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Comparison of Community-Associated and Healthcare-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Canada: CANWARD 2007-2010

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ABSTRACT

Background: This study assessed the demographics, antimicrobial susceptibility and molecular epidemiology of CA- and HA-MRSA in Canada.
Methods: From Jan. 2007 – Dec. 2010, 1112 MRSA were collected from patients attending hospital clinics, emergency rooms, medical and surgical wards and intensive care units in tertiary-care centres across Canada as part of the CANWARD surveillance study. Susceptibilities to β-lactams, ciprofloxacin (CIP), clarithromycin (CLR), clindamycin (CLD), daptomycin (DAP), linezolid (LZD), trimethoprim/sulfamethoxazole (SXT) and vancomycin (VAN) were determined by CLSI broth microdilution. Isolates were characterized by *spa* typing and PCR of the Panton-Valentine leukocidin (PVL) gene.
Results: The annual prevalence of MRSA (% MRSA/*S. aureus*) was 26.1% in 2007, 27.0% in 2008, 21.0% in 2009 and 21.2% in 2010. Of the 1112 MRSA, 309 (27.8%) were CA-MRSA and 775 (69.7%) were HA-MRSA. The prevalence of CA-MRSA increased from 19.5% in 2007 to 38.1% in 2010 (p<0.001). CA-MRSA belonged to epidemic types CMRSA10/USA300 (76.1%) and CMRSA7/USA400 (23.9%); HA-MRSA epidemic types included CMRSA2/USA100/800 (83.9%), CMRSA3/6 (10.7%), CMRSA1/USA600 (2.0%), CMRSA5/USA500 (1.3%), CMRSA8 (1.3%), CMRSA4/USA200 (0.4%) and CMRSA9 (0.4%). PVL was detected in 277/309 (89.6%) CA-MRSA and 7/775 (0.9%) HA-MRSA (p<0.001). Resistance rates (CA vs HA) were 64.0 vs 97.2% to CIP, 75.3 vs 95.5% to CLR, 13.3 vs 71.1% to CLD and 0.0 vs 12.5% to SXT. 2.4% (27/1112) and 0.09% (1/1112) of MRSA had a VAN MIC of 2 and 4 μg/mL, respectively. No MRSA were resistant to DAP or LZD.
Conclusions: CA-MRSA were significantly more susceptible to CIP, CLR, CLD and SXT than HA-MRSA. The majority of CA-MRSA were PVL(+) and belonged to epidemic type CMRSA10/USA300 while most HA-MRSA were PVL(-) and belonged to epidemic type CMRSA2/USA100/800. CA-MRSA represented 27.8% of all MRSA and is increasing in prevalence in CANWARD hospital sites.

BACKGROUND

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in prevalence in Canada, the United States and throughout the world, and is well recognized as a leading cause of nosocomial infections. In the past decade, MRSA has also emerged as a significant community-associated (CA) pathogen capable of causing disease in young, otherwise healthy individuals lacking traditional risk factors for MRSA acquisition/infection. Several reports have documented CA-MRSA infections among aboriginals, military recruits, intravenous drug users, correctional facilities, homeless persons, amateur and professional sports teams, daycares and schools. Of particular concern is that CA-MRSA strains, in addition to skin and soft tissue infections, may be associated with severe invasive disease including necrotizing pneumonia, bacteremia and septic shock, resulting in increased morbidity and mortality.
CA-MRSA genotypes continue to spread within the community and to replace healthcare-associated (HA)-MRSA in the hospital setting. Most CA-MRSA strains differ from their healthcare-associated counterparts in their microbiological, epidemiological and molecular characteristics.

PURPOSE

The purpose of this study was to compare the demographics, antimicrobial susceptibilities and molecular epidemiology of community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* genotypes in Canada.

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MATERIALS & METHODS

Methicillin-Resistant *S. aureus* Isolates
1112 isolates of MRSA were collected between January 2007 and December 2010, inclusive, 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) 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, tigecycline, trimethoprim-sulfamethoxazole and vancomycin were determined by broth microdilution in accordance with CLSI guidelines (M7-A8, 2009). MIC interpretive standards were defined according to CLSI breakpoints (M100-S21, 2011). The following interpretive breakpoint (FDA) was used for 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 also 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=27) were screened for the presence of the hVISA phenotype using the Etest macromethod. A randomly selected subset (25% each) of MRSA with vancomycin MICs of 1 μg/ml (n=230) and 0.5 μg/ml (n=31) were included for comparison. MRSA identified as hVISA by the Etest macromethod were further evaluated by population analysis profile-area under the curve (PAP-AUC).

