

Laboratory Characterization of Methicillin-Resistant *Staphylococcus aureus* in Canadian Hospitals: Results of 5 Years of National Surveillance, 1995–1999

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Two thousand seven hundred eighty single-patient, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected between January 1995 and December 1999 at 17 tertiary care hospital sites across Canada were characterized by phenotypic and genotypic techniques. Six clonal types, as defined by pulsed-field gel electrophoresis, comprised 87% of all isolates and were labeled Canadian (C) MRSA-1 through -6. CMRSA-1 was the most prevalent clonal type, representing 45% of all MRSA. CMRSA-2 was indistinguishable from the New York clone and was more likely to be associated with community acquisition. CMRSA-3 was more likely to cause an infection, compared with the other CMRSA types. CMRSA-4 was indistinguishable from epidemic (E) MRSA-16 from the United Kingdom. Both CMRSA-5 and -6 occurred primarily in single-site, multiyear outbreaks. This study confirms that the epidemiology of MRSA in Canada is evolving, but most isolates at this time appear to belong to one of a small number of epidemic clones.

Shortly after the introduction of methicillin for clinical use, methicillin-resistant *Staphylococcus aureus* (MRSA) were identified in the United Kingdom [1, 2]. Since that time, MRSA have been identified in many countries [3–9] and become one of the most common causes of nosocomial infections in the United States, with 35% of nosocomial *S. aureus* being MRSA in hospitals reporting to the National Nosocomial Infection System in 1996 [10]. In Canada, the first report of MRSA occurred in 1981 [11]. Since then, numerous cases of MRSA at Canadian health care and child care centers have been reported, particularly in the 1990s [12–18]. National surveillance for MRSA has been conducted by the Canadian Nosocomial Infection Surveillance Program (CNISP) since 1995. The rate of MRSA increased from 1.0 detection/100 *S. aureus* isolates in 1995 to 6.0 detections/100 *S. aureus* isolates in 1999 (0.5 detection/1000 admissions in 1995 to 4.1 detections/1000 admissions in 1999; $P < .05$) [6].

The development of macrorestriction techniques and interpretation criteria has allowed MRSA surveillance to be conducted at a molecular level [19, 20]. To this end, comparisons have led investigators to speculate that certain molecular sub-

types of MRSA have higher transmission rates, longer persistence, and the ability to disseminate over vast geographic regions. These so-called “epidemic clones” have been reported on virtually every continent [3, 21–27]. Four Canadian “epidemic” MRSA strains have been identified and labeled Canadian (C) MRSA-1 through -4 [24]. Although the exact virulence factors determining the ease of transmission remain to be elucidated, CMRSA-1 exhibited normal transcription of RNIII, expressed a novel cell surface glycoprotein, and exhibited a unique polymorphism within the accessory gene regulator (*agr*) locus, whereas CMRSA-3 displayed attenuated activation of RNIII transcription, which suggests that both CMRSA-1 and -3 favor the colonization phase of infection [28]. The relative roles of the environment, antibiotic selective pressure, and sub-optimal hand hygiene in contributing to the epidemicity of these strains remain unclear.

The present study was undertaken to determine the prevalence and distribution of CMRSA subtypes over a 5-year period (1995–1999). Using a combination of phenotypic and genotypic methods, CMRSA strains were characterized. Herein, we discuss their distribution and potential implications for the health care system.

Materials and Methods

Surveillance Network

The CNISP, which was established in 1995, is a collaborative effort involving sentinel hospitals across Canada. The collaboration involves members of the Canadian Hospital Epidemiology Com-

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mittee (a subcommittee of the Canadian Infectious Diseases Society), the National Microbiology Laboratory, and the Centre for Infectious Disease Prevention and Control, Health Canada. The data presented in this study represent results obtained from the 17 CNISP hospital sites that have participated for the entire 5 years (1995–1999) of surveillance. To maintain site confidentiality, the data were tabulated into 3 geographic regions as follows: western, which included British Columbia (1 site), Alberta (2 sites), Saskatchewan (1 site), and Manitoba (1 site); central, which included Ontario (6 sites) and Quebec (2 sites); and eastern, which included Nova Scotia (2 sites), New Brunswick (1 site), and Newfoundland (1 site).

Study Design

Surveillance for MRSA was laboratory based, and all (non-repeat) strains from the 17 participating sites were sent to a reference laboratory. Hospital infection-control practitioners collected demographic and clinical information by patient interview and chart review. Patients with MRSA isolated from a clinical specimen were classified as infected or not infected by use of standard definitions for infection surveillance [29]. MRSA colonization was defined as the isolation of MRSA without clinical signs or symptoms of infection or when the criteria for infection were not met. An attempt was made to determine whether the MRSA was acquired in a hospital, a long-term care facility, or in the community. For MRSA colonization or infection to be defined as hospital-acquired, there had to be no evidence that the organism was likely to have been present at the time of hospital admission or that it was likely to have been acquired during a previous hospital admission.

