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Changing Epidemiology of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) in Canadian Hospitals from 2007-2013

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ABSTRACT

Background: As part of the CANWARD surveillance study, we compared the epidemiology of CA-MRSA and healthcare-associated (HA)-MRSA genotypes in Canadian hospitals.
Methods: Between 2007 and 2013, 1550 MRSA were collected from patients attending tertiary-care medical centres across Canada. Susceptibility testing was performed by CLSI broth microdilution. Isolates were characterized by *spa* typing and PCR of the Panton-Valentine leukocidin (PVL) gene. Detection of hVISA was performed by the Etest macromethod and confirmed by population analysis profile-area under the curve.
Results: The annual proportion of MRSA genotypes is shown below:

MRSA Type	Study Year							P-value*
	2007	2008	2009	2010	2011	2012	2013	
All MRSA (% of all *S. aureus*)	26.1	27.0	21.0	21.2	19.3	18.2	20.3	0.002
HA-MRSA (% of all MRSA)	79.2	69.1	65.5	58.7	59.7	54.4	57.5	<0.0001
CMRSA1 [USA600]	2.3	1.1	0	1.8	0.6	4.0	0.6	0.2945
CMRSA2 [USA100/800]	64.9	56.3	58.6	49.8	55.8	43.2	48.8	0.0005
CMRSA3/6	10.6	8.8	4.7	3.1	0.6	0	3.8	0.0073
CMRSA4 [USA200]	0	0.4	0	0.9	0	0.8	0.6	0.2936
CMRSA5 [USA500]	1.0	1.5	0	1.3	1.3	2.4	0	0.3261
CMRSA8	0	0.7	1.7	1.8	1.3	4.0	3.8	0.0006
CMRSA9	0.3	0.4	0.4	0	0	0	0	1
CA-MRSA (% of all MRSA)	19.7	27.6	31.9	38.1	36.4	39.2	35.6	0.0001
CMRSA7 [USA400]	6.5	5.5	8.2	6.7	7.8	12.0	8.1	0.5796
CMRSA10 [USA300]	13.2	22.1	23.7	31.4	28.6	27.2	27.5	0.0001
Unique	1.0	3.3	2.6	3.1	3.9	6.4	6.9	0.0005

*P-value determined by Fisher's exact test comparing 2007 vs. 2013 data.

PVL was detected in 86.4% of CA-MRSA and 1.6% of HA-MRSA. The proportion of PVL-negative USA400 (CMRSA7) strains increased from 16.0% in 2007 to 76.9% in 2013 (*P*=0.0004) despite no significant change in the annual numbers of this genotype. Resistance rates (CA vs HA) were 65.5 vs 96.3% to ciprofloxacin, 72.9 vs 93.9% to clarithromycin, 12.4 vs 66.1% to clindamycin and 0.0 vs 10.5% to trimethoprim-sulfamethoxazole. MRSA were 100% susceptible to linezolid and telavancin and 99.9% susceptible to daptomycin and vancomycin. The hVISA phenotype was detected in 34.5% (10/29) of MRSA with a vancomycin MIC of 2 μg/mL.
Conclusions: The most frequent CA-MRSA genotype was USA300 (CMRSA10) while USA100/800 (CMRSA2) was the predominant HA-MRSA genotype. Despite an overall decrease in the numbers of MRSA, the proportion of CA-MRSA in Canadian hospitals has risen significantly between 2007 and 2013.

BACKGROUND

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. While skin and soft tissue infections are the most common infections caused by CA-MRSA, invasive disease such as bacteremia associated with sepsis and necrotizing pneumonia can occur. The individuals most often affected by CA-MRSA typically lack established risk factors for MRSA acquisition/infection. CA-MRSA differ from health care-associated MRSA (HA-MRSA) in that they are generally more susceptible to a variety of non-beta-lactam antimicrobial agents. Of particular concern, however, is the emergence of isolates with reduced susceptibility or heterogeneous resistance to vancomycin, an important antimicrobial for the empiric treatment of severe infections. In addition, the majority of CA-MRSA strains harbor virulence determinants such as the Panton-Valentine leukocidin (PVL) as well as other toxins that may contribute to the increasing morbidity and mortality associated with CA-MRSA infections.
The purpose of this study was to compare the demographics, antimicrobial susceptibilities and molecular epidemiology of community-associated and health care-associated MRSA genotypes in Canada from 2007 to 2013, inclusive.

