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Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Canada

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Objectives: To compare the demographics, antimicrobial susceptibilities and molecular epidemiology of community-associated (CA) and healthcare-associated (HA) methicillin-resistant *Staphylococcus aureus* (MRSA) in Canada.

Methods: Between 2007 and 2011, 1266 MRSA were collected from inpatients and outpatients attending tertiary-care medical centres across Canada. Susceptibility testing was performed using broth microdilution and isolates were characterized by *spa* typing and PCR to detect the Panton–Valentine leucocidin (PVL) gene. Detection of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) was performed using the Etest macromethod and confirmed by population analysis profiling.

Results: The annual proportion of *S. aureus* that were methicillin resistant decreased from 26.1% in 2007 to 19.3% in 2011 ($P=0.0002$). Of 1266 MRSA isolated, 366 (28.9%) were CA-MRSA genotypes and 868 (68.6%) were HA-MRSA genotypes. The proportion of MRSA represented by CA-MRSA genotypes increased from 19.7% to 36.4% between 2007 and 2011 ($P<0.0001$). CMRSA10 (USA300) was the predominant CA-MRSA genotype (22.1%); the most common HA-MRSA genotype was CMRSA2 (USA100/800) (58.1%). PVL was detected in 328/366 (89.6%) of CA-MRSA genotypes and 6/868 (0.7%) of HA-MRSA genotypes. The hVISA phenotype was detected in 7/27 (25.9%) of MRSA with a vancomycin MIC of 2 mg/L.

Conclusions: The most frequent CA-MRSA genotype was CMRSA10 (USA300), while CMRSA2 (USA100/800) was the predominant HA-MRSA genotype. Despite a decrease in the numbers of MRSA, the proportion of CMRSA10 (USA300) CA-MRSA has risen significantly between 2007 and 2011 in Canada.

Keywords: MRSA, surveillance, epidemiology

Introduction

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) account for an increasing proportion of MRSA isolates in hospitals and long-term care facilities across North America.[1,2] The prevalence and epidemiology of these strains often varies from one geographical region to another.[3] In Canada, >20% of MRSA infections in the healthcare setting can be attributed to CA strains, with a single epidemic type as the predominant cause of most CA-MRSA infections.[4–6]

While skin and soft tissue infections are the most common infections caused by CA-MRSA, invasive disease such as sepsis and necrotizing pneumonia can occur.[7] The individuals most often affected by CA-MRSA typically lack established risk factors for the acquisition of MRSA.[8] CA-MRSA differ from healthcare-associated MRSA (HA-MRSA) in that they are generally more susceptible to a variety of non-β-lactam antimicrobial agents.[8] In addition, the majority of CA-MRSA strains harbour virulence determinants such as the Panton–Valentine leucocidin (PVL) as well as other toxins that may contribute to the increasing morbidity and mortality associated with CA-MRSA infections.[9] Of particular concern is the emergence of isolates with reduced susceptibility or heterogeneous resistance to vancomycin, an important antimicrobial for the empirical treatment of severe MRSA infections.[10] The purpose of the present study was to determine the proportion and distribution of MRSA genotypes in Canada

and to compare the clinical and molecular epidemiology of CA-MRSA and HA-MRSA in Canada from 2007 to 2011.

Materials and methods

Bacterial isolates

Between 2007 and 2011, a total of 5443 *S. aureus* isolates were submitted to the CANWARD study, a national surveillance study assessing pathogen prevalence and antimicrobial resistance in Canadian hospitals. Details of the study and collection criteria have been described previously.[11] Briefly, clinically significant isolates from respiratory, urine, wound and bloodstream infections were collected from tertiary-care medical centres that were geographically distributed in a population-based fashion. Screening for methicillin resistance was performed using the CLSI-approved disc diffusion method with cefoxitin,[12] as well as by growth on MRSA Select chromogenic media (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada).

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

Antimicrobial susceptibility testing

In vitro susceptibility testing against selected antimicrobials was performed using broth microdilution according to CLSI guidelines.[12,13] MICs were interpreted using (2011) breakpoints established by the CLSI.[12] For telavancin and tigecycline, MIC interpretations were based on FDA package insert labels (telavancin susceptible breakpoint $\leq$1 mg/L, Vibativ package insert, Astellas Pharma US Inc., Northbrook, IL, USA; tigecycline susceptible breakpoint $\leq$0.5 mg/L, Tygacil package insert, Pfizer Inc., New York, NY, USA).

