Community-associated methicillin-resistant *Staphylococcus aureus*: Continuing to evolve

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In the 2003 issue of *The Canadian Journal of Infectious Diseases*, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) as a community-acquired pathogen in Canada was reviewed (1). This followed reports of severe, invasive community-acquired MRSA (CA-MRSA) infections in children in Minnesota and North Dakota (USA), and recognition of its role as a pathogen in several Canadian outbreaks. The emergence of CA-MRSA as a pathogen of epidemiological significance in Canada prompted a meeting in 2005 sponsored by the Public Health Agency of Canada, the Ontario Ministry of Health and Long-Term Care and the Canadian Committee on Antibiotic Resistance, that made recommendations for its prevention and management; it was published in the *Journal* in 2006 (2). Since then, research has produced evidence that supports previous findings, challenges others and introduces new ones. Of particular interest is the appearance of CA-MRSA as a nosocomial pathogen, and the potential for its genetic intermingling with its hospital-acquired counterpart (HA-MRSA).

The genetic element coding for methicillin resistance is the *mec*A gene, located with additional genetic material in the *mec* element, also known as the staphylococcal chromosomal cassette (SCC*mec*) (3). Five major types (I to V) of SCC*mec* have been identified to date. A variety of molecular techniques are available to characterize bacterial isolates, including the *mec*A probe looking at polymorphisms in the region of the *mec*A gene, pulsed-field gel electrophoresis (PFGE) that examines the macrorestriction pattern of chromosomal DNA, probes to detect transposon (Tn) insertion patterns, multilocus-sequencing type based on DNA sequencing of the internal fragments of seven housekeeping MRSA genes, and *spa* typing based on the DNA sequencing of the polymorphic region of staphylococcal protein A (3). Used separately or together, these techniques have been used to discriminate among strains, and have allowed examination of the evolution of MRSA. Based on *mec*A polymorphisms, Tn554 insertion patterns and PFGE profiles, five major clonal lineages have been defined that account for approximately 70% of MRSA worldwide (3). This is different from the greater diversity of methicillin-susceptible *S aureus* (MSSA) strains, which have been around for centuries compared with MRSA's relatively recent appearance over the past 40 years. These MRSA clones (Iberian, Brazilian, Hungarian, New York/Japan and pediatric pandemic) have been named after the place in which they were first identified. Specific patterns of association between clonal types and SCC*mec* types have been identified (4). For example, the pediatric pandemic (community) MRSA clone has the SCC*mec* type IV element and the Hungarian and Brazilian clones carry SCC*mec* types III and IIIa elements, respectively (3).

Two distinct MRSA strains (USA300 and USA400), as distinguished by PFGE, are responsible for most CA-MRSA infections in the United States (5). In Canada, USA300 corresponds to CMRSA-10 and USA400 corresponds to CMRSA-7 (2). These strains belong to multilocus sequence types ST8 and ST1, and *spa* types t008 and t128, respectively, and both carry Panton-Valentine leukocidin (PVL) genes and the SCC*mec* type IVa element. These CA-MRSA strains, when compared with larger SCC*mec* types (I, II and III) prevalent in HA-MRSA, exhibit a high degree of horizontal transmissibility (6). Sequencing of USA300 and USA400 has identified a number of mobile genetic elements that encode for antimicrobial resistance and virulence, and provide the opportunity to better characterize disease pathogenesis (5,7). Sequencing, among other techniques, also allows for comparison between CA-MRSA and HA-MRSA strains (7). Several observations of differences between MW2, a USA400 isolate associated with invasive infection, and several HA-MRSA strains are of interest. Compared with two hospital strains, MW2 has few Tns (elements that are thought to confer adaptability to an adverse environment) (7). This is speculated to reflect a less hostile community environment, as opposed to the hospital environment. As with USA300, the *mec*A gene is carried on the smaller SCC*mec* type IVa element, which does not carry any of the multiple antibiotic resistance genes seen in the hospital SCC*mec* type II clones (7). Being a community strain, it is suggested that MW2/USA400 has less need for the resistance patterns of a HA-MRSA strain, which is exposed to a wider range of antimicrobials. Its *mec* element encodes for a number of different toxins, including PVL, enterotoxin H (a superantigen), plus 15 unique superantigen genes (7). It has been hypothesized that the smaller size of the SCC*mec* type IV element might facilitate its transfer on a plasmid or a bacteriophage to a susceptible recipient strain (8). Of note, however, is the presence of plasmids encoding resistance to tetracycline, macrolides, lincosamides and mupirocin in some USA300 strains, a harbinger of more difficult-to-treat infections in the future (5). Another interesting feature of the USA300 strain is an arginine deiminase system, which may enhance its ability to survive and grow within the host (5). *Staphylococcus epidermidis* also has this system and may be the source for the transfer of these genes into USA300 (5).

