant linear trends in patient demographics, MRSA proportion, *spa* type distribution and PVL status. For all statistical analysis, isolates were defined as either susceptible or non-susceptible to each antimicrobial agent. Isolates with intermediate (where applicable) susceptibility were included as part of the non-susceptible group. Variables included year of study, patient age group (<18 , 18–65, >65 years), biological sex (male, female), patient location (outpatient [emergency room and clinic/office] or inpatient [ICU, medical/surgical ward]) and specimen type (blood, respiratory wound or urine). For CA-MRSA and HA-MRSA and each antimicrobial agent, an initial Cochran–Armitage test was performed to identify organism-antimicrobial agent combinations demonstrating an overall trend (increasing or decreasing) in the proportion of non-susceptible isolates with a *P* value ≤ 0.1 . Identified combinations were then subjected to a second-degree factorial multivariate logistic regression analysis using available demographic variables to isolate the effect of time on antimicrobial susceptibility from changes in demographic variables that may have occurred in the dataset as well as second-degree interactions among the variables. Initially, a stepwise regression model was created including all the variables and second-degree interactions. In order to exclude variables without significant impact on the model, variables were excluded from the final model in such a way to minimize the Bayesian Information Criterion. Finally, a regression model was created including only the variables and interactions with a significant impact on resistance to an antimicrobial. Statistical analysis was performed using JMP pro version 18 software by SAS (Cary, NC, USA). A *P* value of ≤ 0.05 was considered statistically significant in all comparisons.

Results

MRSA in Canadian hospitals between 2007 and 2023

Of the 12 734 *S. aureus* isolates collected during the 17-year study period, 21.2% ($n=2697$) were MRSA. In total, 38.6% ($n=1042$) of all MRSA were isolated from respiratory tract infections, 34.4% ($n=929$) were from bloodstream infections, 24.8% ($n=668$) were from wound infections and 2.2% ($n=58$) were from urinary tract infections. Overall, the annual proportion of *S. aureus* isolates that were MRSA decreased from 26.0%

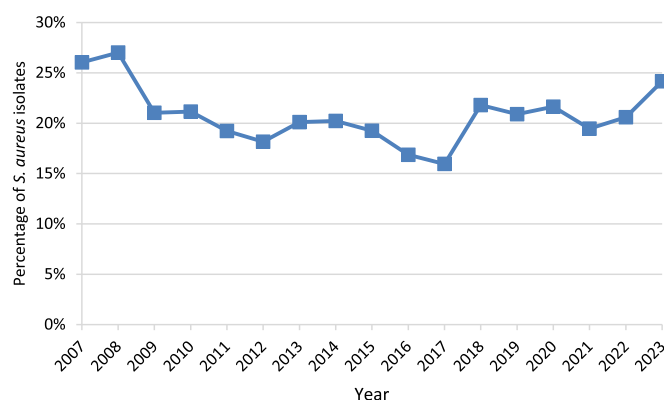


Figure 1. Annual proportion of *S. aureus* isolates identified as MRSA in Canadian hospitals.

(385/1478) in 2007 to 16.0% (115/721) in 2017 before increasing to 24.2% (88/364) in 2023 ($P < 0.0001$) (Figure 1).

Molecular characterization of CA-MRSA and HA-MRSA

Based upon *spa* type, the 2697 MRSA isolates were comprised of 54.8% ($n=1477$) HA-MRSA genotypes and 45.2% ($n=1220$) CA-MRSA genotypes. Figure 2 indicates the annual proportions of MRSA isolates with CA-MRSA and HA-MRSA genotypes. Between 2007 and 2023, CA-MRSA increased significantly from 20.8% (80/385) of MRSA isolates to 75.0% (66/88, $P < 0.0001$). Concurrently, a significant reduction in HA-MRSA from 79.2% (305/385) of MRSA isolates to 25.0% (22/88) was observed during the same period ($P < 0.0001$).

Overall, t008 was the most common CA-MRSA genotype identified in every year from 2007 to 2023; accounting for 24.3% ($n=656$) of all MRSA and 53.8% of all CA-MRSA (Figure 3a). The annual percentage t008 increased significantly over time, rising from 12.0% of MRSA in 2007 to 27.2% of MRSA in 2023 ($P=0.0041$). t128 and t019 were the next most frequently identified *spa* types among CA-MRSA. t128 was identified in every year ($<10\%$ of MRSA in each year) except 2023 and t019 was sporadically identified from 2008 to 2019 but became increasingly common from 2020 to 2023 (as did *spa* types other than t008 and t128). t019 accounted for 12.5% of MRSA in 2023. Other *spa* types, including less common or newly identified *spa* types, increased from 4.4% of MRSA isolates in 2007 to 35.2% in 2023 ($P < 0.0001$).

