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Comparison of Community-Associated and Healthcare-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Canada: CANWARD 2007-2009

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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. 2009, 889 MRSA were collected from patients attending hospital clinics, emergency rooms, medical/surgical wards and intensive care units in Canadian hospitals. Susceptibilities to β-lactams, ciprofloxacin (CIP), clarithromycin (CLR), clindamycin (CLD), trimethoprim/sulfamethoxazole (SXT) and vancomycin (VAN) were determined by CLSI broth microdilution. Isolates were characterized by *spa* typing, SCC*mec* typing, *agr* typing and PCR of the Panton-Valentine leukocidin (PVL) gene. Detection of hVISA was performed by the Etest macromethod.
Results: Of the 889 MRSA, 224 (25.2%) were CA-MRSA and 644 (72.4%) were HA-MRSA. The prevalence of CA-MRSA increased from 19.5% in 2007 to 31.9% in 2009 (p<0.001). CMRSA10/USA300 (73.7%) was the predominant CA-MRSA epidemic type; the most common HA-MRSA epidemic type was CMRSA2/USA100/800 (83.5%). CA-MRSA carried SCC*mec* type IVa (98.2%) and were largely *agr* type I (73.2%). Most HA-MRSA were SCC*mec* type II (81.2%) and *agr* type II (83.4%). PVL was detected in 201/224 (89.7%) CA-MRSA and 3/644 (0.5%) HA-MRSA. Resistance rates (CA vs HA) were 61.4 vs 97.3% to CIP, 72.7 vs 95.7% to CLR, 13.9 vs 72.0% to CLD and 0.0 vs 13.5% to SXT. The hVISA phenotype was detected in 10/21 (47.6%) MRSA with VAN MIC=2 µg/mL and 12/188 (6.4%) MRSA with VAN MIC=1 µg/mL.
Conclusions: CA-MRSA were significantly more susceptible to CIP, CLR, CLD and SXT than HA-MRSA. CA-MRSA were PVL(+), SCC*mec* type IVa and *agr* type I while HA-MRSA were PVL(-), SCC*mec* type II and *agr* type II. CA-MRSA represented 25.2% 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.
Recently, CA-MRSA genotypes have begun to replace healthcare-associated (HA)-MRSA in the hospital setting. These 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

889 isolates of MRSA were collected between January 2007 and December 2009, inclusive, as part of the ongoing CANWARD study assessing antibiotic resistance in Canadian hospitals. Isolates were received from tertiary-care medical centres (12 in 2007, 10 in 2008, 15 in 2009) 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-S20, 2010). 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).
Multiplex PCR was used to identify accessory gene regulator (*agr*) specificity groups (Shopsin et al. 2003. J. Clin. Microbiol. 41:456-459). Staphylococcal chromosome cassette *mec* (SCC*mec*) typing was also performed by multiplex PCR (Zhang et al. 2005. J. Clin. Microbiol. 43:5026-5033).

Detection of Heterogeneous Vancomycin-Intermediate *S. aureus* (hVISA)

MRSA isolates with a vancomycin MIC of 2 µg/ml were screened for the presence of the hVISA phenotype using the Etest macromethod in accordance with the manufacturer's instructions. All methicillin-susceptible *S. aureus* (MSSA) with a vancomycin MIC of 2 µg/ml as well as a random subset of MRSA and MSSA with a vancomycin MIC of 1 µg/ml (~25 and 20%, respectively) were included for comparison.