Of the large number of enzymes and toxins produced by *S aureus* (9), initial interest focused on the PVL gene as the

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major virulence factor. This was based on both epidemiological and clinical observations, as well as theoretical consideration. Leukocidins belong to a group of toxins that kill leukocytes by creating pores in their cell membranes. Laboratory testing of 117 CA-MRSA isolates from several countries across three continents found PVL genes in all, as well as a varied distribution of other genes (10). Gillet et al (11) found that PVL-producing *S aureus* caused rapidly progressive, hemorrhagic, necrotizing pneumonia, mainly in otherwise healthy children and young adults, compared with PVL-negative *S aureus*. Of interest, only one of the PVL-positive strains was methicillin-resistant. Among isolates from 14 cases of MRSA necrotizing fasciitis diagnosed in Los Angeles (USA) between 2003 and 2004, all carried the PVL genes but no other toxin genes (12). This certainly would raise suspicion of a major role for PVL as a virulence factor in severe disease. However, other studies suggest otherwise. Naimi et al (13) found exotoxin genes other than for PVL in their CA-MRSA clinical isolates. Examination of MRSA from purulent skin and soft tissue infections diagnosed in 11 American emergency departments in August 2004 found that USA300 accounted for 97% of isolates, and that the PVL gene was detected in 98% (14). In the same study, 31% of the MSSA strains were USA300 and 42% contained *pvl* genes. Although follow-up was limited in this study, with only 59% of patients contacted 15 to 21 days after their emergency department visit, 96% reported that their infection had resolved or improved. There were no differences in outcomes for patients with MRSA versus other bacterial pathogens, nor according to receipt of the appropriate antimicrobial. In addition to demonstrating that infections with CA-MRSA may not always be as severe as initially thought, this serves as an important reminder that incision and drainage is more important than antimicrobials for many purulent *S aureus* infections. Investigators in Calgary, Alberta, had similar observations regarding PVL. In a retrospective study (15) of MRSA isolates over a six-year period, two clones of the USA400 strain were found, one with and the other without the genes coding for PVL. Clinical characteristics in patients were the same for both these strains. Finally, in a mouse model of sepsis and abscess, MRSA strains lacking PVL were as virulent as those containing it (16). The authors suggested that while the toxin may be a marker for CA-MRSA strains, it is not the major virulence factor. However, the emergency department study (14) where 42% of MSSA had the genes for PVL suggests that it may be a marker with poor specificity. It is likely that the pathogenic potential of CA-MRSA relates to the contributions of different virulence factors (7).

While the literature documents a high proportion of MRSA among staphylococcal infections in the United States, the overall prevalence of MRSA colonization is not clearly known. As part of the 2001–2002 National Health and Nutrition Examination Survey (17), 9622 individuals had a nasal swab for *S aureus*. Only 75 were colonized with MRSA, with SCC*mec* types II and IV equally represented, for a prevalence of 0.84% in the noninstitutionalized American population. In contrast, 31.6% were colonized with MSSA. It is difficult to know how the epidemiology has evolved since 2001, but it should be noted that CA-MRSA had been well recognized as a pathogen before then or shortly thereafter in several American states (18-22). A recent review (23) noted that 7% of healthy children in Nashville (USA) were CA-MRSA colonized, which suggests that the findings from the National Health and Nutrition Examination Survey may no longer reflect the national picture in the United States. In a recent Canadian study, Al-Rawahi et al (24) found that in 2006, 18.6% of 300 injection-drug users tested were carrying MRSA, the majority of which were USA300, representing a steep and surprising rise from the 7.4% shown to be 'colonized' with the drug-resistant form of the bacteria in 2000 (24).