The most common HA-MRSA genotype identified in every year from 2007 to 2022 was t002 (Figure 3b). It accounted for 36.1% ($n=974$) of all MRSA and 65.9% of all HA-MRSA. The annual percentage of t002 decreased significantly from 53.8% of MRSA in 2007 to 5.7% of MRSA in 2023 ($P=0.026$). In 2023, both t1081 and t002 each accounted for 5.7% of MRSA genotypes. HA-MRSA *spa* types other than t002, t037 and t1081 remained relatively consistent from 2007 to 2023, accounting for approximately 2%–15% of MRSA genotypes per year.

Demographic comparison of CA-MRSA and HA-MRSA

A comparison of the demographic characteristics of patients with infections attributed to CA-MRSA versus HA-MRSA is presented in

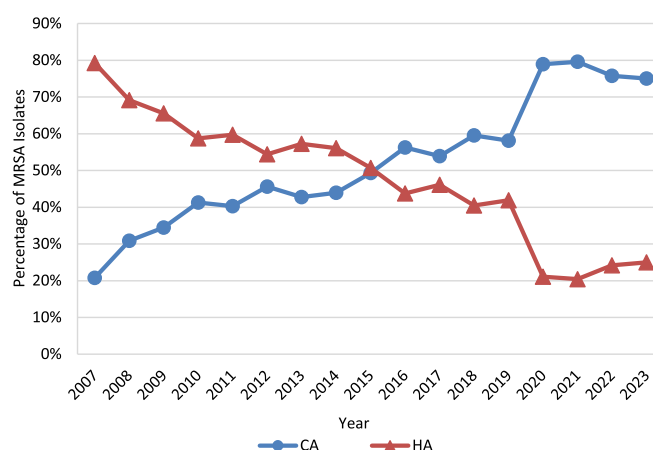


Figure 2. Annual proportion of CA-MRSA and HA-MRSA genotypes in Canadian hospitals.

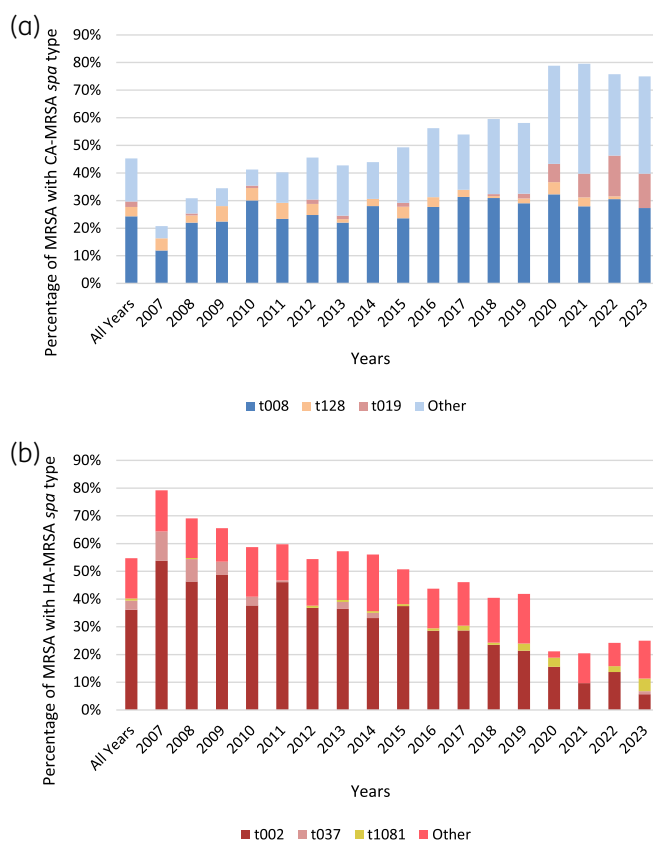


Figure 3. Annual proportion of MRSA isolates with (a) CA-MRSA and (b) HA-MRSA *spa* types.

