

A preliminary guideline for the assignment of methicillin-resistant *Staphylococcus aureus* to a Canadian pulsed-field gel electrophoresis epidemic type using *spa* typing

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BACKGROUND: Increasing rates of methicillin-resistant *Staphylococcus aureus* (MRSA) infections on a global scale is a major health concern. In Canada, there are 10 known epidemic types of MRSA as determined by pulsed-field gel electrophoresis (PFGE). Despite the excellent discriminatory power of PFGE, there are several disadvantages of using this technique, such as high degree of labour intensity and the inability to easily develop an MRSA typing database due to the subjective interpretation of results.

OBJECTIVES: The purpose of the present study was to determine whether *spa* typing, an established DNA sequence-based typing method, could be used as an alternative to PFGE for the typing of Canadian MRSA (CMRSA) epidemic isolates.

RESULTS: *spa* types were determined for 1488 CMRSA isolates, and the method was analyzed for its ability to identify and cluster CMRSA1-10 strains. Minimal spanning tree analysis of 1452 *spa* types revealed individual clonal clusters for PFGE epidemic types CMRSA1, 2, 7 and 8, but *spa* typing could not distinguish CMRSA5 from CMRSA9 and CMRSA10, and CMRSA3 from CMRSA4 and CMRSA6. However, specific *spa* types were generally associated with only one PFGE epidemic type. Based on these results, a *spa* typing guideline for CMRSA isolates was developed and tested using the first 300 MRSA isolates received in 2007 through the Canadian Nosocomial Infection Surveillance Program.

CONCLUSIONS: The high concordance of *spa* types with PFGE epidemic types using this guideline demonstrated the feasibility of *spa* typing as a more rapid and less technically demanding alternative typing method for MRSA in Canada.

Key Words: Canada; CNISP; Methicillin-resistant *Staphylococcus aureus*; Pulsed-field gel electrophoresis, *spa* typing

Methicillin-resistant *Staphylococcus aureus* (MRSA) has had a dramatic impact on the health care systems of many countries worldwide. With rates of MRSA infections steadily increasing each year, it has become important to quickly distinguish strains of MRSA for the purpose of epidemiological investigation. MRSA isolates are generally characterized by pulsed-field gel electrophoresis (PFGE), which is a proven and very powerful technique for determining

Directive préliminaire pour la désignation *Staphylococcus aureus* méthicillino-résistant d'un type épidémique par électrophorèse en champ pulsé canadien utilisant le typage du gène *spa*

HISTORIQUE : Le taux croissant d'infections à *Staphylococcus aureus* méthicillino-résistant (ou MRSA, pour Methicillin-Resistant *Staphylococcus Aureus*) à l'échelle mondiale est un important problème de santé publique. Au Canada, on dénombre dix types épidémiques connus de MRSA déterminés par électrophorèse en champ pulsé (ÉCP). Malgré l'excellent pouvoir discriminant de l'ÉCP, cette technique comporte plusieurs inconvénients, notamment son coefficient de main-d'œuvre et la difficulté de mettre facilement au point une base de données pour le typage du MRSA en raison de l'interprétation suggestive des résultats.

OBJECTIF : Le but de la présente étude était de déterminer si le typage du gène *spa*, méthode éprouvée établie selon les séquences d'ADN, pourrait être utilisé à titre de solution de rechange à l'ÉCP pour le typage des isolats épidémiques de MRSA au Canada (CMRSA).

RÉSULTATS : Les types *spa* ont été déterminés pour 1 488 isolats de CMRSA et la méthode a été analysée sur le plan de sa capacité à identifier et à regrouper les souches 1 à 10 de CMRSA. L'analyse de l'arbre maximal-minimal de 1 452 types *spa* a révélé des complexes clonaux individuels pour les types épidémiques de CMRSA 1, 2, 7 et 8, déterminés par ÉCP, mais le typage *spa* n'a pas permis de distinguer le CMRSA5, du CMRSA9 et du CMRSA10, ni le CMRSA3 du CMRSA4 et du CMRSA6. Par contre, les types *spa* spécifiques ont en général été associés à un seul type épidémique déterminé par ÉCP. Selon ces résultats, des directives pour le typage du gène *spa* appliqués aux isolats de CMRSA ont été mises au point et testées sur les 300 premiers isolats de MRSA reçus en 2007 par l'entremise du Programme canadien de surveillance des infections nosocomiales