Laboratory Methods

Bacterial strains. All isolates were identified at participating sites by routine methods performed at each laboratory. MRSA isolates were sent to a central laboratory, where they were subcultured to Columbia blood agar to ensure viability and purity. Stock cultures were stored at -70°C in Microbank vials (Pro-Lab Diagnostics). Epidemic MRSA clones that were used as controls for pulsed-field gel electrophoresis (PFGE) included the Iberian, Brazilian, Pediatric, Portuguese, Archaic, Hungarian, and New York clones [30] (provided by A. Tomasz, Rockefeller University, New York) and the epidemic (E) MRSA-15 and EMRSA-16 strains (provided by J. A. Lindsay, St. George's Hospital Medical School, London, United Kingdom).

Antimicrobial susceptibility testing. Resistance to oxacillin was confirmed by growth on an oxacillin agar screen plate (Mueller-Hinton agar supplemented with 4% NaCl and oxacillin, $6\ \mu\text{g}/\text{mL}$) incubated at 35°C for 24 h [31]. MICs to oxacillin, clindamycin, erythromycin, ciprofloxacin, fusidic acid, mupirocin, rifampin, vancomycin, teicoplanin, tetracycline, and trimethoprim-sulfamethoxazole were determined by broth microdilution in accordance with National Committee for Clinical Laboratory Standards guidelines [31].

Detection of the *mecA* and *nuc* genes. All isolates were confirmed as MRSA by detection of the *mecA* gene by polymerase chain reaction (PCR). Primer sequences designed to detect the *mecA* gene and PCR conditions were as described elsewhere [32]. Thermocycling conditions, using a GeneAmp 9600 thermocycler

(Perkin-Elmer Cetus), were as follows: 95°C for 2 min followed by 25 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 15 s. PCR amplicons were visualized on a 1% agarose gel after staining with ethidium bromide and were photographed under UV illumination.

Molecular typing by PFGE. Isolates were typed by PFGE following DNA extraction and digestion with *SmaI* [33]. PFGE-generated DNA profiles were digitized into the GelCompar computer software program (version 4.1; GelCompar) for analysis. DNA fragments on each gel were normalized using the molecular weight standard run on each gel to allow for comparisons between different gels. A 1.8% tolerance was selected for use during comparisons of DNA profiles. Cluster analysis was performed by the unweighted pair group method, using arithmetic averages, and DNA relatedness was calculated on the basis of the Dice coefficient. Isolates were considered to be genetically related if their macrorestriction DNA patterns differed by <7 bands [20], and the Dice coefficient of correlation was $\geq 75\%$.

Phage typing. For the first 3 years of the study (1995–1997), MRSA strains were phage typed according to the method of Blair and Williams [34], using the basic international set of typing phages. All phages were used at $100\times$ routine test dilution.

Definition of Epidemic Strains

A clonally related group of isolates was described as a CMRSA strain if the following conditions were met: the isolates were recognized to be clinically or epidemiologically significant (e.g., associated with outbreaks of infection in health care facilities), the strain was identified in patients from ≥ 5 hospital sites or from ≥ 3 geographic regions in Canada, and the strain has been characterized by standard typing methods [24].

Statistical Analysis

Categorical variables were compared with either the Fisher's exact test or the χ^2 test. The extended Mantel-Haenszel χ^2 test for trend was used to determine changes in proportions over time. Differences of $P < .05$ were considered to be statistically significant.

Results

MRSA isolates. A total of 2780 MRSA strains was submitted by the 17 sites over the 5-year period. All strains were confirmed as MRSA by detection PCR of the *mecA* gene. Over the course of the 5-year study, the western region submitted 1033 isolates, the central region submitted 1661, and the eastern region submitted 86. The breakdown of isolates by region and by year is shown in figure 1. The central region submitted the greatest percentage of isolates (60% [1661]), followed by the western (37% [1033]) and eastern (3% [86]) regions. Of 2587 case subjects, 1078 (42%) were reported as having infections. The western region reported the highest percentage (48% [428/895]), followed by the central (39% [625/1608]) and eastern (30% [25/84]) regions.