Table 1. Male patients accounted for 60.4% of CA-MRSA and 58.7% of HA-MRSA. The majority of CA-MRSA infections (68.6%) occurred in patients aged 18–64 years, whereas most of HA-MRSA infections (57.1%) were observed in patients aged ≥ 65 years. The median and mean ages of patients with

Table 1. Demographic characteristics of patients with infections caused by CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2023

Characteristic	CA-MRSA (n=1220)	HA-MRSA (n=1477)	P ^a
Biological sex, n (%) ^b			0.24
male	737 (60.4)	867 (58.7)	
female	482 (39.5)	610 (41.3)	
Mean age, years	44.3	63.9	
Median age, years (range)	46 (1–97)	68 (1–105)	
Age group, years, n (%)			
≤17	152 (12.5)	52 (3.5)	<0.0001
18–64	837 (68.6)	581 (39.3)	<0.0001
≥65	231 (18.9)	844 (57.1)	<0.0001
Region, n (%)			
western Canada	692 (56.7)	447 (30.3)	<0.0001
Ontario	379 (31.1)	504 (34.1)	0.0034
Quebec	80 (6.6)	398 (26.9)	<0.0001
maritimes	69 (5.7)	128 (8.7)	0.0029
Patient location, n (%)			
emergency room	338 (27.7)	214 (14.5)	<0.0001
clinic/office	181 (14.8)	199 (13.5)	0.91
ICU	247 (20.2)	285 (19.3)	0.059
medical/surgical ward	454 (37.2)	779 (52.7)	<0.0001
Infection site, n (%)			
bloodstream	423 (34.7)	506 (34.3)	0.8
respiratory tract	370 (30.3)	672 (45.5)	<0.0001
wound	419 (34.3)	249 (16.9)	<0.0001
urinary tract	8 (0.7)	50 (3.4)	<0.0001

^aP value determined using Fisher’s exact test.

^bBiological sex not available for one isolate.

CA-MRSA and HA-MRSA infection were 46 and 44.3 years, and 68 and 63.9 years, respectively. CA-MRSA were more likely than HA-MRSA to have been isolated in western Canada (56.7% versus 30.3%, $P<0.0001$), whereas HA-MRSA were more frequently isolated than CA-MRSA in Ontario (34.1% versus 31.1%, $P<0.0034$), Quebec (26.9% versus 6.6%, $P<0.0001$) and the maritime provinces (8.7% versus 5.7%, $P=0.0029$).

HA-MRSA (52.7%) and CA-MRSA (37.2%) were both most commonly recovered from patients on medical/surgical wards than other clinical settings. The proportion of CA-MRSA isolates recovered from emergency room patients decreased from 44.7% to 24.2% between 2007 and 2023 ($P=0.0056$). This trend was accompanied by an increase in the proportion of CA-MRSA from patients admitted to medical/surgical wards (23.8% in 2007 to 49.9% in 2023, $P=0.013$).

Bloodstream (34.7%), wound (34.3%) and respiratory tract (30.3%) infections each accounted for approximately one-third of CA-MRSA isolates. The proportion of CA-MRSA from respiratory sources increased from 17.1% in 2007 to 40.1% in 2023 ($P=0.0028$). HA-MRSA was most commonly recovered from respiratory tract (45.5%) and bloodstream (34.2%) infections than other infection sites. The proportion of HA-MRSA from respiratory sources increased significantly from 36.1% in 2007 to 72.7% in

2023 ($P=0.0011$). Concurrently, over the same time period, the proportion of HA-MRSA from bloodstream infections decreased from 45.0% to 18.2% ($P=0.0145$).

PVL genes were detected in 36.0% ($n=970$) of all MRSA isolates. By genotype, 76.3% (931/1220) of CA-MRSA were PVL-positive compared to 2.6% (39/1477) of HA-MRSA (Table 2). Between 2007 and 2023, the PVL-positive rate among HA-MRSA isolates increased from 0.3% to 9.1% ($P=0.005$). In contrast, the proportion of PVL-positive CA-MRSA decreased from 92.5% to 68.2% during the same time period ($P=0.0002$). No significant changes in PVL status were observed among the dominant *spa* types for both CA-MRSA and HA-MRSA (Figure 3). When compared with PVL-negative isolates, PVL-positive strains were more likely to be recovered from patients admitted to a medical/surgical ward ($P<0.0001$) or presenting to an emergency room ($P<0.0001$) (data not shown) than from patients in other locations. Additionally, PVL-positive isolates were more frequently associated with wound infections ($P<0.0001$) than other infection types (data not shown).