Molecular typing

The *lukF*-PV and *lukS*-PV genes encoding the PVL toxin were detected as part of a triplex real-time PCR assay that also targeted the *nuc* and *mecA* genes for confirmation of MRSA identification.[14] Isolates were characterized by sequence-based typing of the staphylococcal protein A (*spa*) gene as previously described[15] and Canadian epidemic PFGE strain types were inferred from the observed *spa* type. A high degree of concordance between *spa* types and Canadian epidemic clones has been documented,[15] demonstrating the suitability of *spa* typing for the assignment of PFGE types. For the purpose of this study, CA-MRSA and HA-MRSA were defined genotypically on the basis of known epidemic types. Isolates designated as PFGE types CMRSA7 (resembling USA400) or CMRSA10 (resembling USA300) following *spa* typing were labelled as CA-MRSA, while isolates designated as PFGE types CMRSA1 (resembling USA600), CMRSA2 (resembling USA100/800), CMRSA4 (resembling USA200), CMRSA5 (resembling USA500), CMRSA3/6, CMRSA8 or CMRSA9 were labelled as HA-MRSA.[16] Isolates that were not assigned an epidemic type by *spa* typing were labelled as unique (non-CMRSA1–10).

Detection of heterogeneous vancomycin-intermediate *S. aureus* (hVISA)

All MRSA isolates with a vancomycin MIC of 2 mg/L (n=27) were screened for the presence of the hVISA phenotype using the Etest macromethod in accordance with the manufacturer's instructions (AB Biodisk, Solna, Sweden). A randomly selected subset (20%) of MRSA with vancomycin MICs of 1 mg/L (n=230) and 0.5 mg/L (n=31) were included for comparison. MRSA identified as hVISA using the Etest macromethod were further evaluated by population analysis profiling using the area under the curve method (PAP-AUC).[17] *S. aureus* reference strains Mu3 (ATCC 700698, hVISA), Mu50 (ATCC 700699, VISA) and ATCC 29213 (vancomycin-susceptible *S. aureus*) were included as controls.

Statistical analysis

Differences in the proportions among categorical variables were assessed using Fisher's exact test or the χ^2 test, as appropriate. Multivariate logistic regression analysis was performed to determine the impact of study year, patient demographics (including age, gender, hospital location and specimen type) and MRSA genotype on antimicrobial resistance (defined as resistant and intermediate). A second-degree factorial analysis was performed to account for the effect of interaction variables. Stepwise regression analysis was used to exclude insignificant variables and second-degree interactions in such a way that the model had the lowest Akaike information criterion. Only the remaining variables were included in the multivariate logistic regression analysis. A P value of $\leq$0.05 was considered to be statistically significant. All analyses were performed using JMP® software version 10 (SAS Institute Inc., Cary, NC, USA).

Results

Demographic comparison of CA-MRSA and HA-MRSA genotypes

Among all *S. aureus*, 23.3% (1266/5443) were identified as MRSA (385 in 2007, 272 in 2008, 232 in 2009, 223 in 2010 and 154 in 2011) (Table 1). As determined by *spa* typing, 28.9% (366/1266) of MRSA were classified as CA-MRSA, while 68.6% (868/1266) were classified as HA-MRSA. The remainder (32/1266, 2.5%) could not be assigned to one of the 10 characterized Canadian epidemic PFGE types (CMRSA1–10) by *spa* type alone and were therefore classified as unique (data not shown). The annual proportion of *S. aureus* that were methicillin resistant decreased from 26.1% (385/1475) in 2007 to 19.3% (154/799) in 2011 (P=0.0002). Despite a decrease in the annual numbers of MRSA, the proportion of CA-MRSA increased significantly from 19.7% (76/385) in 2007 to 36.4% (56/154) in 2011 (P<0.0001). HA-MRSA decreased from 79.2% (305/385) to 59.7% (92/154) during this same period (P<0.0001). In both cases, there was relatively little change in the rates from 2010 to 2011. The basic demographic and clinical characteristics of the patients with CA-MRSA and HA-MRSA infections are summarized in Table 2. Overall, 58.5% (741/1266) of MRSA were isolated from male patients, with no significant difference in the gender distribution between HA-MRSA and CA-MRSA genotypes. The median patient age for CA-MRSA was 43 years and was significantly lower than for HA-MRSA at 68 years (P<0.0001). Although MRSA were identified in all geographical regions of the country, CA-MRSA were more likely than HA-MRSA to have been isolated in western Canada (60.1%) and from patients presenting to hospital emergency rooms (38.3%). The majority of HA-MRSA genotypes were recovered from patients on medical/surgical wards (52.2%). Common sites of HA-MRSA infection included the bloodstream (40.3%) and respiratory tract (38.1%). CA-MRSA genotypes were isolated most often from wound/intravenous (IV) sites (44.0%) followed by bloodstream infections (35.8%).