CONCLUSION : La forte concordance des types *spa* et des types épidémiques déterminés par ÉCP à l'aide de cette directive a démontré l'applicabilité du typage *spa* à titre de méthode de rechange plus rapide et moins exigeante sur le plan technique pour le typage du MRSA au Canada.

the degree of relatedness among isolates (1,2). The Canadian PFGE typing scheme and identification of Canadian 'epidemic' strains has allowed comparison with international MRSA clones (1,3).

In Canada, a 13-year national surveillance study through the Canadian Nosocomial Infection Surveillance Program (CNISP) has revealed 10 Canadian MRSA (CMRSA1-10) epidemic PFGE fingerprint clusters (1,3). In the early stages of surveillance,

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CMRSA1 (resembling USA600) was the most prevalent (1), but it is quickly being replaced by CMRSA2 (resembling USA100/USA800/New York), which accounted for approximately 55% of all strains in 2004 (3). CMRSA3 has virtually disappeared, being replaced by the closely related CMRSA6 (1,3). CMRSA8 is genetically similar to EMRSA15, a common European epidemic strain type (4). PFGE patterns of CMRSA7 and CMRSA10 were indistinguishable from USA400/MW2 and USA300, respectively, which are strain types linked with community-associated MRSA (CA-MRSA) outbreaks in Canada and the United States (5,6). The occurrence of CMRSA7 and CMRSA10 has increased in Canada over the past few years; however, CMRSA10 has become the more prevalent of the two (3). This has also been observed in the United States, where USA300 is more prevalent than USA400 (7).

Despite the excellent discriminatory power of PFGE, there are several disadvantages of using this technique, such as long wait times to obtain final results, a high degree of labour intensity, lack of standardization between laboratories and the inability to easily develop an MRSA typing database due to the subjective interpretation of results associated with gel-based systems (8,9). The desire to quickly and easily type MRSA led to the development of *spa* typing, a DNA sequencing technique that examines the polymorphic X or short-sequence repeat (SSR) region of the protein A gene (*spa*) (8). The SSR region consists of a variable number of short-sequence repeats, usually 24 base pairs long, and is located immediately upstream of the region encoding the C-terminal cell wall attachment sequence. Changes in the SSR region arise due to deletions, duplications and point mutations, which results in a diverse collection of '*spa* types', where each *spa* type consists of a specific combination of SSRs (10). Advantages of DNA sequencing as a method for strain typing include speed, ease of use, unambiguous data interpretation and the ability to easily develop an MRSA typing database.

The present study examines the validity and feasibility of using *spa* typing for national surveillance by comparing PFGE and *spa* types for 1488 CMRSA isolates obtained from across Canada over a 13-year time period. Results from the present study were used to create a preliminary guideline for Canadian laboratories interested in using *spa* typing as a technically less demanding method to identify CMRSA isolates.

METHODS

Bacterial isolates

A total of 1488 CMRSA isolates were selected from a collection of over 14,000 MRSA isolates obtained through the CNISP (1) and through routine services performed at the National Microbiology Laboratory (Winnipeg, Manitoba). Isolates were chosen on the basis of unique PFGE patterns that were associated with one of the 10 CMRSA strains (3). When possible, multiple isolates of the same epidemic PFGE pattern were chosen by varying geographical distribution and/or time of isolation. The samples were collected over a 13-year time span, between 1995 and 2007, and from across Canada (245 from British Columbia, 191 from Alberta, 83 from Saskatchewan, 119 from Manitoba, 471 from Ontario, 189 from Quebec, 78 from Newfoundland, 48 from New Brunswick, 50 from Nova Scotia and 14 from Prince Edward Island).