Macrorestriction analysis. All confirmed MRSA strains were characterized by macrorestriction analysis of *SmaI*-digested genomic DNA separated by PFGE. Of the 2780 DNA

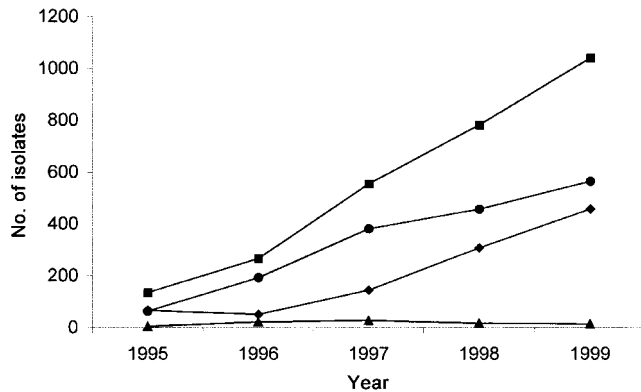


Figure 1. The no. of methicillin-resistant *Staphylococcus aureus*, by year, during a 5-year national surveillance program in Canada (1995–1999). The nos. of strains from the western (◆), central (●), and eastern (▲) regions and total nos. (■) are shown.

macrorestriction patterns analyzed, 360 unique pattern types were identified. Most isolates could be grouped into 6 major clusters that comprised 87% (2423) of all isolates. Four of these clusters have been described elsewhere as CMRSA strains (CMRSA-1 through -4) [24]. Representative CMRSA PFGE DNA macrorestriction patterns are shown in figure 2, and dendrograms representing the patterns from the different regions depicting the epidemic clusters are shown in figure 3.

CMRSA-1 was the most abundant of the epidemic Canadian strains, and, after it was initially identified in Ontario in early 1995, the strain was identified at other sites across Canada. There were changes over time; CMRSA-1 represented 5.9% of the isolates in 1995 and increased to 58% in 1997 ($P < .001$). There was a decrease in CMRSA-1 isolation rates after 1997. The CMRSA-1 cluster was represented by 56 PFGE subtypes, with most isolates (80%) displaying 1 of 2 PFGE patterns. All other patterns each comprised $<3\%$ of the total CMRSA-1 subtypes. The proportion of CMRSA-1 infections has significantly increased over time ($P < .001$), compared with CMRSA-2, -3, and -4. CMRSA-2 was the most widespread of the Canadian epidemic clones; it was isolated at all but 1 site in the present study (figure 3). The number of cases has continued to increase since it was first identified in Ontario in January 1995, the first month of the study. The CMRSA-2 cluster was composed of 81 different macrorestriction patterns, with most PFGE subtypes grouped into 2 different types representing 36% of CMRSA-2 isolates. The remainder of the PFGE types did not comprise $>5\%$ of the total CMRSA-2 isolated. The proportion of CMRSA-2 infections has decreased significantly over time ($P = .02$), compared with CMRSA-1, -3, and -4. After being first identified in early 1995, CMRSA-3 has decreased significantly over time ($P < .001$), from 33% (45) in 1995 to 1% (11) in 1999. CMRSA-3 constituted 19 different macrorestriction patterns, with 3 of them comprising 79% of isolates. The remainder of the PFGE types each represented $<5\%$ of the total. The proportion of CMRSA-3 infections

has decreased significantly over time ($P < .001$), compared with CMRSA-1, -2, and -4.

The number of CMRSA-4 cases has continued to increase since the strain was first isolated in May 1995 and comprised $\sim 5\%$ of all isolates in Canada over the 5-year period; however, the proportion of CMRSA-4 strains has significantly decreased over time ($P < .001$) from 12.5% of all cases in 1995 to 5.1% of all cases in 1999. CMRSA-4 consisted of a cluster of strains composed of 21 unique PFGE types, with the majority of isolates (64%) comprised of 2 PFGE types. No other type composed $\geq 5\%$ of the total isolates of CMRSA-4. The proportion of infections caused by CMRSA-4 has decreased significantly over time ($P < .001$), with the exception of an increase in 1996.

CMRSA-5 was characterized by a single DNA macrorestriction pattern. Although the strain has been observed at 5 sites in the western and central regions, which meets the proposed definition of a Canadian epidemic strain [24], 94% of the isolates have been identified at a single site in the western region. CMRSA-5 was virtually nonexistent for the first 2 years of the study, but infections rapidly increased in 1997 and reached a maximum in 1998, representing 9.5% of the total MRSA isolates for the year. For the years 1997–1999, in which CMRSA-5 was observed, there was a significant decrease in infections ($P < .001$) over time. CMRSA-6 is composed of strains with a single macrorestriction pattern, CDN-type 068, that may have descended from the CMRSA-3 type strain (CDN-type 060) (figure 2). Although the patterns differ by >7 bands, there was a subtype, labeled CDN-type 067, that shares matching patterns

