Comparison of community-associated and health care-associated methicillin-resistant *Staphylococcus aureus* in Canada: Results from CANWARD 2007

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BACKGROUND: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) differ from health care-associated MRSA (HA-MRSA) in their genotypic and phenotypic characteristics. The purpose of the present study was to compare the demographics, antimicrobial susceptibilities and molecular epidemiology of CA-MRSA and HA-MRSA in Canada.

METHODS: In 2007, 385 MRSA isolates were collected from Canadian patients attending hospital clinics, emergency rooms, medical/surgical wards and intensive care units. Susceptibilities to beta-lactams, clarithromycin, clindamycin, daptomycin, levofloxacin, linezolid, moxifloxacin, tigecycline, trimethoprim-sulfamethoxazole and vancomycin were determined by Clinical and Laboratory Standards Institute broth microdilution. Strain typing was performed by pulsed-field gel electrophoresis (PFGE) and the mecA, nuc and pvl genes were detected by polymerase chain reaction.

RESULTS: Of the 385 MRSA, 19.5% were CA-MRSA and 79.2% were HA-MRSA as determined by PFGE. CA-MRSA belonged to PFGE types CMRSA10/USA300 (66.7%) and CMRSA7/USA400 (33.3%); PFGE types identified among HA-MRSA included CMRSA2/USA100/800 (81.6%), CMRSA6 (13.1%), CMRSA1/USA600 (1.3%), CMRSA5/USA500 (1.3%), CMRSA1 (0.3%) and CMRSA9 (0.3%). Panton-Valentine leukocidin (PVL) was detected in 94.7% of CA-MRSA and 0.7% of HA-MRSA. Resistance rates to all antibiotics tested were lower among CA-MRSA than among HA-MRSA. Of the 385 MRSA, 19.5% were CA-MRSA and 79.2% were HA-MRSA as determined by PFGE. CA-MRSA belonged to PFGE types CMRSA10/USA300 (66.7%) and CMRSA7/USA400 (33.3%); PFGE types identified among HA-MRSA included CMRSA2/USA100/800 (81.6%), CMRSA6 (13.1%), CMRSA1/USA600 (1.3%), CMRSA5/USA500 (1.3%), CMRSA1 (0.3%) and CMRSA9 (0.3%). Panton-Valentine leukocidin (PVL) was detected in 94.7% of CA-MRSA and 0.7% of HA-MRSA. Resistance rates to all antibiotics tested were lower among CA-MRSA than among HA-MRSA. Resistance rates to all antibiotics tested were lower among CA-MRSA than among HA-MRSA. Resistance rates to all antibiotics tested were lower among CA-MRSA than among HA-MRSA.

CONCLUSIONS: CA-MRSA represented 19.5% of all MRSA. CA-MRSA was significantly more susceptible to levofloxacin, clarithromycin, clindamycin and trimethoprim-sulfamethoxazole than HA-MRSA. Of CA-MRSA, 94.7% were PVL-positive while 99.3% of HA-MRSA were PVL-negative. CA-MRSA is an emerging pathogen in Canadian hospitals.

Key Words: Community-associated; Health care-associated; Methicillin-resistant *S. aureus*; Panton-Valentine leukocidin; Pulsed-field gel electrophoresis; SCCmec

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Since it was first identified in 1961, methicillin-resistant Staphylococcus aureus (MRSA) has been increasing in prevalence in the United States, Canada and throughout the world, and is now endemic in most areas. Traditionally, MRSA has been well recognized as a leading cause of nosocomial infections. Established risk factors for health care-associated MRSA (HA-MRSA) infection include recent hospitalization or surgery, stay in a long-term care facility, hemodialysis, comorbid conditions, the presence of a catheter and other indwelling medical devices and previous isolation of MRSA, as well as recent antibiotic use (particularly treatment with fluoroquinolones or cephalosporin antibiotics) (1,2). In the past decade, however, MRSA has also emerged as a significant community-associated (CA-MRSA) pathogen capable of causing disease in young, otherwise healthy individuals lacking traditional risk factors for MRSA acquisition/infection (3-5). Several reports have documented CA-MRSA infections among aboriginals, military recruits, intravenous drug users, correctional facilities, homeless persons, amateur and professional sports teams, day-cares and schools (6-9). Of particular concern is that CA-MRSA strains, in addition to skin and soft tissue infections, may be associated with severe disease including necrotizing pneumonia, bacteremia and septic shock, resulting in increased morbidity and mortality (2).