Table 1. Molecular characterization of CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2011

Characteristic, *n* (%)	Study year					Total	*P* value[a]
	2007	2008	2009	2010	2011		
All MRSA (% of all *S. aureus*)	385 (26.1)	272 (27.0)	232 (21.0)	223 (21.2)	154 (19.3)	1266 (23.3)	0.0002
HA-MRSA genotypes (% of all MRSA)	305 (79.2)	188 (69.1)	152 (65.5)	131 (58.7)	92 (59.7)	868 (68.6)	<0.0001
CMRSA1 (USA600)	9 (2.3)	3 (1.1)	0 (0.0)	4 (1.8)	1 (0.6)	17 (1.3)	0.3
CMRSA2 (USA100/800)	250 (64.9)	153 (56.3)	136 (58.6)	111 (49.8)	86 (55.8)	736 (58.1)	0.06
CMRSA3/6	41 (10.6)	24 (8.8)	11 (4.7)	7 (3.1)	1 (0.6)	84 (6.6)	<0.0001
CMRSA4 (USA200)	0 (0.0)	1 (0.4)	0 (0.0)	2 (0.9)	0 (0.0)	3 (0.2)	1.0
CMRSA5 (USA500)	4 (1.0)	4 (1.5)	0 (0.0)	3 (1.3)	2 (1.3)	13 (1.0)	1.0
CMRSA8	0 (0.0)	2 (0.7)	4 (1.7)	4 (1.8)	2 (1.3)	12 (0.9)	0.08
CMRSA9	1 (0.3)	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	3 (0.2)	1.0
CA-MRSA genotypes (% of all MRSA)	76 (19.7)	75 (27.6)	74 (31.9)	85 (38.1)	56 (36.4)	366 (28.9)	<0.0001
CMRSA7 (USA400)	25 (6.5)	15 (5.5)	19 (8.2)	15 (6.7)	12 (7.8)	86 (6.8)	0.58
CMRSA10 (USA300)	51 (13.2)	60 (22.1)	55 (23.7)	70 (31.4)	44 (28.6)	280 (22.1)	<0.0001
PVL gene							
% of HA-MRSA							1.0
positive	1 (0.3)	1 (0.5)	0 (0.0)	4 (3.1)	0 (0.0)	6 (0.7)	
negative	304 (99.7)	187 (99.5)	152 (100)	127 (96.9)	92 (100)	862 (99.3)	
% of CA-MRSA							0.3223
positive	72 (94.7)	64 (85.3)	66 (89.2)	76 (89.4)	50 (89.3)	328 (89.6)	
negative	4 (5.3)	11 (14.7)	8 (10.8)	9 (10.6)	6 (10.7)	38 (10.4)	

[a]*P* value determined by Fisher's exact test comparing 2007 data with 2011 data.

Molecular characterization of CA-MRSA and HA-MRSA genotypes

Table 1 shows the distribution of HA-MRSA and CA-MRSA PFGE types by study year. The predominant epidemic type overall was CMRSA2 (USA100/800), accounting for 58.1% of all MRSA and 84.8% of HA-MRSA genotypes. Other HA-MRSA-associated PFGE types accounted for a further 10.4% of all MRSA and included epidemic types CMRSA1 (USA600) (1.3%), CMRSA3/6 (6.6%), CMRSA4 (USA200) (0.2%), CMRSA5 (USA500) (1.0%), CMRSA8 (0.9%) and CMRSA9 (0.2%). The only significant change among HA-MRSA occurred with CMRSA3/6 isolates, which decreased over time from 10.6% in 2007 to 0.6% in 2011 ($P<0.0001$). CA-MRSA genotypes CMRSA7 (USA400) and CMRSA10 (USA300) represented 6.8% and 22.1% of MRSA, respectively. CMRSA10 (USA300), which accounted for 76.5% of all CA-MRSA isolates, was the second most commonly identified epidemic type overall. Between 2007 and 2011, the proportion of CMRSA10 (USA300) isolates increased significantly from 13.2% to 28.6% ($P<0.0001$). Although the majority of CMRSA10 (USA300) (203/280, 72.5%) and CMRSA7 (USA400) (57/86, 66.3%) infections occurred in patients between the ages of 18 and 64 years, CMRSA10 (USA300) were more likely to have been isolated from adults over the age of 64 years [16.1% (45/280) versus 7.0% (6/86), $P=0.03$] and less likely than CMRSA7 (USA400) to have been recovered from individuals <18 years of age [11.4% (32/280) versus 26.7% (23/86), $P=0.001$]. While CMRSA10 (USA300) isolates were recovered from across Canada, CMRSA7 (USA400) genotypes were predominantly (82/86, 95.3%) isolated from centres in the western provinces ($P<0.0001$).

The PVL gene was detected in 89.6% of CA-MRSA isolates compared with 0.7% of HA-MRSA (Table 1). Most (33/38, 86.8%) PVL-negative CA-MRSA belonged to the CMRSA7 (USA400) genotype (data not shown). No significant change in the proportion of PVL-positive and PVL-negative isolates within each genotypic group (CA-MRSA versus HA-MRSA) was observed over the course of the study.