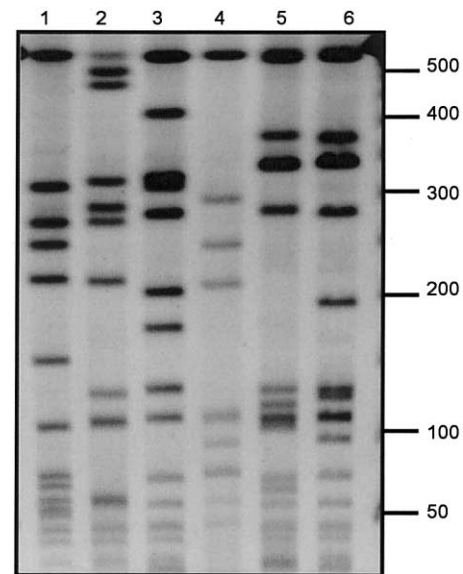


Figure 2. Representative DNA macrorestriction patterns of the various Canadian epidemic methicillin-resistant *Staphylococcus aureus* (CMRSA). Lanes: 1, CMRSA-1; 2, CMRSA-2; 3, CMRSA-5; 4, CMRSA-4; 5, CMRSA-3; and 6, CMRSA-6. Nos. at the side of the figure represent sizes of λ concatamers, in kilobases.