Recently, CA-MRSA has begun to replace HA-MRSA in the health care setting. The purpose of the present study was to compare the demographics, antimicrobial susceptibilities and molecular epidemiology of CA-MRSA and HA-MRSA in Canada.

METHODS

Study isolates

Three hundred eighty-five isolates of MRSA were selected from among 1482 S aureus collected between January and December 2007 as part of the ongoing Canadian Ward Surveillance Study (CANDWARD 2007) assessing pathogen prevalence and antibiotic resistance in Canadian hospitals. Isolates were received from 12 sentinel hospital sites that were geographically distributed in a population-based fashion in major cities in seven of the 10 Canadian provinces. Each centre was asked to submit pathogens (consecutive, one per patient per infection site) from blood, respiratory specimens, urine and wound/intravenous (IV) sites. Isolates were collected from Canadian patients affiliated with hospital clinics, emergency rooms, medical/surgical wards and intensive care units. 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, Manitoba) using the Clinical and Laboratory Standards Institute (CLSI)-approved disk diffusion method with cefoxitin, as well as by growth on MRSA Select chromogenic media (Bio-Rad Laboratories, Canada).

Antimicrobial susceptibility testing

The in vitro activities of cefazolin, clarithromycin, clindamycin, daptomycin, levofloxacin, linezolid, moxifloxacin, tigecycline, trimethoprim-sulfamethoxazole and vancomycin were determined by broth microdilution in accordance with CLSI guidelines (10). Minimum inhibitory concentration (MIC) interpretive standards were defined according to CLSI breakpoints (11). The following interpretive breakpoint (Food and Drug Administration, United States) was used for tigecycline: susceptible, 0.5 µg/mL or less (Tygacil package insert, Wyeth Pharmaceuticals Inc, USA).

Molecular characterization

MRSA status was confirmed by real-time polymerase chain reaction (PCR) of the meA and nuc genes (12). This triplex PCR assay also included primers for the detection of the lukF-PV and lukS-PV genes encoding the components of the Panton-Valentine leukocidin (PVL) toxin (12). Typing of the staphylococcal cassette chromosome mec (SCCmec) was performed by multiplex PCR as previously described (13).

MRSA strains were typed by pulsed-field gel electrophoresis (PFGE) of Smal digests following the Canadian standardized protocol (14). PFGE profiles were digitized for analysis with BioNumerics software (v3.5, Applied Maths Inc, USA), and strain relatedness was determined following established criteria (15). PFGE patterns were also compared with the national MRSA fingerprint database and were grouped into one of 10 Canadian epidemic PFGE strain types (CMRSA1 to CMRSA10) (16). For the purpose of the present study, CA-MRSA and HA-MRSA were defined genotypically (ie, on the basis of their PFGE epidemic type) and not epidemiologically as per Centers for Disease Control and Prevention criteria for distinguishing CA-MRSA from HA-MRSA, because epidemiological information was not available. Any MRSA with a CMRSA7 (USA400) or CMRSA10 (USA300) genotype was labelled as CA-MRSA while all other genotypes corresponding to a characterized epidemic type (eg, CMRSA1 [USA600], CMRSA2 [USA100/800], CMRSA3, CMRSA5 [USA500], CMRSA6, CMRSA9, etc) were labelled as HA-MRSA. Isolates that could not be defined as community- or health care-associated based on PFGE patterns were further characterized by staphylococcal protein A (spa) typing as described elsewhere (17). There has previously been shown to be good correlation between PFGE fingerprint clusters and spa types (17), allowing for further classification of strains as either CA-MRSA or HA-MRSA. MRSA with a PFGE pattern or spa type not associated with one of the known Canadian epidemic types were labeled as unique (non-CMRSA).