Antimicrobial susceptibilities of CA-MRSA and HA-MRSA

Percent susceptible values for all MRSA isolates were $\geq 99\%$ for ceftobiprole, dalbavancin, daptomycin, linezolid, nitrofurantoin and vancomycin (Table 3). The modal vancomycin MIC for all MRSA isolates was 1 mg/L and remained unchanged over time. No vancomycin-resistant isolates were detected, but an intermediate MIC interpretation (4 mg/L) was observed in two isolates, both of which were HA-MRSA, including one with *spa* type t002. One isolate that was not susceptible to dalbavancin (MIC, 0.5 mg/L) was also identified and confirmed; it was a CA-MRSA t019 *spa* type. The antimicrobial susceptibility testing profile for isolates of the emerging CA-MRSA *spa* type t019 ($n=34$) was not significantly different from the profile for t008 isolates, the most common CA-MRSA *spa* type ($n=320$). Ceftobiprole resistance (MIC, 4 mg/L) was confirmed in two isolates of HA-MRSA. Less than 25% of all MRSA isolates were susceptible to clarithromycin, ciprofloxacin, levofloxacin and moxifloxacin. Percent susceptible values for clindamycin (HA-MRSA, 38.9%; CA-MRSA, 86.9%) ($P<0.0001$), doxycycline (HA-MRSA, 96.6%; CA-MRSA, 98.8%) ($P=0.0003$) and trimethoprim/sulfamethoxazole (HA-MRSA, 91.4%; CA-MRSA, 98.8%) ($P<0.0001$) were significantly lower for HA-MRSA than CA-MRSA.

Over time, resistance to clarithromycin, clindamycin and fluoroquinolones declined significantly ($P<0.0001$) (data not shown). Clarithromycin resistance was highest in Quebec, more frequent in isolates from patients aged ≥ 65 years and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Similarly, clindamycin resistance was associated with older adults and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Fluoroquinolone resistance was higher in eastern Canada, predominantly observed in isolates from patients aged ≥ 65 years and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Fluoroquinolone resistance was also elevated in medical/surgical wards compared to intensive care units ($P=0.0005$) and higher in outpatient isolates compared to isolates from hospitalized patients ($P<0.0001$). Additionally, fluoroquinolone resistance was more frequent in urinary and respiratory tract infection isolates than in wound or bloodstream isolates ($P=0.0001$). Resistance to trimethoprim/

Table 2. Annual proportion of PVL-positive or PVL-negative CA-MRSA and HA-MRSA genotypes in Canadian hospitals

PVL gene, n (%)	Study year																Total	P ^a
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	
HA-MRSA																		0.005
positive	1 (0.3)	1 (0.5)	0 (0.0)	4 (3.1)	0 (0.0)	1 (1.5)	9 (9.9)	2 (2.3)	0 (0.0)	3 (6.1)	1 (1.9)	3 (5.5)	5 (10.2)	2 (10.5)	3 (15.8)	2 (8.7)	2 (9.1)	
negative	304 (99.7)	187 (99.5)	152 (100)	127 (96.9)	92 (100)	67 (98.5)	82 (90.1)	86 (97.7)	73 (100)	46 (93.9)	52 (98.1)	52 (94.5)	44 (89.8)	17 (89.5)	16 (84.2)	21 (91.3)	20 (90.9)	1438 (97.3)
CA-MRSA																		0.0002
positive	74 (92.5)	66 (78.6)	68 (85.0)	78 (84.8)	52 (83.9)	39 (68.4)	46 (67.6)	54 (78.3)	50 (70.4)	42 (66.7)	43 (69.4)	61 (75.3)	47 (69.1)	59 (83.0)	52 (70.3)	55 (76.4)	45 (68.2)	931 (76.3)
negative	6 (7.5)	18 (21.4)	12 (15.0)	14 (15.2)	10 (16.1)	18 (31.6)	22 (32.4)	15 (21.7)	21 (29.6)	21 (33.3)	19 (30.6)	20 (24.7)	21 (30.9)	12 (17)	22 (29.7)	17 (23.6)	21 (31.8)	289 (23.7)

^aP value determined using Fisher's exact test.

sulfamethoxazole was higher in western Canada than other regions of Canada ($P<0.0001$); no other significant trends were identified for trimethoprim/sulfamethoxazole.

Discussion

MRSA is a common HA pathogen² and is also an important pathogen in the community. Over time, CA-MRSA has accounted for an increasing proportion of MRSA isolates in hospitals and long-term care facilities in Canada¹⁸ and worldwide.^{19–22} In the current study, the prevalence of CA-MRSA among all MRSA in Canada increased from 20.8% of isolates in 2007 to 75.0% in 2023 (Figure 2). This rise coincided with both a stepwise decrease in the annual proportion of *S. aureus* isolates that were MRSA from 26.0% in 2007 to 16.0% in 2017 followed by a stepwise increase to 24.2% in 2023 (Figure 1). The observed increase in MRSA prevalence beginning in 2018 is concerning given previous decreases from 2007 to 2017. Explanations for this observation are likely multifactorial but may include reduced healthcare spending on hospital infection control and prevention and antimicrobial stewardship programmes, the emergence of other priorities (e.g. SARS-CoV-2 pandemic) as well as the admission of sicker patients to hospital, longer hospital and ICU stays, greater numbers of invasive clinical procedures, an aging patient population and significantly higher MRSA colonization rates among the elderly than among the general population.^{2,22}