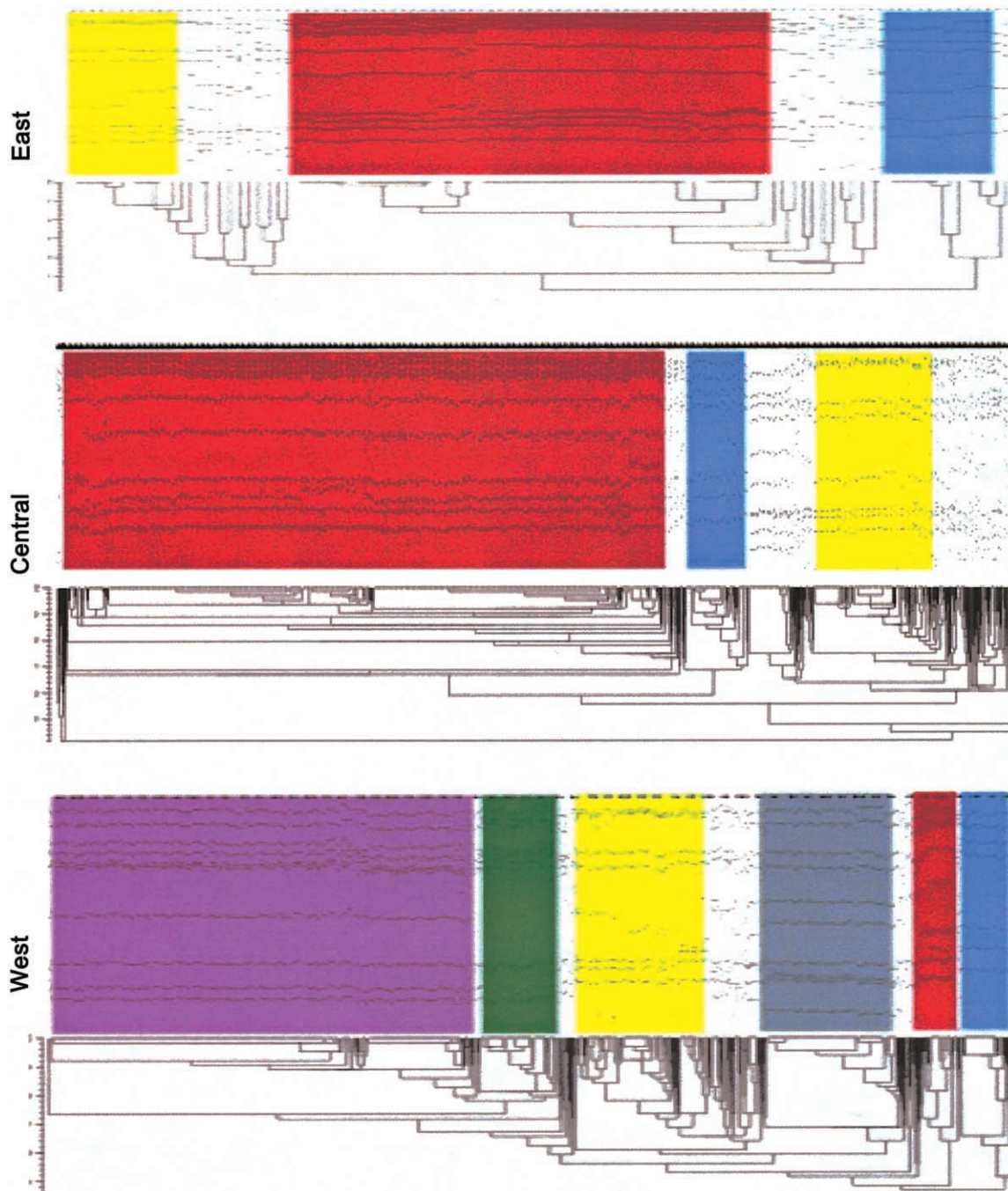


Figure 3. Dendrograms depicting the DNA macrorestriction patterns obtained for Canadian epidemic methicillin-resistant *Staphylococcus aureus* (CMRSA) during a 5-year hospital surveillance in the 3 regions of Canada. Colored blocks indicate clonal epidemic clusters as follows: red, CMRSA-1; yellow, CMRSA-2; green, CMRSA-3; blue, CMRSA-4; gray, CMRSA-5; purple, CMRSA-6.

(<7 bands) to both CMRSA-3 (CDN-type 060) and CMRSA-6 (CDN-type 068). CDN-type 067 was identified only 16 times, predominantly in 1995 (14/16 isolates), and was identified at the same site that CMRSA-3 and CMRSA-6 populate. Although the number of cases caused by CMRSA-6 were negli-

gible in 1995 through 1997, there was a statistically significant increase in its numbers and rates, from <1% of infections from 1995 to 1997 to 35% and 27% of infections in 1998 and 1999, respectively ($P < .001$). There was a proportionate decrease of infections from 1998 to 1999 involving CMRSA-6 ($P = .04$).

CMRSA-6 also met the definition of a Canadian epidemic strain, although the vast majority of isolates have been identified from a single site in the western region.

Phage typing and CMRSA strains. A total of 1150 MRSA isolates was phage typed over the initial 3 years of the study. Of the 850 CMRSA-1 isolates typed, most (82% [697]) were phage type (PT) 95. All other CMRSA-1 strains tested reacted with PT95 as well as other phages, with the exception of 50 isolates (5.7%) that were nontypeable (NT). Of the 220 CMRSA-2 isolates tested, 48 (22%) were NT. The remaining isolates fell into a very diverse set of ~100 different PT, although the strains reacted primarily with the group II phages. Of the 101 CMRSA-3 strains examined, only 12 isolates were NT. As with CMRSA-2, CMRSA-3 appears to have a diverse set of types, although all typeable strains reacted with phage 85 and 21 (21%) were PT85. Most isolates reacted with Group III phages. Of the 95 CMRSA-4 isolates typed, 14 (15%) were NT, and the others displayed a wide variety of types that reacted with Group I, II, or III phages. Of the 64 CMRSA-5 isolates tested, 87.5% (56) were NT, with the remainder reacting primarily with PT85. Because of the late onset of CMRSA-6, only 5 of 434 isolates were phage typed: 3 were PT84, 1 was PT85, and 1 was NT.

CMRSA patient demographics. The acquisition patterns (community or facility acquired) of the various CMRSA isolates are shown in table 1. About 94% (1176) of the CMRSA-1 isolates were found in the central region, reporting from 7 sites, followed by the eastern region, which reported 54 (4.2%) isolates from 3 sites, and the western region, which reported 29 (2.2%) isolates from 4 sites. Age was recorded for 1278 cases: 14 were among children 0–2 years old, 10 were among persons 3–20 years old, 394 were among persons 21–64 years old, and 860 were among persons ≥65 years old. Individuals with community-acquired cases were more likely to be <65 years of age than were those with hospital-acquired cases ($P < .001$; relative risk [RR], 1.66; confidence interval [CI], 1.26–2.18). Five hundred ten isolates were from females, and 774 isolates were from males.

CMRSA-2 was widely distributed and was identified at 16 sites. It was more uniformly distributed than CMRSA-1, with 37.5%, 59%, and 3.5% of the cases identified in the western, central, and eastern regions, respectively. Of the 316 cases of CMRSA-2, 202 (64%) were isolated from males. Of the 313 cases in which age was recorded, 17 were reported among children 0–2 years old, 21 were among persons 3–20 years old, 117 were among persons 21–64 years old, and 158 were among persons ≥65 years old. Of cases caused by the 6 epidemic strains, only CMRSA-2 cases were significantly more likely to report community-acquired acquisition ($P < .001$).

CMRSA-3 was primarily identified in the western region, with only 8% of the 123 isolates identified in the central region and a single isolate identified in the eastern region. Seventy-eight of the isolates (63%) were from males. Two isolates were from children 0–2 years old, 3 were from persons 3–20 years old, 42 were from persons 21–64 years old, and 76 were from persons ≥65

Table 1. Acquisition pattern (by community or facility type) of Canadian epidemic methicillin-resistant *Staphylococcus aureus* (CMRSA) clonal types.