Statistical analysis

χ² analysis was used to evaluate statistical significance, as appropriate, using Graphpad Quickcalcs (www.graphpad.com/quickcalcs/index.cfm_).

RESULTS

Based on the genotypic definition of CA-MRSA and HA-MRSA described above, 75 (19.5%) of the 385 MRSA collected during the CANDWARD 2007 study were categorized as CA-MRSA while 305 (79.2%) were classified as HA-MRSA. The remaining five (1.3%) MRSA isolates could not be designated as CMRSA1 to CMRSA10 by either PFGE or spa typing and were therefore deemed unique. The patient demographics of the MRSA strains are shown in Table 1. Both HA-MRSA and CA-MRSA were isolated from all regions of the country. Among the CA-MRSA, 54.7% (41 of 75) were isolated from male patients while 45.3% (34 of 75) were collected from female patients. Of the HA-MRSA cohort, 63.6% (194 of 305) were from males and 36.4% (111 of 305) were from females. A significant trend (P<0.0001) toward younger patient age was
observed for CA-MRSA (mean age 36.9 years) compared with HA-MRSA, where older patients were more commonly affected (mean age 62.4 years).

Table 2 shows the distribution of CA-MRSA and HA-MRSA strains by hospital ward type as well as by specimen source. The majority (35 of 75, 46.7%) of CA-MRSA were isolated from patients presenting to hospital emergency rooms. A further 24.0% (18 of 75) of CA-MRSA were obtained from patients on medical/surgical wards while 14.7% (11 of 75) each were collected from patients admitted to intensive care units or attending hospital clinics. HA-MRSA were isolated predominantly from medical/surgical wards (162 of 305, 53.1%), followed by intensive care units at 20.0% (61 of 305), emergency rooms at 15.4% (47 of 305) and hospital clinics at 11.5% (35 of 305). Sites of CA-MRSA infection included wounds/IV (43 of 305, 14.1%) and the urinary tract (15 of 305, 4.9%).

Molecular analysis by PFGE revealed eight Canadian epidemic strain types and four unique (non-CMRSA) PFGE types among all 385 MRSA isolates (Table 3). CMRSA2 (USA100/800) was the predominant PFGE type among HA-MRSA (249 of 305, 81.6%). Other PFGE types identified among HA-MRSA included CMRSA6 (40 of 305, 13.1%), CMRSA5 (USA500) (10 of 305, 3.3%), CMRSA1 (USA600) (86.2%) isolates while the remainder (13 of 94, 13.8%) carried SCCmec type III. Among CA-MRSA patients, bloodstream and respiratory tract were the most common infection sites at 44.9% (137 of 305) and 36.1% (110 of 305), respectively. Additional sites of CA-MRSA infection included wounds/IV (43 of 305, 14.1%) and the urinary tract (15 of 305, 4.9%).

Table 2 shows the distribution of CA-MRSA and HA-MRSA isolates by hospital ward type and site of infection. CA-MRSA were isolated predominantly from medical/surgical wards (162 of 305, 53.1%), followed by intensive care units at 20.0% (61 of 305), emergency rooms at 15.4% (47 of 305) and hospital clinics at 11.5% (35 of 305). Sites of CA-MRSA infection included wounds/IV (43 of 305, 14.1%) and the urinary tract (15 of 305, 4.9%).

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The antimicrobial susceptibilities of CA-MRSA and HA-MRSA are shown in Table 4. CA-MRSA demonstrated lower MICs30 and MICs90 (MICs needed to inhibit 50% and
**DISCUSSION**

Infections caused by CA-MRSA are being reported with increasing frequency worldwide and represent a growing public health concern. These CA-MRSA strains differ from their health care-associated counterparts in their microbiological, epidemiological and molecular characteristics. HA-MRSA typically carry SCCmec types I to III, which contain the mecA gene responsible for resistance to beta-lactam antibiotics, and may also contain multiple determinants for resistance to other classes of antibiotics such as aminoglycosides, fluoroquinolones, macrolides and clindamycin (1). CA-MRSA, by comparison, usually carry unique SCCmec elements (type IV or V) and are often associated with the presence of the PVL toxin encoding a pore-forming protein involved in primary skin infection and pneumonia (1). Most CA-MRSA strains are resistant to beta-lactams but remain relatively susceptible to clindamycin and other non-beta-lactam antibiotics, with variable resistance to macrolides (1). CA-MRSA has also been found to be genotypically distinct from HA-MRSA, with most strains in Canada belonging to two common PFGE types (8,18).