CONCLUSIONS

1. Overall, 25.2% and 72.4% 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 31.9% in 2009 while HA-MRSA decreased from 79.2% to 65.5% during this same period (p<0.001).
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 23.7% in 2009 (p<0.001).
3. CMRSA2 (USA100/800) was the predominant PFGE epidemic type among HA-MRSA [83.5%].
4. The majority (89.7%) of CA-MRSA were PVL(+). 10.3% of CA-MRSA and 99.5% of HA-MRSA were PVL(-).
5. Most CA-MRSA belonged to *agr* type I (73.2%) or III (26.3%). The majority of HA-MRSA belonged to *agr* type I (16.1%) or II (83.4%).
6. 98.2% of CA-MRSA carried SCC*mec* type IVa. Most HA-MRSA carried SCC*mec* type II (81.2%) or III (12.4%).
7. CA-MRSA strains were more susceptible to clarithromycin, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole than HA-MRSA.
8. Intermediate resistance (MIC, 4 µg/ml) to vancomycin was observed in one HA-MRSA strain. No MRSA were resistant to daptomycin, linezolid or tigecycline.
9. Among all MRSA tested, 10.5% were identified as hVISA. Since the majority (86.4%) of these hVISA strains were recovered from patients in Ontario (77.3% from a single hospital), clonal spread of hVISA may have occurred.

RESULTS

Figure 1. Proportion of MRSA strains identified as community- or healthcare-associated by *spa* typing.

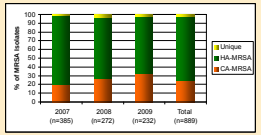

Figure 2. Distribution of PVL(+) and PVL(-) community- and healthcare-associated MRSA.

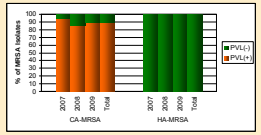

Figure 3. Distribution of *agr* types among community- and healthcare-associated MRSA.

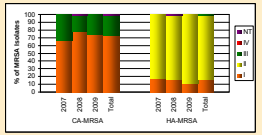

Figure 4. Distribution of SCC*mec* types among community- and healthcare-associated MRSA.

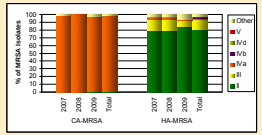

Figure 5. Distribution of community- and healthcare-associated PFGE epidemic types.

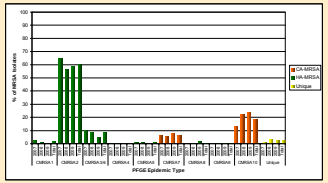

Figure 6. Distribution of PVL(+) and PVL(-) community- and healthcare-associated PFGE epidemic types.

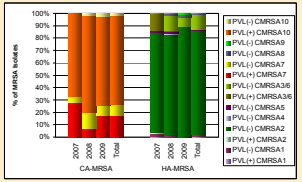

Table 1. Demographics of patients with community- and healthcare-associated MRSA infections.

Characteristic	HA-MRSA (n=644)	CA-MRSA (n=224)	Total (n=889)
Sex, n (%)			
Male	390 (60.6)	126 (55.8)	523 (58.8)
Female	254 (39.4)	99 (44.2)	366 (41.2)
Mean age, years	64.9	40.8	58.1
Median age (range)	67 (1-105)	42 (1-89)	61 (1-105)
Age group, n (%)			
≤ 17	7 (1.1)	30 (13.4)	44 (5.0)
18-64	268 (41.6)	167 (74.1)	444 (49.9)
≥ 65	369 (57.3)	28 (12.5)	401 (45.1)
Region, n (%)			
British Columbia/Alberta	132 (20.5)	68 (30.4)	202 (22.7)
Saskatchewan/Manitoba	46 (7.1)	72 (32.1)	123 (13.8)
Ontario	201 (31.2)	59 (26.3)	270 (30.4)
Quebec/Maritimes	265 (41.1)	25 (11.2)	294 (33.1)
Hospital ward type, n (%)			
Emergency room	100 (15.5)	91 (40.6)	198 (22.3)
Clinic/office	71 (11.0)	37 (16.5)	111 (12.5)
Intensive care unit	122 (18.9)	36 (16.1)	160 (18.0)
Medical/surgical ward	351 (54.5)	60 (26.8)	420 (47.2)
Infection site, n (%)			
Bloodstream	261 (40.5)	89 (39.7)	356 (40.0)
Respiratory tract	248 (38.5)	41 (18.3)	293 (33.0)
Urinary tract	33 (5.1)	1 (0.5)	35 (3.9)
Wounds/IV sites	102 (15.8)	93 (41.5)	205 (23.1)

Table 2. Detection of hVISA in *S. aureus* strains from across Canada .