spa sequencing

DNA was prepared using a glass bead DNA extraction method. Briefly, a 1 µL loop was used to collect colonies from an

overnight culture grown on Tryptic Soy agar plates containing 5% sheep blood, and then emulsified in a microfuge tube containing 600 µL neutralization buffer (500 mL stock solution [15 mL 1 M Tris-HCl pH 8.4, 2 mL 0.5 M EDTA pH 9 and 483 mL deionized distilled water]) and 50 µL of cell disruption media (0.1 mM) (Scientific Industries Inc, USA). The samples were heated for approximately 3 min at 95°C to 100°C in a heating block, followed by disruption of cells for 3 min using a Genie vortex adaptor (Ambion, USA). The samples were then centrifuged at 3000 rpm for 1 min. Five microlitres of the supernatant was used as the template for the polymerase chain reactions (PCRs). PCR amplification of the *spa* repeat region was performed as previously described using primers, *spa*-1113f (5'-TAA AGA CGA TCC TTC GGT GAG C-3') and *spa*-1514r (5'-CAG CAG TAG TGC CGT TTG CTT-3') (10). For individual PCRs, amplicons were purified using YM-100 Microcon filters (Millipore, USA). For high throughput, Agencourt AMPure Reagent (Agencourt Bioscience Corporation, USA) was used to purify amplicons in a 96-well format. Purified amplicons were sequenced in-house by the DNA core facility (National Microbiology Laboratory). The DNA sequences of the *spa* repeat region in both directions were imported as ABI files and analyzed using the *spa* typing program provided with BioNumerics version 4.6 (Applied Maths Inc, USA). DNA sequences were compared using the *spa* typing Web sites <<http://tools.eugenomics.com/public/login.aspx>> and <<http://www.spaserver.ridom.de>>, the latter of which was developed by Ridom GmbH (Germany) and curated by SeqNet.org <<http://www.SeqNet.org/>> (10).

Cluster analysis was performed using the *spa* typing module of BioNumerics version 4.6. The default cost matrix was used to correct for the evolutionary distances between the repeats. Cluster analysis settings were set to 400% gap creation cost, 70% gap extension cost, 25% duplicate creation cost, 25% duplicate extension and a maximum duplication length of three repeats. The minimal spanning tree was calculated from the distance matrix using the default distance bin size of 100%. A total of 36 isolates had repeat successions (successions less than five), and were excluded from the cluster analysis as previously discussed (11).

PFGE

PFGE was performed as previously described (12). CMRSA1-10 were determined using guidelines as previously described (1,3).

RESULTS

spa typing

In total, 1488 CMRSA isolates representing 802 unique PFGE patterns (Figure 1) were chosen for *spa* typing in the present study. Multiple isolates (range two to 119) from 256 indistinguishable PFGE patterns were chosen by varying geographical distribution and/or time of isolation, when possible (refer to Table S1 provided online <www.pulsus.com>).

For the 1488 CMRSA isolates tested, *spa* typing revealed a total of 114 unique *spa* types averaging eight to nine repeats (Table 1). Comparison of *spa* types obtained from multiple isolates of 256 indistinguishable PFGE patterns revealed that 167 were associated with only one *spa* type. The remaining isolates from 89 indistinguishable PFGE patterns contained anywhere between two and five different *spa* types (Table S1).

Cluster analysis, based on the degree of similarity between *spa* repeat successions, was performed for 1452 isolates using

the *spa* minimal spanning tree application of BioNumerics version 4.6. This analysis revealed six *spa* clonal complexes (*spaCC*), which were arbitrarily named *spaCC*1-6 (Figure 2). *spaCC*1 contained only isolates with PFGE epidemic fingerprint types CMRSA1, *spaCC*2 contained only isolates with PFGE epidemic fingerprint types CMRSA2, *spaCC*3 contained only isolates with PFGE epidemic fingerprint types CMRSA7 and *spaCC*4 contained only isolates with PFGE epidemic fingerprint types CMRSA8. The remaining two clusters consisted of a mixture of PFGE types, which included isolates of PFGE epidemic fingerprint types CMRSA3, 4, and 6 in *spaCC*5 and CMRSA5, 9 and 10 in *spaCC*6 (Figure 1).

Despite the inability to clearly differentiate all PFGE epidemic types using *spa* clustering (Figure 2), the majority of individual *spa* types within these clusters (110 of 114) were only associated with a single PFGE epidemic type, which was even noted for *spaCC*4 (Table 1). On the basis of these results, a table is provided to be used as a preliminary guideline for *spa* typing of CMRSA epidemic isolates (Table 1).