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MATERIALS & METHODS

Methicillin-Resistant *S. aureus* Isolates
1550 isolates of MRSA were collected between 2007 and 2013 as part of the ongoing CANWARD surveillance study assessing antibiotic resistance in Canadian hospitals. Isolates were received from tertiary-care medical centres (12 in 2007, 10 in 2008, 15 in 2009, 14 in 2010, 15 in 2011, 12 in 2012 and 15 in 2013) that were geographically distributed in a population-based fashion in 8 of the 10 Canadian provinces. All *S. aureus* were identified at the originating centre using local site criteria. Resistance to methicillin was confirmed at the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) using the CLSI-approved disk diffusion method with cefoxitin, as well as by growth on MRSA*Select* chromogenic media.

Antimicrobial Susceptibility Testing
The *in vitro* activities of cefazolin, clarithromycin, clindamycin, ciprofloxacin, daptomycin, levofloxacin, linezolid, moxifloxacin, telavancin, tigecycline, trimethoprim-sulfamethoxazole and vancomycin were determined by broth microdilution in accordance with CLSI guidelines (M7-A9, 2012). MIC interpretive standards were defined according to CLSI breakpoints (M100-S23, 2013). The following interpretive breakpoints (FDA) were used: telavancin susceptible, ≤1 μg/ml; tigecycline susceptible, ≤0.5 μg/ml.

Molecular Characterization of MRSA
MRSA status was confirmed by real-time PCR of the *mecA* and *nuc* genes (McDonald et al. 2005. J. Clin. Microbiol. 43:6147-6149). This triplex PCR assay included primers for the detection of the *lukF-PV* and *lukS-PV* genes encoding the Panton-Valentine leukocidin (PVL) toxin (McDonald et al. 2005. J. Clin. Microbiol. 43:6147-6149).
MRSA strains were characterized by staphylococcal protein A (*spa*) typing as previously described (Golding et al. 2008. Can. J. Infect. Dis. Med. Microbiol. 19:273-281). For the purpose of this study, community-associated (CA)-MRSA and healthcare-associated (HA)-MRSA were defined genotypically (ie. on the basis of their *spa* type) and not epidemiologically as per CDC criteria for distinguishing CA-MRSA from HA-MRSA, because epidemiologic information was not available. There has previously been shown to be good correlation between *spa* types and Canadian epidemic PFGE strain types CMRSA1-10 (Golding et al. 2008. Can. J. Infect. Dis. Med. Microbiol. 19:273-281), allowing for classification of strains as either CA-MRSA or HA-MRSA. Any MRSA with a *spa* type associated with a CMRSA7 (USA400) or CMRSA10 (USA300) genotype were labeled as CA-MRSA while all other *spa* types corresponding to a characterized epidemic type (eg. CMRSA1 [USA600], CMRSA2 [USA100/800], CMRSA4 [USA200], CMRSA5 [USA500], CMRSA3/6, CMRSA8, CMRSA9, etc.) were labeled as HA-MRSA. MRSA with a *spa* type not associated with one of the known Canadian epidemic types were labeled as unique (non-CMRSA).

Detection of Heterogeneous Vancomycin-Intermediate *S. aureus* (hVISA)
All MRSA isolates with a vancomycin MIC of 2 μg/ml (n=29) were screened for the presence of the hVISA phenotype using the Etest macromethod. MRSA identified as hVISA by the Etest macromethod were further evaluated by population analysis profile-area under the curve (PAP-AUC). *S. aureus* reference strains Mu3 (ATCC 700698, hVISA), Mu50 (ATCC700699, VISA) and ATCC 29213 (vancomycin-susceptible *S. aureus*) were included as controls.

CONCLUSIONS

1. Overall, 30.5% and 66.3% of MRSA strains from Canadian hospitals were identified by *spa* typing as CA-MRSA and HA-MRSA, respectively. The prevalence of CA-MRSA increased significantly from 19.7% in 2007 to 35.8% in 2013 while HA-MRSA decreased from 79.2% to 57.2% during this same period (*P*<0.0001).
2. CA-MRSA genotypes CMRSA7 (USA400) and CMRSA10 (USA300) represented 7.4% and 23.1% of all MRSA, respectively. The prevalence of CMRSA10 (USA300) increased significantly from 13.2% in 2007 to 27.7% in 2013 (*P*=0.0001).
3. CMRSA2 (USA100/800) was the predominant HA-MRSA genotype, accounting for 55.9% of all MRSA and 84.4% of HA-MRSA.
4. The majority (86.4%) of CA-MRSA were PVL(+) whereas 98.4% of HA-MRSA were PVL(-).
5. Although the annual proportion of CMRSA7 (USA400) strains did not change significantly over the study period, the proportion of PVL(-) [versus PVL(+)] CMRSA7 (USA400) increased from 16.0% in 2007 to 76.9% in 2013 (*P*=0.0004).
6. CA-MRSA strains were more susceptible to clarithromycin, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole than HA-MRSA.
7. 0.8% of CA-MRSA had a vancomycin MIC of 2 μg/ml compared to 2.4% of HA-MRSA (*P*=0.04). Intermediate resistance (MIC, 4 μg/ml) to vancomycin was observed in one MRSA with a PVL-negative CMRSA2 (USA100/800) genotype. MRSA were 100% susceptible to linezolid and telavancin and 99.9% susceptible to daptomycin and vancomycin.
8. Detection of hVISA by PAP-AUC was common in isolates with a vancomycin MIC of 2 μg/ml (34.5%).