The current study depicts the emergence of new or less common *spa* types among CA-MRSA (identified as the Other category in Figure 3) over time in Canada. Notably, t019, which was not detected in 2007, became the second most dominant *spa* type by 2023, reaching a prevalence of 12.5%. The Other category for CA-MRSA increased from 4.4% in 2007 to 35.2% in 2023. In comparison, the Other category for HA-MRSA did not demonstrate an increased prevalence over time; however, it did increase as a percentage of HA-MRSA from 2007 to 2023 as the contribution of t002 decreased. The majority of CA-MRSA in Canada continue to harbour PVL which is known to contribute to the morbidity and mortality associated with CA-MRSA infection.²³ Interestingly, we also observed that the PVL-positive rate among HA-MRSA isolates increased from 0.3% to 9.1% ($P=0.005$) between 2007 and 2023 as the proportion of PVL-positive CA-MRSA decreased from 92.5% to 68.2% ($P=0.0002$) suggesting that a strict separation between CA-MRSA and HA-MRSA based upon this virulence factor may no longer be possible as genetic exchange and clonal adaptation blur their boundaries and that epidemiological criteria previously used to differentiate them may also be less useful.²⁴ These observations highlight the need for a more nuanced approach to studying and managing MRSA infections, as emphasized in a recent study that underscores the evolving epidemiology of MRSA.²⁵

CA-MRSA is more susceptible to many non-β-lactam antimicrobial agents (clarithromycin, clindamycin, doxycycline, tigecycline, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin and moxifloxacin) than are HA-MRSA in Canada (Table 3). In the current study, we also observed percent susceptible values for clarithromycin, clindamycin and fluoroquinolones decreased significantly over time from 2007 to 2023. This finding is in concordance with a report from the SENTRY surveillance program that collected consecutive, clinically significant isolates of

Table 3. Antimicrobial susceptibility of CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2023

Organism (no. tested)/antimicrobial agent	MIC (mg/L)			MIC interpretation		
	MIC ₅₀	MIC ₉₀	MIC range	% Susceptible	% Intermediate	% Resistant
MRSA (n = 2697)						
ceftobiprole	≤1	2	≤1–4	99.9	–	0.1
ciprofloxacin	>16	>16	0.25 to >16	22.2	0.5	77.3
clarithromycin	>16	>16	0.25 to >16	18.7	0.6	80.7
clindamycin	0.25	>8	0.25 to >8	60.6	0.1	39.3
dalbavancin	0.06	0.06	≤0.03–0.5	99.9	–	–
daptomycin	0.25	0.5	0.06–4	99.9	–	–
doxycycline	≤0.12	1	≤0.12–16	97.8	1.0	1.2
levofloxacin	>32	>32	0.12 to >32	14.1	–	85.9
linezolid	2	2	≤0.12–16	100	–	–
moxifloxacin	8	>16	≤0.06 to >16	19.1	4	76.9
nitrofurantoin	16	16	8–128	99.7	0.1	0.2
tigecycline	0.25	0.5	0.03–2	98.2	–	–
trimethoprim/sulfamethoxazole	≤0.12	0.25	≤0.12 to >8	94.7	–	5.3
vancomycin	1	1	≤0.12–4	99.9	0.1	0
HA-MRSA (n = 1477)						
ceftobiprole	≤1	2	≤1–4	99.9	–	0.1
ciprofloxacin	>16	>16	0.25 to >16	8.6	0.3	91.1
clarithromycin	>16	>16	0.25 to >16	8.2	0.5	91.3
clindamycin	>8	>8	0.25 to >8	38.9	–	61.1
dalbavancin	0.06	0.06	≤0.03–0.25	100	–	–
daptomycin	0.25	0.5	0.06–4	99.9	–	–
doxycycline	≤0.12	2	≤0.12–16	96.6	1.0	2.4
levofloxacin	>32	>32	0.12 to >32	2.9	–	97.1
linezolid	2	4	≤0.12–4	100	–	–
moxifloxacin	8	>16	≤0.06 to >16	6.7	0.5	92.8
nitrofurantoin	16	16	8–128	99.5	0.2	0.3
tigecycline	0.25	0.5	0.03–2	97.5	–	–
trimethoprim/sulfamethoxazole	≤0.12	0.5	≤0.12 to >8	91.4	–	8.6
vancomycin	1	1	≤0.12–4	99.9	0.1	0
CA-MRSA (n = 1220)						
ceftobiprole	≤1	2	≤1–2	100	–	0
ciprofloxacin	16	>16	0.25 to >16	38.6	0.8	60.6
clarithromycin	>16	>16	0.25 to >16	31.4	0.7	67.9
clindamycin	0.25	>8	0.25 to >8	86.9	0.1	13.0
dalbavancin	0.06	0.12	≤0.03–0.5	99.9	–	–
daptomycin	0.25	0.5	0.12–2	99.8	–	–
doxycycline	≤0.12	0.5	≤0.12–16	98.8	1.0	0.2
levofloxacin	4	8	0.12 to 32	63.1	–	36.9
linezolid	2	2	0.5 to >16	99.9	–	0.1
moxifloxacin	2	2	≤0.06 to >16	38.1	9.2	52.7
nitrofurantoin	16	16	8–128	99.9	–	0.1
tigecycline	0.25	0.5	0.03–2	99.1	–	–
trimethoprim/sulfamethoxazole	≤0.12	≤0.12	≤0.12 to >8	98.8	–	1.2
vancomycin	1	1	0.5–2	100	0	0