Validation of the *spa* guideline

The first 300 MRSA isolates received in 2007 through the CNISP were used to validate the *spa* typing guideline. Resolved *spa* types and PFGE banding patterns were independently assigned CMRSA epidemic types and were then compared (refer to Table S2 provided online <www.pulsus.com>). Of the 300 isolates, 285 were assigned Canadian epidemic types (CMRSA1-10) using *spa* typing and the guideline alone. Of the 15 isolates that were not assigned epidemic types, eight contained *spa* types that were not listed in the guideline, but based on similarities of repeat successions; Panton-Valentine leukocidin (PVL) results were assigned to their corresponding PFGE epidemic types (CMRSA10 [t068, t1767, t2067, t3135 and t3154], CMRSA2 [t688] and CMRSA7 [t2593 and t555]) (Tables 1 and S2). Six isolates did not cluster with any of the 10 Canadian epidemic *spa* types, which included four isolates with *spa* types t216, t186 and t901. The other two were *spa* type t437, which is associated with a ST59 pandemic CA-MRSA strain (also referred to as USA1000/WA MRSA15), and one with t044, which is associated with a ST80 pandemic CA-MRSA strain (also referred to as the 'European CA-MRSA clone') (Table S2). The last nonassigned epidemic isolate was *spa* type t008 and was PVL-negative and, therefore, required PFGE for designation.

Of the same 300 isolates, 283 were assigned Canadian epidemic types (CMRSA1-10) using PFGE. PFGE was consistent for the six non-CMRSA epidemic isolates predicted using *spa* typing (Table S2). However, the PFGE banding pattern for the remaining 11 isolates clustered within a PFGE epidemic type, but they were not assigned to that epidemic cluster because they differed by seven or more bands from the epidemic type strain. These 11 nonepidemic-assigned isolates clustered within the same PFGE epidemic fingerprint types that were predicted using *spa* typing (Table S2).

Cost and time comparison of *spa* and PFGE

To compare the costs and required time for either *spa* typing or PFGE, a number of assumptions had to be made. For PFGE, the availability of two PFGE units for MRSA was assumed and for *spa* typing, in-house capability of DNA sequencing was assumed. Using these assumptions, the cost and time required to process 48 MRSA isolates were compared. For *spa* typing, the time

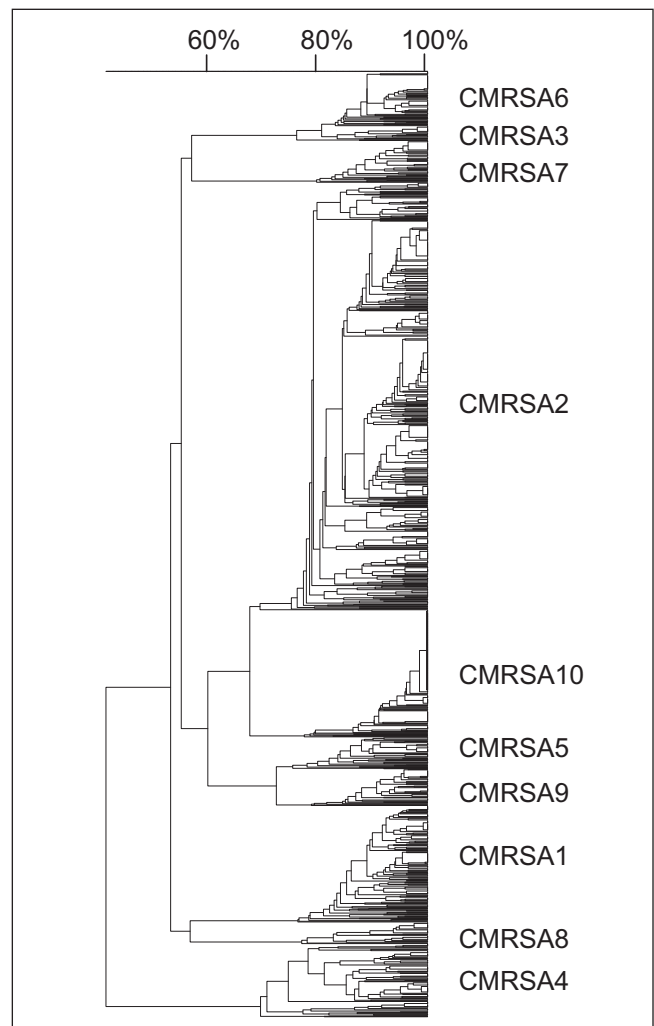


Figure 1 Pulsed-field gel electrophoresis dendrogram of 1488 Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) isolates (types 1 to 10)