Epidemic strain	No. of isolates	No. (%) with identified acquisition	Place of acquisition, no. (%) of isolates		
			ACF	LTC	Community
CMRSA-1	1259	1004 (79.7)	821 (81.8)	139 (13.8)	44 (4.4)
CMRSA-2	316	236 (74.1)	158 (67.5)	37 (15.8)	41 (17.5)
CMRSA-3	123	99 (80.5)	90 (90.9)	2 (2)	7 (7.1)
CMRSA-4	152	125 (82.2)	109 (87.2)	7 (5.6)	9 (7.2)
CMRSA-5	139	94 (67.6)	87 (92.6)	3 (3.2)	4 (4.3)
CMRSA-6	434	369 (85.0)	367 (99.5)	0 (0)	2 (0.5)

NOTE. ACF, acute-care facility; LTC, long-term care. Percentages were determined using the value in column 3.

years old. PFGE CDN-type 060 was significantly more likely (63%) to cause an infection than were other CMRSA PFGE type strains (39%; $P < .001$). When analysis was conducted at only the sites where CDN-type 060 was isolated, patients had a 2 times greater risk of being infected versus colonized when they had this strain rather than other strains found at these sites ($P = .001$; RR, 2.03; CI, 1.29–3.21). In addition, CMRSA-3 infections were more likely than all the other epidemic strains to occur in persons ≥65 years of age ($P < .05$). Although this strain was primarily isolated in the western region, there was no statistically significant difference in infection rates between the different regions, suggesting that the increased infection rates were not due to a bias in reporting from one region.

CMRSA-4 was also widely distributed. Fourteen sites reported this strain: 35.5% were in the western region, 59.9% were in the central region, and 4.6% were in the eastern region. CMRSA-4 was isolated from males 60.5% of the time. Of the 150 cases in which age was recorded, 5 were reported among children 0–2 years old, 4 among persons 3–20 years old, 56 among persons 21–64 years old, and 85 among persons ≥65 years old.

CMRSA-5 was isolated almost exclusively from a single site in the western region; however, 5% were identified at 3 sites in the central region, and the strain was also identified at 1 other site in the western region. Fifty-six percent of the patients were male. Of the 136 cases for which age was recorded, 3 were among children 0–2 years old, 5 were among persons 3–20 years old, 69 were among persons 21–64 years old, and 59 were among persons ≥65 years old.

CMRSA-6 is similar to CMRSA-5 in terms of distribution. Almost all cases were reported from the same site in the western region; 1 case was reported in the central region. Sixty-seven percent of cases were reported among males. Of the 430 cases in which age was recorded, 5 were among children 0–2 years old, 11 were among persons 3–20 years old, 238 were among persons 21–64 years old, and 181 were among persons ≥65 years old.

Antimicrobial susceptibility testing. All MRSA isolates were tested for susceptibility to a panel of antimicrobials. Overall, 93% of the isolates were resistant to erythromycin and clin-

damycin, 87% were resistant to ciprofloxacin, 46% were resistant to trimethoprim-sulfamethoxazole, 42% were resistant to tetracycline, 3% were resistant to fusidic acid and rifampin, and 2% were resistant to mupirocin. No isolates had reduced susceptibilities to vancomycin or teicoplanin. Isolates of all clonal types were resistant, with some exceptions, to erythromycin, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and tetracycline. CMRSA-1 were typically susceptible to tetracycline (95%), CMRSA-2 were susceptible to trimethoprim-sulfamethoxazole (92%), and ~50% of CMRSA-3 isolates were resistant to mupirocin.

Comparison to international epidemic strains. DNA macrorestriction patterns of all Canadian isolates were compared with the Archaic clone (ATCC BAA-38), Iberian clone (ATCC BAA-44), Brazilian clone (ATCC BAA-43), Pediatric clone (ATCC BAA-42), Portuguese clone (ATCC BAA-40), Hungarian clone (ATCC BAA-39), New York clone (ATCC BAA-41), and 2 United Kingdom clones labeled EMRSA-15 and EMRSA-16. A dendrogram of the analysis is shown in figure 4.

The Hungarian and Portuguese clones showed a ≥ 7 band difference to all Canadian patterns examined. The Brazilian clone displayed some similarity to CDN-type 284 (82% similarity; 4 band difference), which was identified 4 times in the central region, at a single location in November 1998. Strains with PFGE CDN-type 284 were PT84 or NT, and all were isolated from colonized males >65 years of age and displaying resistance to the antimicrobials oxacillin, erythromycin, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole.