bacterial pathogens (including *S. aureus*) in North America, Europe, Latin America and the Asia-Pacific region from 2007 to 2016.²⁶ The SENTRY surveillance program also reported lower percent susceptible values for HA-MRSA compared with CA-MRSA for clindamycin, doxycycline and trimethoprim/sulfamethoxazole,²⁶ similar to our study (Table 3).

Vancomycin remains the most common parenteral treatment for MRSA infections.²⁷ In the current study, its activity remained stable over time and only two isolates with an intermediate MIC interpretation (both MICs 4 mg/L) were identified from 2007 to 2023 (Table 3). The modal vancomycin MICs for all MRSA isolates were 1 mg/L in all study years of the CANWARD

study (2007–23) and we did not observe vancomycin MIC creep, confirming the findings of earlier investigators.²⁸ In our study, we also identified and confirmed one isolate (CA-MRSA) that were not susceptible to dalbavancin (isolate MIC of 0.5 mg/L and corresponding vancomycin MIC of 1 mg/L) and two isolates (both HA-MRSA) that were not susceptible to ceftobiprole (both isolate MICs were 4 mg/L). In Canada, dalbavancin is primarily used alone to treat acute bacterial skin and skin structure infections²⁹ and ceftobiprole to treat severe MRSA infections particularly in the case of failing initial antimicrobial therapy.³⁰ Ceftobiprole's utility is further enhanced by the addition of other agents such as vancomycin and daptomycin for synergistic activity for persistent infections.³⁰ The need for judicious use and targeted application of newer agents like dalbavancin and ceftobiprole is crucial to preserve the effectiveness of these last-resort therapies.

Limitations of the CANWARD study relevant to reporting MRSA data have been previously described and remain relevant.⁴ Importantly, we cannot guarantee that the present study accurately reflects the true proportion of community- and healthcare-associated MRSA cases in Canadian hospitals. In addition, there is growing evidence that traditional molecular definitions can no longer consistently distinguish between CA-MRSA and HA-MRSA strains.³¹ Our data may also be confounded by minor inconsistencies in the annual number of contributing sites and changes in the number of isolates, and their associated specimen sources, collected each year.⁵ Despite this variability, our data describe MRSA as a proportion of *S. aureus*, and as such we believe that the observed trends represent true changes in the burden of MRSA in Canada as well as the distribution of MRSA genotypes.

In conclusion, the changing epidemiology of MRSA in Canadian hospitals warrants continued national surveillance efforts, including genomic surveillance, as a resource to support therapeutic guidelines and infection control and prevention programmes.

Acknowledgements

We would like to thank the participating centres, investigators and laboratory site staff from the CANWARD sites for their continued support and cooperation. We would also like to thank the Public Health Agency of Canada-National Microbiology Laboratory (PHAC-NML) for their support of this project. CANWARD sites are detailed in Zhanel *et al.*³² CANWARD data can also be found at www.can-r.ca, the official website of the Canadian Antimicrobial Resistance Alliance (CARA).

Funding

The CANWARD Study was supported in part by the University of Manitoba, Shared Health, the National Microbiology Laboratory, Avir, Iterum, Paladin Labs, Pfizer, Sandoz, Shionogi, Sunovion, Venatorx, Verity and Xediton. This study was also supported in part by a research grant from Investigator-Initiated Studies Program of Merck Canada Inc. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Canada Inc.