Antimicrobial susceptibilities of CA-MRSA and HA-MRSA

Antimicrobial susceptibility results for CA-MRSA and HA-MRSA genotypes are presented in Table 3. CA-MRSA were associated with lower rates of resistance to clarithromycin, clindamycin, fluoroquinolones and trimethoprim/sulfamethoxazole than HA-MRSA ($P<0.0001$ in all comparisons). Interestingly, a comparison of the susceptibility patterns for CA-MRSA genotypes CMRSA7 (USA400) and CMRSA10 (USA300) showed that isolates with a CMRSA10 (USA300) genotype had significantly higher rates of resistance than their CMRSA7 (USA400) counterparts to clarithromycin (92.8% versus 17.4%, $P<0.0001$), clindamycin (15.5% versus 4.7%, $P=0.009$) and the fluoroquinolones (74.6%–83.9% versus 3.4%–5.8%, $P<0.0001$).

Among all MRSA, fluoroquinolone (specifically ciprofloxacin) resistance decreased over time ($P=0.01$) (data not shown). Resistance to this agent occurred least often in MRSA isolated from patients under the age of 18 years ($P<0.001$) and was less common in bloodstream isolates than among isolates recovered from urinary and respiratory tract infections ($P=0.002$). Clindamycin resistance also decreased over the course of the study

Table 2. Demographic characteristics of patients with infections caused by CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2011

Characteristic	CA-MRSA ($n=366$)	HA-MRSA ($n=868$)	*P* value
Sex, *n* (%)			0.34
male	208 (56.8)	520 (59.9)	
female	158 (43.2)	348 (40.1)	
Mean age, years	41.4	65.4	
Median age (range)	43 (1–95)	68 (1–105)	
Age group, *n* (%)			
≤17 years	55 (15.0)	10 (1.2)	<0.0001
18–64 years	260 (71.0)	349 (40.2)	<0.0001
≥65 years	51 (13.9)	509 (58.6)	<0.0001
Region, *n* (%)			
West	220 (60.1)	226 (26.0)	<0.0001
Ontario	108 (29.5)	284 (32.7)	0.28
Quebec	17 (4.6)	291 (33.5)	<0.0001
Maritimes	21 (5.7)	67 (7.7)	0.23
Hospital ward type, *n* (%)			
emergency room	140 (38.3)	133 (15.3)	<0.0001
clinic/office	73 (19.9)	112 (12.9)	0.002
intensive care unit	52 (14.2)	170 (19.6)	0.03
medical/surgical ward	101 (27.6)	453 (52.2)	<0.0001
Infection site, *n* (%)			
bloodstream	131 (35.8)	350 (40.3)	0.14
respiratory tract	72 (19.7)	331 (38.1)	<0.0001
urinary tract	2 (0.5)	44 (5.1)	<0.0001
wounds/IV sites	161 (44.0)	143 (16.5)	<0.0001

($P=0.01$) and was lowest among isolates from patients <18 years of age ($P<0.001$). Resistance rates for clindamycin were lower among MRSA recovered from patients presenting to emergency rooms or clinics than other hospital locations ($P=0.02$) and were higher in intensive care units than in medical/surgical wards ($P<0.001$). Like ciprofloxacin, resistance to clarithromycin was lowest in MRSA isolated from patients under the age of 18 years ($P=0.008$) and occurred less frequently in bloodstream isolates than among isolates recovered from urinary or respiratory tract infections ($P=0.01$). Clarithromycin resistance was lower among isolates recovered from patients in intensive care units than among MRSA isolated from patients presenting to an emergency room ($P=0.003$). In contrast to other agents, resistance to trimethoprim/sulfamethoxazole occurred least often among isolates recovered from older adults (≥65 years of age, $P=0.003$).

The modal vancomycin MIC of all MRSA was 1 mg/L and did not change over time. In total, 27 MRSA had a vancomycin MIC of 2 mg/L, while 1 had an MIC of 4 mg/L. Only 0.5% (2/366) of CA-MRSA had a vancomycin MIC of ≥2 mg/L compared with 3.0% (26/868) of HA-MRSA ($P=0.009$). Isolates with a vancomycin MIC ≥2 mg/L were more likely to have been isolated from respiratory tract infections ($P=0.02$), be PVL negative ($P=0.02$), belong to the HA-MRSA genotype CMRSA2 (USA100/800) ($P=0.003$) and demonstrate resistance to ciprofloxacin ($P=0.02$), clarithromycin ($P=0.04$) and clindamycin ($P=0.0009$) (data not shown). Intermediate resistance (MIC=4 mg/L) to vancomycin was observed in one HA-MRSA isolate with a PVL-negative CMRSA2 (USA100/800) genotype. No temporal change in the distribution of vancomycin MICs was observed, regardless of genotype. Among all MRSA, 99.8% were susceptible to tigecycline and 100% were susceptible to daptomycin, linezolid and telavancin.