The Archaic clone displayed some similarity to CMRSA-5 (80% similarity; 5 band difference). The Iberian clone was iden-

tical to a single Canadian strain with PFGE CDN-type 160. This strain was identified in the western region in 1995 and was phage typed as PT85. The DNA macrorestriction pattern of Pediatric clone was indistinguishable from that of PFGE CDN-type 348, a Canadian strain isolated in the western region in 1999 from a patient of unknown age. This isolate was sensitive to most antimicrobials tested, displaying resistance to only oxacillin and erythromycin. The Canadian PFGE CDN-type 030 (CMRSA-2; 120 isolates) was indistinguishable from the New York clone, and EMRSA-16 was indistinguishable from PFGE CDN-type 050 (CMRSA-4; 81 isolates). CMRSA-1 (PFGE CDN-type 001) and CMRSA-3 (PFGE CDN-type 060) did not show a close relationship (<7 band differences) to the other epidemic clones examined.

Discussion

In a previous report from this surveillance system, we demonstrated that the incidence rates of MRSA in Canadian hospitals increased from 0.5 cases/1000 admissions in 1995 to 4.1 cases/1000 admissions in 1999, with a concordant increase in infection rates from 0.25 infections/1000 admissions in 1995 to 1.11 infections/1000 admissions in 1999 [6]. In the present study, we analyzed all nonrepeat MRSA strains identified over a 5-year period at 17 hospital sites across Canada. Because these sites represent a majority of medical teaching centers across the country, we believe that the data reported accurately reflect the epidemiology of MRSA in Canadian tertiary-care hospitals even though some differences may exist in the screening criteria of patients at individual hospital sites.

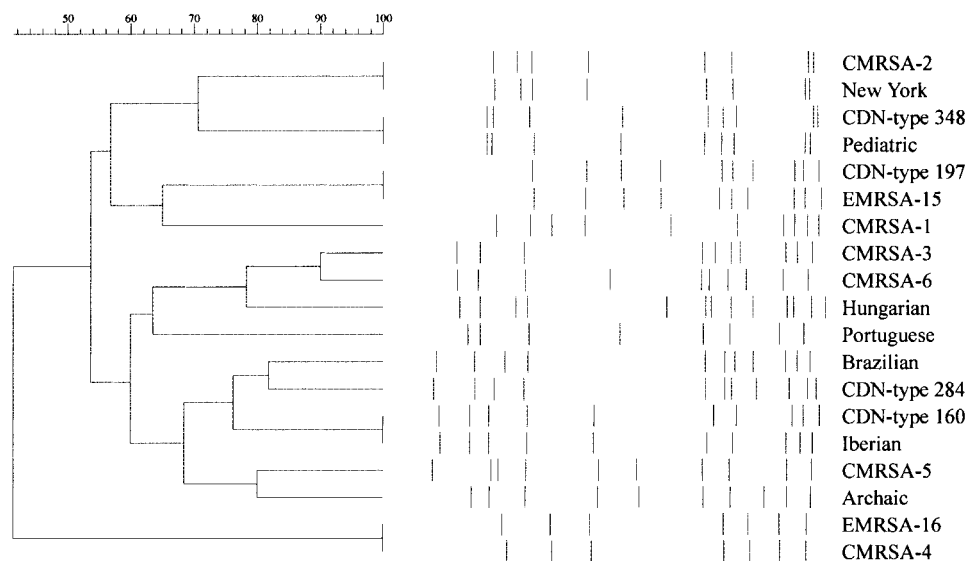


Figure 4. Dendrogram comparing internationally identified epidemic clones and closely related Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) isolates. Names of the epidemic clones or Canadian pulsed-field gel electrophoresis types are listed on the right side of the dendrogram.

This study has continued to document the dissemination of the 4 CMRSA strains and the emergence of 2 novel epidemic strains, CMRSA-5 and -6. In addition, some of the CMRSA strains are indistinguishable from epidemic strains in other countries. The PFGE subtypes of CMRSA-1 are not similar to the other epidemic clones studied in this report (figure 4); however, a Swiss group, using random amplification polymorphic DNA typing, determined that CMRSA-1 clustered with a number of epidemic strains from Belgium, Switzerland, and Germany, which suggests that this group of closely related isolates may be more fit to disseminate in a population [25].

CMRSA-2, the most widespread of the Canadian epidemic clones, was indistinguishable from the New York clone, which has been identified in a number of metropolitan hospitals [21, 35] and from health care facilities in Connecticut, New Jersey, and Pennsylvania [5]. In addition, this clonal type has been identified in a hospital in Tokyo [36], suggesting the global dissemination of this epidemic clone. We determined that CMRSA-2 is significantly more likely than other epidemic strains to be community acquired. A recent study conducted in New York City did not identify a significant number of individuals carrying MRSA in the community; however, this study focused on pediatric community acquisition [37], whereas most patients with community-acquired CMRSA-2 isolates in this study were between 21 and 64 years old.