RESULTS

Figure 1. Proportion of *S. aureus* strains identified as MSSA or MRSA.

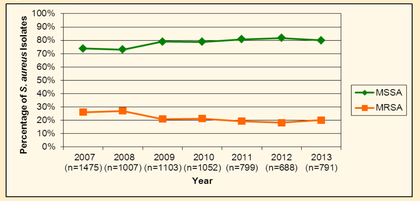


Figure 2. Proportion of MRSA strains identified as CA-MRSA or HA-MRSA.

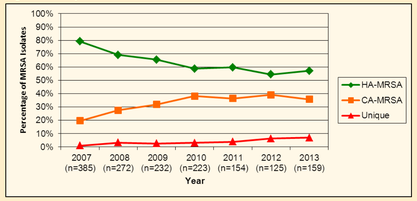


Figure 3. Distribution of CA-MRSA and HA-MRSA PFGE epidemic types.

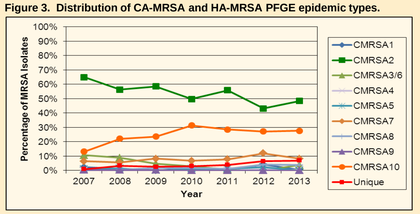


Figure 4. Distribution of PVL(+) and PVL(-) CA-MRSA and HA-MRSA.

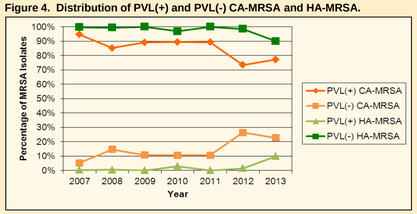


Figure 5. Proportion of PVL(+) and PVL(-) CMRSA7 strains.

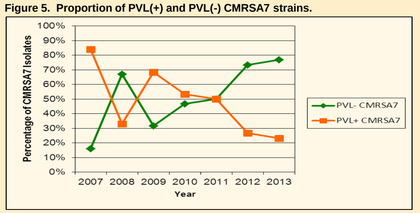
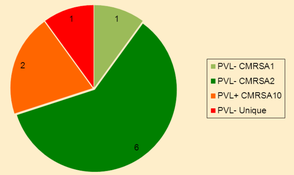


Figure 6. Distribution of hVISA genotypes.



10/29 MRSA with a vancomycin MIC of 2 μg/ml were identified as hVISA.

Table 1. Demographics of patients with MRSA infections.

Characteristic	CA-MRSA (n=472)	HA-MRSA (n=1027)	Total (n=1550)
Sex, n (%)			
Male	272 (57.6)	617 (60.1)	912 (58.8)
Female	200 (42.4)	410 (39.9)	638 (41.2)
Mean age, years	42.3	65.1	57.4
Median age (range)	44 (1-95)	68 (1-105)	61 (1-105)
Age group, n (%)			
≤ 17	70 (14.8)	19 (1.9)	99 (6.4)
18-64	331 (70.1)	404 (39.3)	766 (49.4)
≥ 65	71 (15.0)	604 (58.8)	685 (44.2)
Region, n (%)			
West	278 (58.9)	274 (26.7)	570 (36.8)
Ontario	149 (31.6)	345 (33.6)	519 (33.5)
Quebec	19 (4.0)	324 (31.5)	348 (22.5)
Maritimes	26 (5.5)	84 (8.2)	113 (7.3)
Hospital ward type, n (%)			
Emergency room	167 (35.4)	153 (14.9)	333 (21.5)
Clinic/office	86 (18.2)	134 (13.0)	233 (15.0)
Intensive care unit	75 (15.9)	207 (20.2)	287 (18.5)
Medical/surgical ward	144 (30.5)	533 (51.9)	697 (45.0)
Infection site, n (%)			
Bloodstream	168 (35.6)	392 (38.2)	575 (37.1)
Respiratory tract	106 (22.5)	425 (41.4)	543 (35.0)
Urinary tract	2 (0.4)	44 (4.3)	47 (3.0)
Wounds/IV sites	196 (41.5)	166 (16.2)	385 (24.8)

Table 2. Vancomycin MIC distributions for CA-MRSA and HA-MRSA.