Detection of hVISA among CA-MRSA and HA-MRSA genotypes

The hVISA phenotype was detected by PAP-AUC in 25.9% (7/27) of all MRSA isolates with a vancomycin MIC of 2 mg/L and one isolate (1/230, 0.4%) with an MIC of 1 mg/L. No hVISA were identified among MRSA with a vancomycin MIC of 0.5 mg/L. hVISA were predominantly (87.5%) isolated from medical centres in Ontario ($P=0.002$) and most (75.0%) belonged to PFGE epidemic type CMRSA2 (USA100/800), although the latter was not statistically significant (Table 4). hVISA were identified in all study years except 2011. The majority of hVISA isolates were resistant to clarithromycin (8/8, 100%), clindamycin (6/8, 75%) and the fluoroquinolones (7/8, 87.5%), while all hVISA were susceptible to daptomycin, linezolid, telavancin and tigecycline. No additional differences in the epidemiological and microbiological features associated with hVISA and non-hVISA isolates were observed.

Discussion

The rapid emergence and introduction of CA-MRSA into the healthcare setting has led to a significant change in the epidemiology of MRSA infections. Although HA strains remain the leading cause of MRSA disease in most acute-care facilities within the USA and Canada, increasing rates of CA-MRSA infection have been reported among hospitalized patients.[2,5] In a national surveillance study of MRSA among paediatric patients in Canada, the proportion of CA-MRSA cases increased from 21% (1995–99) to 50% (2004–07).[4] Another study of MRSA colonization/infection among both adult and paediatric patients in Canadian hospitals from 1995 to 2007 found that the percentage of patients with CA-MRSA increased from 6% (1995–97) to 21% (2004–07) and was 23% in the final year of surveillance.[5] Our study also demonstrates that the proportion of CA-MRSA in Canadian hospitals has risen significantly, with an increase from 19.7% of MRSA in 2007 to 36.4% in 2011 ($P<0.0001$).

CA-MRSA is a common cause of skin and soft tissue infections and often affects children, young adults and otherwise healthy individuals with no healthcare risk factors.[7,18] More recently, CA-MRSA has also been reported to cause invasive diseases, such as bacteraemia, osteomyelitis, endocarditis, necrotizing fasciitis and pulmonary infections with rapid and sometimes fatal progression to necrotizing pneumonia.[7,9,18] In our study, CA-MRSA genotypes were associated with patients in the younger (≤17 and 18–64 years) age groups and were most often (44%) isolated from wounds. Additional isolates were recovered from bloodstream and respiratory tract specimens, which is consistent with the ability of this organism to cause invasive infections. While the majority (58.2%) of CA-MRSA were isolated from patients presenting to emergency rooms or ambulatory care settings, a number of CA-MRSA genotypes were

Table 3. Antimicrobial susceptibilities of CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2011