In our study, 19.5% and 79.2% of MRSA strains from Canadian hospitals were identified by PFGE as CA-MRSA and HA-MRSA, respectively. CA-MRSA infection was strongly associated with patients in the younger (17 years or less) and the 18 to 64 years age groups, an observation that has been well documented in the literature. Although the majority of CA-MRSA were isolated from wounds and IV sites, CA-MRSA strains were also isolated from bloodstream and respiratory tract specimens, which is likely reflective of the ability of this organism to cause invasive infections including bacteremia and necrotizing pneumonia in addition to skin and soft tissue infections. HA-MRSA carried SCCmec type II or III, were almost exclusively PVL-negative and belonged primarily to the MRSA7 (USA400) genotype. By comparison, CA-MRSA carried only SCCmec type IV, were predominantly PVL-positive and belonged to either the CMRSA7 (USA400) or CMRSA10 (USA300) genotypes.

CMRSA7 (USA400) and CMRSA10 (USA300) are the predominant CA-MRSA strains circulating in North America and, although not as prevalent as they are in many centres in the United States, have been increasingly isolated in Canada since 2004 (8,18,19). Presence of the PVL toxin has frequently been linked to CA-MRSA infections and is hypothesized to

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MIC range</th>
<th>% of isolates per category</th>
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<tr>
<td>CMRSA7 (USA400)</td>
<td></td>
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<tr>
<td>Cefazolin</td>
<td>128</td>
<td>256</td>
<td>1-256</td>
<td>100.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Clarithromycin</td>
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<td>64</td>
<td>0.25-64</td>
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<td>32</td>
<td>0.12-16</td>
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<td>0.25</td>
<td>0.12-1</td>
<td>100.0</td>
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<td>128</td>
<td>0.12-64</td>
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<tr>
<td>TMP-SMX</td>
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<td>0.12</td>
<td>0.06-0.25</td>
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<td>Tigecycline</td>
<td>0.25</td>
<td>0.5</td>
<td>0.12-0.5</td>
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<tr>
<td>Vancomycin</td>
<td>1</td>
<td>2</td>
<td>0.5-1</td>
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| CMRSA10 (USA300) | | | | |
| Cefazolin | 128 | 256 | 1-256 | 100.0<sup>a</sup> |
| Clarithromycin | 32 | 64 | 0.25-64 | 97.7 |
| Clindamycin | 16 | 32 | 0.12-16 | 73.3 |
| Daptomycin | 0.12 | 0.25 | 0.12-1 | 100.0 |
| Levofloxacin | 64 | 128 | 0.12-64 | 7.7 |
| TMP-SMX | 0.12 | 0.12 | 0.06-0.25 | 100.0 |
| Tigecycline | 0.25 | 0.5 | 0.12-0.5 | 100.0 |
| Vancomycin | 1 | 2 | 0.5-1 | 100.0 |

<sup>a</sup>Based on cefoxitin disk test; I Intermediate; MIC Minimum inhibitory concentration in µg/mL; MIC<sub>50</sub>/<sub>90</sub> MICS necessary to inhibit 50%/90% of organisms; R Resistant; S Susceptible; TMP-SMX Trimethoprim-sulfamethoxazole