The many reports of CA-MRSA outbreaks identify similar risk factors for infection (2). These include young age, belonging to a minority population, incarceration, injection drug use and involvement in team sports. Many of these risks are associated in some way with overcrowding, close contact and potential for poor hygiene. It has long been recognized, however, that outbreaks may not reflect the usual situation in terms of transmission of infection. In one study (22) that looked at endemic community-acquired *S aureus* infections in Atlanta (USA), independent risk factors for CA-MRSA compared with MSSA were being of black ethnicity, female sex and hospitalization within the previous 12 months. Risk factors for CA-MRSA among patients presenting to 11 emergency departments were being of black ethnicity, use of antibiotics in the previous month, history of MRSA infection and close contact with a person with a similar infection (14). Curiously, CA-MRSA patients were also more likely to have reported a spider bite as the inciting event. While the authors reported these risk factors, they also pointed out that most patients without MRSA had at least one of these factors, and almost one-half without a risk factor had MRSA, laying doubt to the ability to correctly predict CA-MRSA as the pathogen, clearly impacting on empirical antimicrobial choices (14).

Molecular examination points to a very different genetic background for CA-MRSA and HA-MRSA strains, suggesting that CA-MRSA did not emerge from HA-MRSA (10). Furthermore, the genetic similarity of CA-MRSA and CA-MSSA clinical isolates suggests that they are related (14,25). Initial publications (18-20,25) pointed to the absence of prior hospitalization in patients with CA-MRSA. This is in support of the notion that CA-MRSA represents neither transfer of HA-MRSA into the community, nor transfer of HA-MRSA genetic elements to community strains of MSSA. However, as CA-MRSA has become more established as a pathogen, this hospital versus community distinction has become somewhat blurred. In the previously noted emergency department study (14), 27% of patients with CA-MRSA infections had at least one established risk factor for health care-associated MRSA and 18% had household contact with health care exposure (14). Hospitalization within the previous 12 months was an independent risk factor for infection with MRSA USA300 in the Atlanta cohort (22).

It is, therefore, not surprising that hospitals have begun to examine the role that CA-MRSA might be playing as a cause of nosocomial infections. Gonzalez et al (26) examined the clinical and laboratory features of MRSA associated with nosocomial blood stream infections at their Veterans Affairs hospital in Texas, between July 2003 and June 2004. They found that 65% of 37 MRSA isolates were SCC*mec* type IV, and 22 of these 24 isolates belonged to the USA300 clone. Thirty of these 37 infections were health care-associated, and 60% were caused by a CA-MRSA strain. In contrast to other studies of CA-MRSA, these authors found that one-quarter of their isolates were clindamycin- and tetracycline-resistant and more than one-half were fluoroquinolone-resistant. They

hypothesized that the antimicrobial selection pressures seen in hospitals may have contributed to a higher rate of resistance than usually seen in CA-MRSA strains (13,14,20,21,27). This finding is supported by a study (28) examining the clinical and molecular epidemiology of MRSA infections in a cohort of 100 patients hospitalized in Detroit, USA. The SCC*mec*-IV HA-MRSA strains had higher rates of susceptibility to clindamycin and levofloxacin compared with the HA-MRSA SCC*mec*-II/III strains, but lower susceptibility rates compared with true community strains of SCC*mec*-IV. Clinical outcomes were similar for the two groups. These authors commented that in patients with health care contact, SCC*mec*-IV MRSA strains exhibit characteristics similar to traditional HA-MRSA strains. Finally, the proportion of MRSA infections that were CA-MRSA increased from 11.8% in 2000 to 25% in 2005 in a Detroit dialysis cohort (29). Clinical outcomes were the same for patients with CA-MRSA and HA-MRSA. This suggests that the prevalence of CA-MRSA strains in health care facilities may well increase over time.

It is evident that this story of CA-MRSA has not ended. It remains to be seen how well established CA-MRSA will become as a cause of both community- and hospital-acquired infections in Canada. More will be learned about its virulence factors and under what circumstances they may be expressed. It shall be seen whether we become better at predicting who has an infection with a CA-MRSA strain, and whether the genetic elements of CA-MRSA and HA-MRSA mix in such a way as to create a new *S aureus* strain in this most adaptable of microorganisms.

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