CMRSA-3 was first identified in 1993 in British Columbia, Canada, and was linked to the Punjab region of India [38]. This strain was more likely than the other CMRSA strains to be associated with documented infections. The genetic determinants of the increased infection risks, if any, remain to be elucidated. However, hospital epidemiologists and infectious disease specialists should be aware of this when dealing with CMRSA-3 type isolates. Comparisons with other epidemic strains revealed that the predominant CMRSA-4 strain (PFGE CDN-type 050) was indistinguishable from EMRSA-16, an epidemic strain isolated in the United Kingdom [39].

This study also revealed that hospitals in Canada have been exposed to other reported epidemic strains, but these exposures were not followed by the widespread dissemination of clones that other countries have experienced under similar circumstances. For example, a single isolate (PFGE CDN-type 160), indistinguishable from the type strain representing the Iberian clone, was identified. The Iberian clone was first identified in Spain and Portugal and has since been reported in a number of other European countries [40]. It is not known why the Iberian clone did not spread after it was first identified in Canada in 1995; however, a similar observation has been reported in New York City, where the clone was detected in 2 affiliated hospitals [41] but has not spread to facilities in neighboring states as was observed with the New York clone [5]. In addition, the Brazilian clone (PFGE CDN-type 284) was identified in Canada 4 times during a single site outbreak in November 1998, 1 isolate was found to be indistinguishable from the previously

described pediatric clone (PFGE CDN-type 348), and an isolate (PFGE CDN-type 197) with a macrorestriction pattern indistinguishable from the EMRSA-15 strain was identified in 1997 (figure 4). It is not known why these previously reported epidemic strains have failed to disseminate in Canada. Perhaps the Canadian isolates, although displaying an indistinguishable macrorestriction patterns, do not harbor the same genetic determinants as the epidemic strains. A more likely explanation may be that after a single introduction, the infection-control measures that were implemented were sufficient to limit the spread of these epidemic strains.

We have identified 2 new epidemic strains, CMRSA-5 and -6, both of which emerged over the last 3 years of the study. Although both have been identified primarily from the same site in the western region, molecular epidemiologists should be aware of these emerging strains and the potential for rapid dissemination at a hospital site.

MRSA has long been considered a primarily nosocomial pathogen; however, as the prevalence of this drug-resistant organism increases in the community, a domestic focus may be of increasing importance to the hospital infection-control practitioner [42]. Endemic community-acquired strains of EMRSA-15 have led to confusion over the extensiveness of an outbreak. At a large teaching hospital in Manchester, United Kingdom, a retrospective study determined that a perceived large outbreak of EMRSA-15 was really from numerous sporadic cases and smaller clusters of EMRSA-15 variants [43]. Less than 15% of the cases reported in the present study were thought to have been acquired outside the hospital setting; however, a significantly higher proportion of CMRSA-2 isolates was identified from community sources. As CMRSA-2 rates continue to increase, it may become progressively more difficult to distinguish between nosocomial transmission and sporadic cases [43].

The genes responsible for the rapid spread of certain clonal types identified by PFGE over large geographic areas have not been determined. Blanc et al. [25] suggested that the spread of certain MRSA clones may be related to intrinsic factors (strain survivability, colonization ability, and antimicrobial resistance) and/or extrinsic opportunities, such as population migration, infection-control practices, and duration of stay. Laurent et al. [44] recently reported that a gentamicin-sensitive MRSA clone that has steadily increased in prevalence over the past 7 years in France has a shorter generation time than the gentamicin-resistant MRSA clones. The authors suggested that the fitness of the gentamicin-sensitive MRSA strains could account for the steady increase over the gentamicin-resistant MRSA strains. It will be interesting to compare the growth rates of the CMRSA strains with those of sporadic Canadian MRSA to determine if fitness is a common factor involved in the spread of epidemic strains.

In summary, Canada has experienced a rapid increase in MRSA over the last 5 years. Clones responsible for epidemics in other countries have been identified in Canada, although not

all of them have caused outbreaks. MRSA surveillance in Canada is continuing, and it is hoped that information gained will aid in improved infection control and the identification of virulence and fitness genes responsible for epidemicity of strains. With the development of a standardized MRSA PFGE protocol and implementation of quality-control programs in Canada [33] and by the use of server-based software for comparing DNA macrorestriction patterns by using the Internet (BioNumerics; Applied Maths), a real-time, molecular based surveillance system for MRSA is possible. The strains characterized in this study will provide a solid foundation for a database of unique pattern types for Canadian laboratories monitoring MRSA.

Canadian Nosocomial Infection Surveillance Program

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