90% of organisms, respectively) values than HA-MRSA to all beta-lactams, including cefazolin, cefepime, ceftriaxone, meropenem and piperacillin-tazobactam (data shown only for cefazolin). CA-MRSA were also more susceptible to clarithromycin (resistance, 73.3% versus 96.7%), clindamycin (resistance, 12.0% versus 74.8%), fluoroquinolones (levofloxacin resistance, 58.7% versus 97.7%; and moxifloxacin resistance, 57.3% versus 97.4%) and trimethoprim-sulfamethoxazole (resistance, 0.0% versus 15.4%) than HA-MRSA (P<0.0001 in all comparisons). A comparison of antimicrobial susceptibility patterns of 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 (100% versus 20.0%, P<0.0001), levofloxacin (86.0% versus 4.0%, P<0.0001) and moxifloxacin (84.0% versus 4.0%, P<0.0001). Both CMRSA7 (USA400) and CMRSA10 (USA300) isolates had similar resistance rates to clindamycin (8.0% versus 14.0%, P=0.71) and trimethoprim-sulfamethoxazole (0.0% versus 0.0%). No MRSA, regardless of genotype, were resistant to daptomycin, linezolid, tigecycline or vancomycin.
play a significant role in increased disease severity (2,20). Recently, however, it has been shown that PVL is not present in all CA-MRSA strains and cannot be used as a definitive marker of CA-MRSA infection (20,21). In this study we found that 5.3% of CA-MRSA were PVL-negative while 0.7% of HA-MRSA were PVL-positive. The isolation of PVL-negative CA-MRSA and PVL-positive HA-MRSA, although rare, indicates that the epidemiology of MRSA in Canadian hospitals continues to change.

As previously described, CA-MRSA strains were more susceptible to beta-lactams, macrolides, clindamycin and fluoroquinolones compared with HA-MRSA. Both CA-MRSA and HA-MRSA displayed low rates of resistance (0.0% to 15.4%) to trimethoprim-sulfamethoxazole. This agent therefore remains a reasonable therapeutic option for empirical treatment of mild to moderate infections caused by CA-MRSA or HA-MRSA. As expected, all MRSA regardless of genotype were susceptible to daptomycin, linezolid, tigecycline and vancomycin. Interestingly, we observed a significant difference between the susceptibility of CMRSA7 (USA400) CA-MRSA and CMRSA10 (USA300) CA-MRSA. With high rates of resistance to clarithromycin and fluoroquinolones, CMRSA10 (USA300) isolates displayed an antimicrobial susceptibility profile intermediate between those of CMRSA7 (USA400) strains and those of HA-MRSA. This raises concerns that some community-associated strains may be able to acquire additional resistance determinants, resulting in an organism capable of causing serious disease that carries the PVL gene and displays a multidrug-resistant phenotype. Similarly, the acquisition of the PVL gene in existing HA-MRSA strains could likewise result in multidrug-resistant organisms with increased virulence, and is of equal concern.

Our study is not without its limitations. The Centers for Disease Control and Prevention criteria for distinguishing CA-MRSA from HA-MRSA state that a CA-MRSA infection can be defined as one that occurs in an individual with no established health care-associated risk factors and who has a positive MRSA culture within 48 h of hospitalization, has no previous history of MRSA infection or colonization, has no history of hospitalization, surgery, dialysis or residence in a long-term care facility within the past year, and has no permanent indwelling catheters or medical devices (22). Because the CANWARD study is a passive surveillance study involving microbiology laboratories, clinical and epidemiological information were not available. Therefore, CA-MRSA and HA-MRSA were defined genotypically based on PFGE epidemic types. Consequently, some CA-MRSA may have been misclassified as HA-MRSA and, conversely, some HA-MRSA may have been incorrectly labelled as CA-MRSA. Thus, the present study may not fully reflect the true proportion of CA- and HA-MRSA in Canadian hospitals. Secondly, due to limited resources, we did not perform SCCmec typing for all HA-MRSA isolates.

CONCLUSIONS

CA-MRSA appears to be an emerging pathogen in Canadian hospitals. As strains that were once thought to be associated only with infections in the community continue to disseminate into the hospital setting and, similarly, as health care-associated strains spread to the community, the distinction between CA-MRSA and HA-MRSA will continue to blur. Ongoing surveillance of the clinical, epidemiological and biological characteristics of CA-MRSA is necessary to increase our understanding of this important pathogen so that effective therapeutic options and infection control measures for combating the spread of CA-MRSA in both the community and the hospital can be established.

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