required for DNA extraction, PCR, amplicon cleanup, DNA sequencing and the analysis of 48 isolates was estimated to be approximately 13 h. Whereas for PFGE, the time required to prepare and digest plugs, load and run gels, stain and destain gels, and the analysis of 48 isolates was estimated to be approximately 58 h. Estimated 'hands-on' technical times were approximately 6 h for *spa* typing and 12 h for PFGE. The estimated cost for *spa* typing of a single isolate was approximately between \$9.50 and \$12.00. Nonaccess to in-house DNA sequencing would obviously add to the cost and turnaround time of *spa* typing, but could be worth the increased objectivity of the analysis. The estimated cost of PFGE for a single isolate was approximately between \$9.50 and \$10.00. The following estimates will vary depending on laboratory capabilities and availability of equipment, but were used as baselines to compare the two methods.

DISCUSSION

spa typing and PFGE comparison

CMRSA isolates (n=1488) representing 802 unique PFGE patterns (Figure 1) were chosen for *spa* typing in the present study. A comparison of *spa* types and unique PFGE patterns

TABLE 1

Preliminary guideline for the assignment of Canadian epidemic types using *spa* typing (see also Table S1 <www.pulsus.com>). *spa* types indicated in bold were identified as Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) pulsed-field gel electrophoresis (PFGE) epidemic types from the 300 isolates used for validation from the 2007 Canadian Nosocomial Infection Surveillance Program and were subsequently added to the table (see also Table S2 <www.pulsus.com>)