Genotype, Study Year	Number (%) at each Vancomycin MIC				
	≤0.25	0.5	1	2	4
CA-MRSA					
2007 (n=76)		17 (22.4)	59 (77.6)		
2008 (n=75)		17 (22.7)	58 (77.3)		
2009 (n=74)		8 (10.8)	64 (86.5)	2 (2.7)	
2010 (n=85)		11 (12.9)	74 (87.1)		
2011 (n=56)		22 (39.3)	34 (60.7)		
2012 (n=49)		27 (55.1)	29 (40.8)	2 (4.1)	
2013 (n=57)		41 (71.9)	16 (28.1)		
HA-MRSA					
2007 (n=305)	5 (1.6)	22 (7.2)	274 (89.8)	4 (1.3)	
2008 (n=188)		18 (9.6)	161 (85.6)	8 (4.3)	1 (0.5)
2009 (n=152)		14 (9.2)	131 (86.2)	7 (4.6)	
2010 (n=131)		9 (6.9)	116 (88.5)	6 (4.6)	
2011 (n=92)		13 (14.1)	79 (85.9)		
2012 (n=68)		18 (26.5)	50 (73.5)		
2013 (n=91)		37 (40.7)	54 (59.3)		

Table 3. Comparison of antibiotic resistance rates among CA-MRSA and HA-MRSA.

CA-MRSA (n=472)

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category		
				S	I	R
Cefazolin	8	64	1 - >128	-	-	100.0%[a]
Ciprofloxacin	16	>16	0.12 - >16	33.8%	0.6%	65.5%
Clarithromycin	>16	>16	≤0.25 - >16	26.7%	0.4%	72.9%
Clindamycin	≤0.25	>8	≤0.25 - >8	87.6%	0.0%	12.4%
Daptomycin	0.25	0.25	0.12 - 2	99.8%	-	0.2%
Levofloxacin	4	8	0.12 - 32	40.0%	0.0%	60.0%
Linezolid	2	2	1 - 4	100.0%	-	-
Moxifloxacin	2	2	≤0.06 - 16	35.1%	7.9%	57.0%
Telavancin	0.25	0.5	0.12 - 1	100.0%	-	-
Tigecycline	0.25	0.25	0.06 - 0.5	100.0%	-	-
TMP-SMX	≤0.12	≤0.12	≤0.12 - 2	100.0%	-	0.0%
Vancomycin	1	1	0.5 - 2	100.0%	0.0%	0.0%

HA-MRSA (n=1027)

MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category		
			S	I	R
128	>128	1 - >128	-	-	100.0%[a]
>16	>16	0.25 - >16	3.7%	0.0%	96.3%
>16	>16	≤0.25 - >16	5.8%	0.3%	93.9%
>8	>8	≤0.25 - >8	33.8%	0.1%	66.1%
0.25	0.25	0.06 - 1	100.0%	-	-
>32	>32	0.12 - >32	2.9%	0.0%	97.1%
2	4	≤0.12 - 4	100.0%	-	0.0%
8	>16	≤0.06 - >16	3.9%	0.4%	95.7%
0.25	0.5	≤0.06 - 1	100.0%	-	-
0.25	0.5	0.06 - 2	99.1%	-	0.9%
≤0.12	8	≤0.12 - >8	89.5%	-	10.5%
1	1	≤0.25 - 4	99.9%	0.1%	0.0%

All MRSA (n=1550)

MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category		
			S	I	R
64	>128	1 - >128	-	-	100.0%[a]
>16	>16	0.12 - >16	15.6%	0.3%	84.1%
>16	>16	≤0.25 - >16	14.1%	0.3%	85.5%
≤0.25	>8	≤0.25 - >8	51.8%	0.1%	48.1%
0.25	0.25	0.06 - 2	99.9%	-	0.1%
>32	>32	0.12 - >32	14.1%	0.0%	85.9%
2	2	≤0.12 - 4	100.0%	-	-
8	>16	≤0.06 - >16	16.3%	2.6%	81.1%
0.25	0.5	≤0.06 - 1	100.0%	-	-
0.25	0.5	0.06 - 2	99.4%	-	0.6%
≤0.12	≤0.12	≤0.12 - >8	93.0%	-	7.0%
1	1	≤0.25 - 4	99.9%	0.1%	0.0%

[a] Based on cefoxitin disk test.