CONCLUSIONS

1. Overall, 27.8% and 69.7% 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 19.5% in 2007 to 38.1% in 2010 while HA-MRSA decreased from 79.2% to 58.7% during this same period (p<0.0001).
2. CA-MRSA belonged to PFGE types CMRSA7 (USA400) [26.3%] and CMRSA10 (USA300) [73.7%]. Among all MRSA, the prevalence of CMRSA10 (USA300) increased significantly from 13.0% in 2007 to 31.4% in 2010 (p<0.0001).
3. CMRSA2 (USA100/800) was the predominant PFGE epidemic type among HA-MRSA [83.9%].
4. The majority (89.6%) of CA-MRSA were PVL(+). 10.4% of CA-MRSA and 99.1% of HA-MRSA were PVL(-).
5. CA-MRSA strains were more susceptible to clarithromycin, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole than HA-MRSA.
6. Only 0.6% of CA-MRSA had a vancomycin MIC of 2 μg/ml compared to 3.2% (including 4.6% in 2009 and 2010) of HA-MRSA (p=0.015). Intermediate resistance (MIC, 4 μg/ml) to vancomycin was observed in one (0.09%) HA-MRSA strain. No MRSA were resistant to daptomycin, linezolid or tigecycline.
7. Detection of hVISA by PAP-AUC was rare overall (2.8%), but was common in isolates with a vancomycin MIC of 2 μg/ml (25.9%).

RESULTS

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

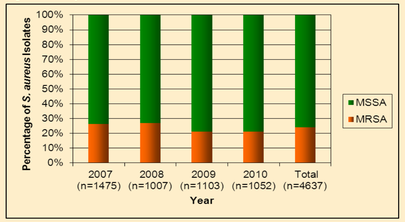

Figure 2. Proportion of MRSA strains identified as CA-MRSA or HA-MRSA.

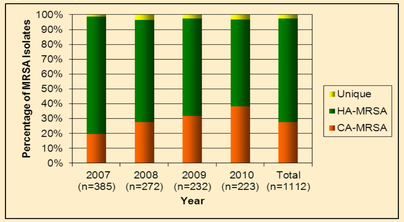

Figure 3. Distribution of CA-MRSA and HA-MRSA PFGE epidemic types.

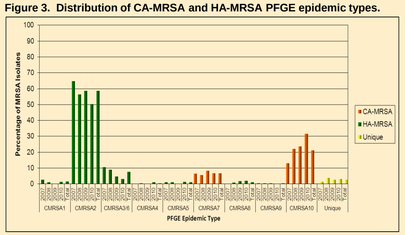

Figure 4. Distribution of PVL(+) and PVL(-) CA-MRSA and HA-MRSA.

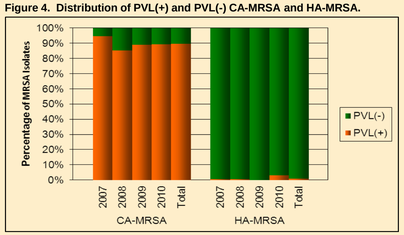

Table 1. Demographics of patients with MRSA infections.

Characteristic	HA-MRSA (n=775)	CA-MRSA (n=309)	Total (n=1112)
Sex, n (%)			
Male	468 (60.4)	174 (56.3)	653 (58.7)
Female	307 (39.6)	135 (43.7)	459 (41.3)
Mean age, years	65	41.7	57.8
Median age (range)	67 (1-105)	43 (1-95)	61 (1-105)
Age group, n (%)			
≤ 17	9 (1.2)	45 (14.6)	61 (5.5)
18-64	323 (41.7)	222 (71.8)	560 (50.4)
≥ 65	443 (57.2)	42 (13.6)	491 (44.2)
Region, n (%)			
British Columbia/Alberta	156 (20.1)	93 (30.1)	251 (22.6)
Saskatchewan/Manitoba	55 (7.1)	95 (30.7)	158 (14.2)
Ontario	251 (32.4)	85 (27.5)	349 (31.4)
Quebec/Maritimes	313 (40.4)	36 (11.7)	354 (31.8)
Hospital ward type, n (%)			
Emergency room	118 (15.2)	115 (37.2)	242 (21.8)
Clinic/office	100 (12.9)	65 (21.0)	170 (15.3)
Intensive care unit	147 (19.0)	43 (13.9)	192 (17.3)
Medical/surgical ward	410 (52.9)	86 (27.8)	508 (45.7)
Infection site, n (%)			
Bloodstream	318 (41.0)	113 (36.6)	439 (39.5)
Respiratory tract	289 (37.3)	56 (18.1)	349 (31.4)
Urinary tract	39 (5.0)	2 (0.6)	42 (3.8)
Wounds/IV sites	129 (16.6)	138 (44.7)	282 (25.4)

Table 2. Vancomycin MIC distributions of MRSA strains.