Transparency declarations

G.G.Z. has received research grants from Avir Pharma Inc., Iterum Therapeutics, Merck Canada Inc., Paladin Labs, Pfizer, Sandoz Canada,

Shionogi Inc., Sunovion Pharmaceuticals Canada Inc., Venatorx Pharmaceuticals Inc., Verity Pharmaceuticals and Xediton Pharmaceuticals Inc. All other authors have no declarations.

Disclaimer

The opinions expressed in this paper are those of the authors, and do not necessarily represent those of Avir Pharma Inc., Iterum Therapeutics, Merck Canada Inc., Paladin Labs, Pfizer, Sandoz Canada, Shionogi Inc., Sunovion Pharmaceuticals Canada Inc., Venatorx Pharmaceuticals Inc., Verity Pharmaceuticals and Xediton Pharmaceuticals Inc.

References

- 1 Anderson DJ, Spelman D, Hall KK. *Methicillin-resistant Staphylococcus aureus (MRSA) in Adults: Epidemiology*. UpToDate, 2024. <https://www.wolterskluwer.com/en/solutions/uptodate>.
- 2 Damji S, Perrott J, Shajari S *et al.* Pattern of acquisition of hospital-associated pathogens in the ICU of an academic tertiary care hospital. *J Assoc Med Microbiol Infect Dis Can* 2022; **7**: 108–16. <https://doi.org/10.3138/jammi-2021-0025>
- 3 Nichol KA, Adam HJ, Roscoe DL *et al.* Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Canada. *J Antimicrob Chemother* 2013; **68**: i47–55. <https://doi.org/10.1093/jac/dkt026>
- 4 Nichol KA, Adam HJ, Golding GR *et al.* Characterization of MRSA in Canada from 2007 to 2016. *J Antimicrob Chemother* 2019; **74**: iv55–63. <https://doi.org/10.1093/jac/dkz288>
- 5 Zhanel GG, Adam HJ, Baxter M *et al.* Antimicrobial susceptibility of 42,936 pathogens from Canadian hospitals: 10-year results of the CANWARD 2007–16 study. *J Antimicrob Chemother* 2018; **74**: iv5–21. <https://doi.org/10.1093/jac/dkz283>
- 6 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Twelfth Edition: M07*. 2024.
- 7 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-Fourth Edition: M100*. 2024.
- 8 EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 15.0. 2025. <http://eucastrg.org>.
- 9 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests—Fourteenth Edition: M02*. 2024.
- 10 McDonald RR, Antonishyn NA, Hansen T *et al.* Development of a triplex real-time PCR assay for detection of Panton-Valentine leukocidin toxin genes in clinical isolates of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; **43**: 6147–9. <https://doi.org/10.1128/JCM.43.12.6147-6149.2005>
- 11 Harmsen D, Claus H, Witte W *et al.* Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 2003; **41**: 5442–8. <https://doi.org/10.1128/JCM.41.12.5442-5448.2003>
- 12 Golding G, Campbell J, Spreitzer D *et al.* A preliminary guideline for the assignment of methicillin-resistant *Staphylococcus aureus* to a Canadian pulsed-field gel electrophoresis epidemic type using spa typing. *Can J Infect Dis Med Microbiol* 2008; **19**: 273–81. <https://doi.org/10.1155/2008/754249>
- 13 Bharat A, Petkau A, Avery BP *et al.* Correlation between phenotypic and *in silico* detection of antimicrobial resistance in *Salmonella enterica* in Canada using Staramr. *Microorganisms* 2022; **10**: 292. <https://doi.org/10.3390/microorganisms10020292>
- 14 Bortolaia V, Kaas RS, Ruppe E *et al.* ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500. <https://doi.org/10.1093/jac/dkaa345>