Epidemic type	Ridom <i>spa</i> type	Number of isolates observed	Unique PFGE types	Ridom repeat succession	Kreiswirth repeat succession
CMRSA1 (n=183)	t004	123	85	r09r02r16r13r13r17r34r16r34	A2AKEEMBKB
	t015	2	2	r08r16r02r16r34r13r17r34r16r34	XKAKBEMBKB
	t026	3	2	r08r16r34	XKB
	t040	1	1	r09r02r16r13r17r34r16r34	A2AKEEMBKB
	t050	1	1	r08r16r02r16r34r34r17r34r16r34	XKAKBBMBKB
	t061	5	4	r09r02r16r13r34r17r34r16r34	A2AKEBMBKB
	t065	8	7	r09r02r16r34r13r17r34r16r34	A2AKBEMBKB
	t130	3	2	r09r34r13r17r34r16r34	A2BEMBKB
	t132	1	1	r09r34r16r34	A2BKB
	t1946	1	1	r09r34r13r13r17r34r16r34	A2BEEMBKB
	t1996	1	1	r08r16r02r16r34r34r34r16r34	XKAKBBBKB
	t266	1	1	r09r02r16r13r13r13r17r34r16r34	A2AKEEMBKB
	t2757	1	1	r08r18r1r34	X??B
	t2758	1	1	r08r16r18r2r16r34r17r34r16r34	XK??KBMBKB
	t282	1	1	r09r02r16r34	A2AKB
	t2988	1	1	r09r08r16r13r17r34	A2AKEMB
	t3031	1	1	r09r02r16r13r13r16r34	A2AKEEBK
	t330	2	2	r09r02r16r34r34r17r34r16r34	A2AKBBMBKB
	t362	13	13	r09r34	A2B
	t390	1	1	r08r16r16r34	XKKB
	t553	1	1	r09r13r13r17r34r16r34	A2EEMBKB
	t589	2	2	r08r16r02r16r34r34r13r17r34r16r34	TK
	t630	7	4	r08r16r02r16r34r17r34r16r34	XKAKBMBKB
t779	1	1	r08	X	
CMRSA2 (n=674)	t002	495	381	r26r23r17r34r17r20r17r12r17r16	TJMBMDMGMK
	t003	14	13	r26r17r20r17r12r17r17r16	TMDMGMMK
	t010	4	3	r26r17r34r17r20r17r12r17r16	TMBMDMGMK
	t014	4	4	r26r17r20r17r12r17r17r16	TMDMGMMMK
	t045	23	13	r26r17r20r17r12r17r16	TMDMGMK
	t062	7	4	r26r23r17r12r17r16	TJMGMK
	t067	11	9	r26r23r17r34r17r20r17r12r17	TJMBMDMG
	t088	7	6	r26r23r17r34r17r20r17r12r12r17r16	TJMBMDMGGMK
	t105	3	3	r26r23r17r34r17r20r17r17r16	TJMBDMMK
	t1080	1	1	r26r23r17r34r17r20r17r34r17r16	TJMBDMMBK
	t111	1	1	r26r23r17r16	TJMK
	t1154	1	1	r26r20r17r12r17r16	TDMGMK
	t1265	1	1	r26r23r17r34r17r20r17r12r12r16	TJMBMDMGGGK
	t1781	2	2	r26r16r16	TKK
	t179	2	2	r26r23r17r34r17r20r17r12r12r16	TJMBMDMGGK
	t1791	1	1	r26r23r17r34r17r20r17r20r17r12r17r16	TJMBMDMDMGMK
	t2066	1	1	r26r23r17r34r17r20r17r12r17r12r17r16	TJMBMDMGGMK
	t214	2	2	r26r23r17r34r17r20r17r12r17r16r16	TJMBMDMGKMK
	t2212	2	2	r26r23r17r34r22r17r12r17r16	TJMBLMGMK
	t2235	5	5	r26r23	TJ
	t2302	2	2	r26r23r17r34	TJMB
	t2358	1	1	r26r23r17r34r17r20r17r12r17r20r17r12r17r16	TJMBMDMGMDMGMK
	t242	29	28	r26r23r17r13r17r20r17r12r17r16	TJMEMDMGMK
t2528	2	2	r26r23r17r34r12r12r12r16	TJMBGGGK	
t306	1	1	r26r23r17r34r17r20r17r12r17r17r16	TJMBMDMGMMK	
t311	21	19	r26r23r17r34r20r17r12r17r16	TJMBDMGMK	

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TABLE 1 – CONTINUED

Preliminary guideline for the assignment of Canadian epidemic types using *spa* typing (see also Table S1 <www.pulsus.com>). *spa* types indicated in bold were identified as Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) pulsed-field gel electrophoresis (PFGE) epidemic types from the 300 isolates used for validation from the 2007 Canadian Nosocomial Infection Surveillance Program and were subsequently added to the table (see also Table S2 <www.pulsus.com>)