Organism (no. tested)/antimicrobial agent	MIC (mg/L)			MIC interpretation		
	MIC_{50}	MIC_{90}	MIC range	% S	% I	% R
HA-MRSA (n=868)						
cefazolin	128	>128	1 to >128			100.0[a]
ciprofloxacin	>16	>16	0.25 to >16	2.8		97.2
clarithromycin	>16	>16	≤0.25 to >16	4.6		95.4
clindamycin	>8	>8	≤0.25 to >8	30.4	0.1	69.5
daptomycin	0.25	0.25	0.06–1	100.0		
levofloxacin	>32	>32	0.12 to >32	3.0		97.0
linezolid	2	4	≤0.12–4	100.0		
moxifloxacin	8	>16	≤0.06 to >16	2.9	0.1	97.0
telavancin	0.25	0.5	≤0.06–1	100.0		
tigecycline	0.25	0.5	0.06–2	99.6		0.4
trimethoprim/sulfamethoxazole	≤0.12	8	≤0.12 to >8	88.6		11.4
vancomycin	1	1	≤0.25–4	99.9	0.1	
CA-MRSA (n=366)						
cefazolin	16	64	1 to >128			100.0[a]
ciprofloxacin	16	>16	0.12 to >16	33.4	0.8	65.8
clarithromycin	>16	>16	≤0.25 to >16	24.8	0.3	74.9
clindamycin	≤0.25	>8	≤0.25 to >8	87.0		13.0
daptomycin	0.25	0.25	0.12–1	100.0		
levofloxacin	4	8	0.12–32	39.7		60.3
linezolid	2	2	1–4	100.0		
moxifloxacin	2	2	≤0.06–16	35.1	6.8	58.1
telavancin	0.25	0.5	0.12–1	100.0		
tigecycline	0.25	0.25	0.06–0.5	100.0		
trimethoprim/sulfamethoxazole	≤0.12	≤0.12	≤0.12–2	100.0		
vancomycin	1	1	0.5–2	100.0		
CMRSA7 (n=86)						
cefazolin	16	64	1–128			100.0[a]
ciprofloxacin	0.5	1	0.12 to >16	93.0	1.2	5.8
clarithromycin	0.25	32	0.12 to >32	82.6		17.4
clindamycin	≤0.12	0.25	≤0.12 to >8	95.3		4.7
daptomycin	0.25	0.25	0.12–0.5	100.0		
levofloxacin	0.25	0.5	0.12–8	96.6		3.4
linezolid	2	2	1–4	100.0		
moxifloxacin	≤0.06	≤0.06	≤0.06–2	96.5		3.5
telavancin	0.25	0.5	0.12–1	100.0		
tigecycline	0.25	0.25	0.12–0.5	100.0		
trimethoprim/sulfamethoxazole	≤0.12	≤0.12	≤0.12–1	100.0		
vancomycin	1	1	0.5–1	100.0		
CMRSA10 (n=280)						
cefazolin	8	64	1 to >128			100.0[a]
ciprofloxacin	16	>16	0.25 to >16	15.4	0.7	83.9
clarithromycin	>32	>32	0.12 to >32	6.8	0.4	92.8
clindamycin	≤0.12	>8	≤0.12 to >8	84.5		15.5
daptomycin	0.25	0.25	0.12–1	100.0		
levofloxacin	4	8	0.12–32	19.9		80.1
linezolid	2	2	1–4	100.0		
moxifloxacin	2	2	≤0.06–16	16.4	8.9	74.6
telavancin	0.25	0.5	0.12–1	100.0		
tigecycline	0.25	0.25	0.06–1	99.3		0.3
trimethoprim/sulfamethoxazole	≤0.12	≤0.12	≤0.12–2	100.0		
vancomycin	1	1	0.5–2	100.0		

S, susceptible; I, intermediate; R, resistant.
[a]Based on cefoxitin disc test.

Table 4. Demographic and microbiological characteristics of hVISA and non-hVISA strains

Characteristic	hVISA ($n=8$)	Non-hVISA ($n=280$)	*P* value
Sex, *n* (%)			0.71
male	6 (75.0)	173 (61.8)	
female	2 (25.0)	107 (38.2)	
Mean age, years	55.5	59.5	
Median age (range)	56.5 (41–65)	62 (1–105)	
Age group, *n* (%)			
≤17 years	0 (0.0)	11 (3.9)	1.0
18–64 years	6 (75.0)	140 (50.0)	0.28
≥65 years	2 (25.0)	129 (46.1)	0.3
Region, *n* (%)			
West	1 (12.5)	105 (37.5)	0.27
Ontario	7 (87.5)	88 (31.4)	0.002
Quebec	0 (0.0)	66 (23.6)	0.21
Maritimes	0 (0.0)	21 (7.5)	1.0
Hospital ward type, *n* (%)			
emergency room	1 (12.5)	43 (15.4)	1.0
clinic/office	2 (25.0)	46 (16.4)	0.36
intensive care unit	2 (25.0)	52 (18.6)	0.65
medical/surgical ward	3 (37.5)	139 (49.6)	0.72
Infection site, *n* (%)			
bloodstream	4 (50.0)	104 (37.1)	0.48
respiratory tract	2 (25.0)	90 (32.1)	1.0
urinary tract	0 (0.0)	12 (4.3)	1.0
wounds/IV sites	2 (25.0)	74 (26.4)	1.0
PFGE type, *n* (%)			0.48
CMRSA2	6 (75.0)	166 (59.3)	
other	2 (25.0)	114 (40.7)	
PVL gene, *n* (%)			0.21
positive	0 (0.0)	70 (25.0)	
negative	8 (100)	210 (75.0)	

recovered from medical/surgical wards and intensive care units. The circulation of CA-MRSA in hospitals has previously been described and several reports suggest that CA-MRSA may be displacing traditional HA-MRSA strains in the healthcare setting.[1,8]

In contrast to CA-MRSA, HA-MRSA are typically associated with older individuals and those with recent exposure to the healthcare setting.[18] HA-MRSA strains usually cause pneumonia, bacteraemia and other invasive infections.[18] In this study, HA-MRSA genotypes were significantly associated with older adults (≥65 years), were predominantly isolated from bloodstream and respiratory tract infections and were most frequently recovered from patients admitted to medical/surgical wards and intensive care units.