- 15 Seemann T. ABRicate: Mass Screening of Contigs for Antimicrobial Resistance or Virulence Genes, v1.0.1. 2016. <https://bioweb.pasteur.fr/packages/pack@abricate@1.0.1>.
- 16 Chen LH, Zheng DD, Liu B et al. VFDB 2016: hierarchical and refined dataset for big data analysis-10years on. *Nucleic Acids Res* 2016; **44**: D694–7. <https://doi.org/10.1093/nar/gkv1239>
- 17 Christianson S, Golding GR, Campbell J et al. Comparative genomics of Canadian epidemic lineages of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2007; **45**: 1904–11. <https://doi.org/10.1128/JCM.02500-06>
- 18 Canadian Nosocomial Infection Surveillance Program. Healthcare-associated infections and antimicrobial resistance in Canadian acute care hospitals, 2018–2022. *Can Commun Dis Rep* 2024; **50**: 179. <https://doi.org/10.14745/ccdr.v50i06a02>
- 19 Postiglione U, Batisti Biffignandi G, Corbella M et al. Combining genome surveillance and metadata to characterize the diversity of *Staphylococcus aureus* circulating in an Italian hospital over a 9-year period. *Microbiol Spect* 2023; **11**: e0101023. <https://doi.org/10.1128/spectrum.01010-23>
- 20 Zhong J, Wei M, Yang C et al. Molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* and clinical characteristics of different sites of infection. *Infect Drug Resist* 2023; **16**: 1485–97. <https://doi.org/10.2147/IDR.S401998>
- 21 Kishita M, Matsumura Y, Yamamoto M et al. Increase in the frequency of community-acquired methicillin-resistant *Staphylococcus aureus* clones among inpatients of acute care hospitals in the Kyoto and Shiga regions, Japan. *J Infect Chemother* 2023; **29**: 458–63. <https://doi.org/10.1016/j.jiac.2023.01.013>
- 22 Bloomfield LE, Coombs G, Armstrong P. Community-associated methicillin-resistant *Staphylococcus aureus* in the Kimberley region of Western Australia, epidemiology and burden on hospitals. *Epidemiol Infect* 2024; **152**: e147. <https://doi.org/10.1017/S0950268824001201>
- 23 Hasanpour AH, Sepidarkish M, Mollalo A et al. The global prevalence of methicillin-resistant *Staphylococcus aureus* colonization in residents of elderly care centers: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2023; **12**: 4. <https://doi.org/10.1186/s13756-023-01210-6>
- 24 Jin Y, Zhou W, Ge Q et al. Epidemiology and clinical features of skin and soft tissue infections caused by PVL-positive and PVL-negative methicillin-resistant *Staphylococcus aureus* isolates in inpatients in China: a single-center retrospective 7-year study. *Emerg Microb Infect* 2024; **13**: 2316809. <https://doi.org/10.1080/22221751.2024.2316809>
- 25 Feil EJ, Cooper JE, Grundmann H et al. How clonal is *Staphylococcus aureus*? *J Bacteriol* 2003; **185**: 3307–16. <https://doi.org/10.1128/JB.185.11.3307-3316.2003>
- 26 Junnila J, Hirvijoja T, Rintala E et al. Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in a low endemicity area—new challenges for MRSA control. *Eur J Clin Microbiol Infect Dis* 2020; **39**: 2299–307. <https://doi.org/10.1007/s10096-020-03824-9>
- 27 Diekema DJ, Pfaller MA, Shortridge D et al. Twenty-year trends in antimicrobial susceptibilities among *Staphylococcus aureus* from the SENTRY antimicrobial surveillance program. *Open Forum Infect Dis* 2019; **6**: S47–53. <https://doi.org/10.1093/ofid/ofy270>
- 28 Rodvold KA, McConeghy KW. Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. *Clin Infect Dis* 2014; **58**: S20–7. <https://doi.org/10.1093/cid/cit614>
- 29 Diaz R, Afreixo V, Ramalheira E et al. Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis. *Clin Microbiol Infect* 2018; **24**: 97–104. <https://doi.org/10.1016/j.cmi.2017.06.017>
- 30 Zhanel GG, Silverman M, Malhorta J et al. Real-life experience with IV dalbavancin in Canada; results from the CLEAR (Canadian LEadership on Antimicrobial Real-life usage) registry. *J Glob Antimicrob Resist* 2024; **38**: 154–7. <https://doi.org/10.1016/j.jgar.2024.06.002>
- 31 Zhanel GG, Kosar J, Baxter M et al. How is ceftobiprole used in Canada: the CLEAR study final results. *Expert Rev Anti Infect Ther* 2024; **22**: 681–8. <https://doi.org/10.1080/14787210.2024.2374280>
- 32 Sader H, Mendes RE, Jones RN et al. Antimicrobial susceptibility patterns of community- and hospital-acquired methicillin-resistant *Staphylococcus aureus* from United States hospitals: results from the AWARE ceftaroline surveillance program (2012–2014). *Diagn Microbiol Infect Dis* 2016; **86**: 76–9. <https://doi.org/10.1016/j.diagmicrobio.2016.06.017>
- 33 Zhanel GG, Adam HJ. Seventeen years of the CANWARD study (2007–23). *J Antimicrob Chemother* 2025; **80**(Suppl 2): ii2–4. <https://doi.org/10.1093/jac/dkaf216>