hVISA Phenotype	MRSA [n (%)]			MSSA [n (%)]		
	Van MIC 1 µg/ml	Van MIC 2 µg/ml	Total	Van MIC 1 µg/ml	Van MIC 2 µg/ml	Total
+	12 (6.4)	10 (47.6)	22 (10.5)	7 (3.0)	2 (18.2)	9 (3.7)
-	176 (93.6)	11 (52.4)	187 (89.5)	223 (97.0)	9 (81.8)	232 (96.3)

hVISA were identified predominantly among MRSA strains from middle-aged (mean age, 57.2 years) males (16/22, 72.7%) from Ontario (19/22, 86.4%) and were most often isolated from bloodstream infections (14/22, 63.6%). The majority (19/22, 86.4%) of these hVISA were HA-MRSA (CMRSA2), *agr* type II and SCC*mec* type II.

Table 3. Comparison of antibiotic resistance rates among community-associated and healthcare-associated MRSA strains.

CA-MRSA (n=224)

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category S	I	R
Cefazolin	16	64	2 - >128	-	-	100.0%[a]
Ciprofloxacin	16	>16	0.25 - >16	37.7%	0.9%	61.4%
Clarithromycin	>16	>16	≤0.25 - >16	26.9%	0.5%	72.7%
Clindamycin	≤0.25	>8	≤0.25 - >8	86.1%	0.0%	13.9%
Daptomycin	0.25	0.25	0.12 - 1	100.0%	-	-
Levofloxacin	4	8	0.12 - 32	39.9%	0.0%	60.1%
Linezolid	2	2	1 - 4	100.0%	-	-
Moxifloxacin	2	2	≤0.06 - 8	39.9%	3.6%	56.5%
Tigecycline	0.25	0.25	0.06 - 0.5	100.0%	-	-
TMP-SMX	≤0.12	≤0.12	≤0.12 - 2	100.0%	0.0%	0.0%
Vancomycin	1	1	0.5 - 2	100.0%	0.0%	0.0%

HA-MRSA (n=644)

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category S	I	R
Cefazolin	128	>128	1 - >128	-	-	100.0%[a]
Ciprofloxacin	>16	>16	0.25 - >16	2.7%	0.0%	97.3%
Clarithromycin	>16	>16	≤0.25 - >16	4.3%	0.0%	95.7%
Clindamycin	>8	>8	≤0.25 - >8	27.8%	0.2%	72.0%
Daptomycin	0.12	0.25	0.06 - 1	100.0%	-	-
Levofloxacin	>32	>32	0.12 - >32	2.9%	0.0%	97.1%
Linezolid	2	2	≤0.12 - 4	100.0%	-	-
Moxifloxacin	8	>16	≤0.06 - >16	2.9%	0.2%	97.0%
Tigecycline	0.25	0.5	0.12 - 0.5	100.0%	-	-
TMP-SMX	≤0.12	8	≤0.12 - >8	86.5%	0.0%	13.5%
Vancomycin	1	1	≤0.25 - 4	99.8%	0.2%	0.0%

All MRSA (n=889)

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates per Category S	I	R
Cefazolin	64	>128	1 - 256	-	-	100.0%[a]
Ciprofloxacin	>16	>16	0.25 - 32	13.4%	0.2%	86.4%
Clarithromycin	>16	>16	≤0.25 - >16	11.7%	0.1%	88.2%
Clindamycin	>8	>8	≤0.25 - >8	44.0%	0.1%	55.9%
Daptomycin	0.12	0.25	0.06 - 1	100.0%	-	-
Levofloxacin	>32	>32	0.12 - >32	14.1%	0.0%	85.9%
Linezolid	2	2	≤0.12 - 4	100.0%	-	-
Moxifloxacin	8	>16	≤0.06 - >16	14.1%	1.0%	84.9%
Tigecycline	0.25	0.5	0.06 - 0.5	100.0%	-	-
TMP-SMX	≤0.12	1	≤0.12 - >8	90.3%	0.0%	9.7%
Vancomycin	1	1	≤0.25 - 4	99.9%	0.1%	0.0%

[a] Based on cefoxitin disk test.