Molecular characterization of MRSA has identified a number of unique HA and CA genotypes, each with defined epidemiological traits. HA-MRSA strain types are the most common source of MRSA infections within many healthcare settings, where CMRSA2 (USA100/800) remains the most widespread of the epidemic clones.[9,19,20] Among nearly 1500 MRSA isolates collected in 2004 as part of the Canadian Nosocomial Infection Surveillance Programme (CNISP), Christianson *et al.*[16] found that CMRSA2 (USA100/800) accounted for ∼55% of all isolates. Similarly, Simor *et al.*[5] reported that the most common MRSA genotype identified in CNISP hospitals from 2004 to 2007 was CMRSA2 (USA100/800), accounting for 58% of the characterized strains. In our study, HA-MRSA epidemic types also predominated, with most MRSA isolates (58.1%) belonging to the CMRSA2 (USA100/800) clone. While the second most common HA-MRSA genotype was CMRSA3/6, the proportion of these isolates decreased from >10% to <1% during the 5 year study period. CMRSA3 (genetically similar to CMRSA6) was first identified in western Canada in the early 1990s and was linked to a patient who had previously been hospitalized in Punjab, India.[21] Subsequent MRSA outbreaks occurred shortly thereafter in two tertiary-care institutions in the provinces of British Columbia and Manitoba.[21] Only one CMRSA3/6 strain was isolated east of Manitoba in our current CANWARD study and the apparent elimination of the CMRSA3/6 genotype does not appear to be associated with replacement by another HA-MRSA type. Although the reason for the decline in the proportion of CMRSA3/6 is unknown, it has been hypothesized that altered expression of virulence factors associated with colonization and infection may impact the epidemicity and invasive potential of these strains.[22]

While HA-MRSA isolates often belong to a number of genotypes, the majority of CA-MRSA disease in North American hospitals can be attributed to two common PFGE types: CMRSA7 (USA400) and CMRSA10 (USA300).[9] In Canada, CMRSA7 (USA400) was first identified in the late 1990s, when it was responsible for clusters of CA-MRSA disease in rural Manitoba.[23] It later spread to First Nations populations in northern Manitoba as well as neighbouring communities in east-central and northern Saskatchewan and is still the predominant strain type (accounting for >90% of MRSA infections) in those regions.[24,25] Since the early 2000s, however, CMRSA10 (USA300) has emerged as a highly successful clone that has effectively replaced CMRSA7 (USA400) as the most prevalent CA-MRSA genotype in most regions across North America.[9] Within the USA, CMRSA10 (USA300) is widely disseminated and is by far the predominant epidemic type associated with CA-MRSA infections.[1,9,18,20,26] In Canada, the CMRSA10 (USA300) genotype was initially identified in Alberta in outbreaks of MRSA among individuals with a history of drug use, homelessness and/or recent incarceration.[27] According to CNISP data from 2007, CMRSA10 (USA300) is now the second most common genotype identified among MRSA in Canada, accounting for 27% of characterized strains from hospitalized patients.[5] As expected in our study, CMRSA10 (USA300) accounted for more than three-quarters (76.5%) of all CA-MRSA isolates, was the second most commonly identified epidemic type overall (22.1%) and increased significantly in proportion (13.2%–28.6%) between 2007 and 2011. Interestingly, we observed that CMRSA10 (USA300) isolates were statistically more likely to have been recovered from elderly patients and from across Canada compared with CMRSA7 (USA400) strains, which were more likely to have been isolated from younger individuals (≤17 years of age) and from hospitals in the western region of the country. This serves as evidence that CA-MRSA belonging to the CMRSA10 (USA300) genotype may have descended from known HA-MRSA clones [specifically, CMRSA5 (USA500)] through acquisition of specific virulence factors, including PVL.[9]

PVL is a bicomponent pore-forming cytotoxin that causes rapid necrosis and destruction of leucocytes.[9] Due to its high prevalence in CA strains, PVL is often regarded as an epidemiological marker of CA-MRSA infection.[18] Although several investigators have reported a strong correlation between toxin production and increased disease severity, its role in virulence remains controversial as PVL is not ubiquitous in all CA-MRSA and strains lacking PVL can still cause disease.[18] In the present study, PVL was strongly associated (89.6%) with CA-MRSA infections, while HA-MRSA were almost exclusively (99.3%) PVL negative ($P<0.0001$). Among the CA-MRSA genotypes CMRSA10 (USA300) and CMRSA7 (USA400), PVL was detected in 98.2% and 61.6% of strains, respectively. This observation supports the hypothesis that PVL is not the sole virulence determinant of CA-MRSA, but rather one of several important factors that may play a key role in the successful dissemination of CA-MRSA.[28]