Genotype, study year	Number (cumulative %) at each vancomycin MIC				
	≤0.25	0.5	1	2	4
CA-MRSA					
2007 (n=75)		17 (22.7)	58 (100)		
2008 (n=75)		17 (22.7)	58 (100)		
2009 (n=74)		8 (10.8)	64 (97.3)	2 (100)	
2010 (n=85)		11 (12.9)	74 (100)		
HA-MRSA					
2007 (n=305)	5 (1.6)	21 (8.5)	275 (98.7)	4 (100)	
2008 (n=187)		17 (9.1)	161 (95.2)	8 (99.5)	1 (100)
2009 (n=152)		14 (9.2)	131 (95.4)	7 (100)	
2010 (n=131)		9 (6.9)	116 (95.4)	6 (100)	

Table 3. Detection of hVISA in MRSA strains from across Canada.

Phenotype	Vancomycin MIC (no. tested)			
	0.5 μg/ml (n=31)	1 μg/ml (n=230)	2 μg/ml (n=27)	Total (n=288)
hVISA+ by Etest macromethod [n (%)]	0 (0)	17 (7.4)	15 (55.6)	32 (11.1)
hVISA+ by population analysis profile [n (%)]	0 (0)	1 (0.4)	7 (25.9)	8 (2.8)

Of the 8 hVISA identified by PAP-AUC, 7 (87.5%) had vancomycin MICs of 2 μg/ml and belonged to healthcare-associated *spa* types. hVISA were predominantly isolated from blood cultures (4/8, 50.0%) of male patients (6/8, 75.0%) between the ages of 18 and 64 (6/8, 75.0%; mean age, 55.5 years) from Ontario (7/8, 87.5%).

Table 4. Comparison of antibiotic resistance rates among CA-MRSA and HA-MRSA strains.

Antibiotic	CA-MRSA (n=309)						HA-MRSA (n=775)						All MRSA (n=1112)					
	MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category			MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category			MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category		
				S	I	R				S	I	R				S	I	R
Cefazolin	16	64	1 - >128	-	-	100.0%[a]	128	>128	1 - >128	-	-	100.0%[a]	64	>128	1 - >128	-	-	100.0%[a]
Ciprofloxacin	16	>16	0.25 - >16	35.1%	1.0%	64.0%	>16	>16	0.25 - >16	2.8%	0.0%	97.2%	>16	>16	0.25 - >16	13.9%	0.3%	85.9%
Clarithromycin	>16	>16	≤0.25 - >16	24.4%	0.3%	75.3%	>16	>16	≤0.25 - >16	4.5%	0.0%	95.5%	>16	>16	≤0.25 - >16	11.8%	0.1%	88.1%
Clindamycin	≤0.25	>8	≤0.25 - >8	86.7%	0.0%	13.3%	>8	>8	≤0.25 - >8	28.8%	0.1%	71.1%	>8	>8	≤0.25 - >8	46.3%	0.1%	53.6%
Daptomycin	0.25	0.25	0.12 - 1	100.0%	-	-	0.12	0.25	0.06 - 1	100.0%	-	-	0.25	0.25	0.06 - 1	100.0%	-	-
Levofloxacin	4	8	0.12 - 32	39.9%	0.0%	60.1%	>32	>32	0.12 - >32	2.9%	0.0%	97.1%	>32	>32	0.12 - >32	14.1%	0.0%	85.9%
Linezolid	2	2	1 - 4	100.0%	-	-	2	2	≤0.12 - 4	100.0%	-	-	2	2	≤0.12 - 4	100.0%	-	-
Moxifloxacin	2	2	≤0.06 - 8	37.0%	5.5%	57.5%	8	>16	≤0.06 - >16	2.9%	0.1%	97.0%	8	>16	≤0.06 - >16	14.5%	1.6%	83.9%
Tigecycline	0.25	0.25	0.06 - 0.5	100.0%	-	-	0.25	1	0.12 - 0.5	99.7%	-	-	0.25	0.5	0.06 - 1	99.8%	-	-
TMP-SMX	≤0.12	≤0.12	≤0.12 - 2	100.0%	0.0%	0.0%	≤0.12	8	≤0.12 - >8	87.5%	0.0%	12.5%	≤0.12	0.25	≤0.12 - >8	91.4%	0.0%	8.6%
Vancomycin	1	1	0.5 - 2	100.0%	0.0%	0.0%	1	1	≤0.25 - 4	99.9%	0.1%	0.0%	1	1	≤0.25 - 4	99.9%	0.1%	0.0%

[a] Based on cefoxitin disk test.

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