Prompt and appropriate treatment of MRSA infections is important for achieving a favourable outcome. Consistent with previous data, CA-MRSA genotypes in our study were more susceptible to macrolides, clindamycin, fluoroquinolones and trimethoprim/sulfamethoxazole compared with HA-MRSA genotypes. Interestingly, we observed that CA-MRSA isolates belonging to the CMRSA10 (USA300) genotype were significantly less susceptible than CMRSA7 (USA400) strains. With higher rates of resistance to clarithromycin, clindamycin and the fluoroquinolones, the *in vitro* susceptibility pattern of these isolates more closely resembles that of HA-MRSA than classic CA-MRSA and is consistent with other studies that show increased resistance among CMRSA10 (USA300) isolates. The potential evolution of the CMRSA10 (USA300) genotype from CMRSA5 (USA500) may explain why CMRSA10 (USA300) isolates are more resistant than CMRSA7 (USA400). There is growing concern that CMRSA10 (USA300) isolates may also acquire additional resistance determinants, possibly through direct acquisition from multidrug-resistant HA-MRSA strains, under the selective pressures of the healthcare environment.[29]

Vancomycin is a key antimicrobial agent for the treatment of severe or complicated infections caused by MRSA.[7,18,30] Unfortunately, increasing use of vancomycin has led to the emergence of MRSA strains with reduced susceptibility to this antibiotic.[30] Although increases in vancomycin MICs at some institutions have been attributed to vancomycin MIC creep, not all studies evaluating MIC creep have detected this phenomenon.[30] Over a 12 year span between 1995 and 2006, no significant increase in the proportion of Canadian MRSA isolates with a vancomycin MIC $\geq$2 mg/L was observed.[31] Our data likewise confirm that vancomycin MIC creep has not occurred in Canada.

Several studies have established an association between elevated vancomycin MICs and clinical failures.[7,30] Of particular concern is the isolation of MRSA with heterogeneous resistance to vancomycin, as these strains are thought to be precursors to vancomycin-intermediate *S. aureus*.[30] The prevalence of hVISA varies considerably, with rates ranging from <1% to as high as 74%.[32] In our study, detection of hVISA by PAP was rare overall (2.8% of all MRSA tested), but was common in isolates with a vancomycin MIC of 2 mg/L (25.9%). Of note, most (75%) of these isolates were PVL negative and belonged to the CMRSA2 (USA100/800) genotype. Since the majority (83.3%) of CMRSA2 (USA100/800) genotype hVISA strains with a vancomycin MIC of 2 mg/L were recovered from patients in Ontario (66.7% from a single hospital), clonal spread of hVISA is likely to have occurred. Fortunately, 99.8% of all MRSA (including 100% of hVISA isolates) were susceptible to tigecycline and no resistance to daptomycin, linezolid or telavancin was observed in the present study. Therefore, these agents remain attractive alternatives for the treatment of serious MRSA infections.

A limitation of this study is the variability in the number of participating centres as well as the number of isolates collected in each year of CANWARD. Although the number of isolates requested from each participating site decreased between 2007 and 2011, the data presented in this study describe MRSA as a proportion of *S. aureus*. Therefore, we believe that the observed decrease in the proportion of MRSA represents a true reduction in the burden of MRSA and is not a bias of annual changes in study criteria. An additional limitation of this study is the lack of access to clinical and epidemiologic information for CANWARD isolates, preventing us from classifying CA-MRSA and HA-MRSA strains as per the CDC criteria.[33] Thus, the present study may not accurately reflect the true proportion of CA-MRSA and HA-MRSA genotypes in Canadian hospitals. Characterization of additional markers such as SCC*mec*, *agr* and the arginine catabolic mobile element (ACME) might serve to improve classification, but was beyond the scope of this project. Another potential limitation is the use of traditional 2-fold serial dilutions for MIC testing, which may mask subtle changes or shifts in MICs that might otherwise be detected by measuring incremental dilutions via Etest. There is also debate surrounding the most sensitive measure of vancomycin MIC creep (geometric mean, modal MIC or the proportion of strains for which the vancomycin MIC is above a certain value), as the detection of MIC creep may be dependent on the method used for data analysis. Finally, we could not screen all MRSA for the presence of the hVISA phenotype, so over- or underestimation of hVISA rates cannot be excluded. However, with the exception of strains with vancomycin MICs of 2 mg/L, all other isolates were randomly selected for hVISA screening and we believe that this subpopulation accurately reflects the proportion of hVISA in Canadian hospitals.

In summary, although the overall proportion of MRSA has declined in Canadian hospitals, the proportion of CMRSA10 (USA300) CA-MRSA rose significantly between 2007 and 2011. As these strains continue to become more resistant and cause an increasing proportion of invasive MRSA infections, the treatment and control of MRSA in the healthcare setting will become increasingly difficult. Our study demonstrates that national surveillance programmes are a critical part of the ongoing efforts to monitor and better understand the changing epidemiology of MRSA within both the community and the hospital environment.

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CANWARD data can also be found at www.can-r.ca, the official web site of the Canadian Antimicrobial Resistance Alliance (CARA).

Members of the Canadian Antimicrobial Resistance Alliance (CARA)

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