Epidemic type	Ridom <i>spa</i> type	Number of isolates observed	Unique PFGE types	Ridom repeat succession	Kreiswirth repeat succession
CMRSA2 (n=674)	t450	1	1	r26r23r17r34r16	TJMBK
	t539	15	11	r26r23r17r34r17r12r17r16	TJMBMGMK
	t548	6	6	r26r23r17r34r17r20r17r12r16	TJMBMDMGK
	t586	2	2	r26r16	TK
	t601	1	1	r26r23r17r34r34r17r20r17r12r17r16	TJMBBMDMGMK
	t640	1	1	r26r23r17r12r16	TJMGK
	t653	2	2	r26r17r12r17r16	TMGMK
	t668	1	1	r26r23r17r34r17r20r17r16	TJMBMDMK
	t688	1	1	r26r23r17r34r17r16	TJMBMK
	t854	1	1	r26r23r17r34r17r20r17r12r16r16	TJMBMDMGKK
	t954	1	1	r26r23r17r34r17r17r16	TJMBMMK
CMRSA3 (n=17)	t030*	1	1	r15r12r16r02r24r24	WGKAQQ
	t037*	15	13	r15r12r16r02r25r17r24	WGKAOMQ
	t138	1	1	r08r16r02r25r17r24	XKAOMQ
CMRSA4 (n=116)	t007	3	1	r15r12r16r16r16r16r02r25r17	WGKKKKAOM
	t012	38	25	r15r12r16r02r16r02r25r17r24r24	WGKAKAOMQQ
	t018	35	25	r15r12r16r02r16r02r25r17r24r24r24	WGKAKAOMQQQ
	t019	15	8	r08r16r02r16r02r25r17r24	XKAKAOMQ
	t021	7	6	r15r12r16r02r16r02r25r17r24	WGKAKAOMQ
	t037*	1	1	r15r12r16r02r25r17r24	WGKAOMQ
	t122	1	1	r08r16r02r16r02r25r17r24r24	XKAKAOMQQ
	t238	1	1	r15r21r12r16r02r16r02r25r17r24r24	WFGKAKAOMQQ
	t253	4	4	r15r12r16r02r16r02r25r17r24r24r24r24	WGKAKAOMQQQQ
	t275*	1	1	r15r12r16r02r25r17r24r24	WGKAOMQQ
	t318	3	2	r15r12r16r16r02r16r02r25r17r24	WGKKAKAOMQ
	t323	4	3	r15r12r16r02r16r34r17r24r24r24	WGKAKBMQQQ
	t338	2	1	r15r21r16r02r25r17r24	WFKAOMQ
	t638	1	1	r15r12r16r02r17	WGKAM
CMRSA5 (n=50)	t008*	2	2	r11r19r12r21r17r34r24r34r22r25	YHGFMBQBLO
	t051	1	1	r11r19r21r12r21r17r34r24r34r22r25	YHFGFMBQBLO
	t064	37	27	r11r19r12r05r17r34r24r34r22r25	YHGCMBQBLO
	t068	1	1	r11r19r19r12r21r17r34r24r34r22r25	YHHGFMBQBLO
	t1171	1	1	r11r19r12r17r34r24r34r22r25	YHGMBQBLO
	t190	2	2	r11r17r34r24r34r22r25	YMBQBLO
	t451	4	3	r11r12r05r17r34r24r34r22r25	YGCMBQBLO
	t460	1	1	r11r19r21r17r34r24r24r34r22r25	YHFMQBQBLO
	t951	1	1	r11r10r05r17r34r24r34r22r25	YC2CMBQBLO
	CMRSA6 (n=92)	t030*	1	1	r15r12r16r02r24r24
t037*		87	47	r15r12r16r02r25r17r24	WGKAOMQ
t074		1	1	r15r21r12r16r02r25r17r24	WFGKAOMQ
t129		1	1	r15r12r24	WGQ
t275*		1	1	r15r12r16r02r25r17r24r24	WGKAOMQQ
t388		1	1	r15r12r16r02r25r24	WGKAOQ
CMRSA7 (n=66)	t127	5	5	r07r23r21r16r34r33r13	UJFKBPE
	t1274	1	1	r07r23r21r16r16r33r21r16r33r21r16r33r13	UJFKKPFKPFKPE
	t128	50	21	r07r23r23r21r16r34r33r13	UJFKBPE
	t1508	1	1	r15r16r34r33r13	WKBPE
	t175	7	6	r07r23r21r16r16r33r21r16r33r13	UJFKKPFKPE
	t1784	1	1	r07r34r33r13	UBPE
t2207	1	1	r07r33r13	UPE	

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TABLE 1 – CONTINUED

Preliminary guideline for the assignment of Canadian epidemic types using *spa* typing (see also Table S1 <www.pulsus.com>). *spa* types indicated in bold were identified as Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) pulsed-field gel electrophoresis (PFGE) epidemic types from the 300 isolates used for validation from the 2007 Canadian Nosocomial Infection Surveillance Program and were subsequently added to the table (see also Table S2 <www.pulsus.com>)

Epidemic type	Ridom <i>spa</i> type	Number of isolates observed	Unique PFGE types	Ridom repeat succession	Kreiswirth repeat succession
CMRSA7	t2593	1	1	r07r23r21r34r33r13	UJFBPE
(n=66)	t555	1	1	r07r23r23r23r21r16r34r33r13	UJJFKBPE
CMRSA8	t005	1	1	r26r23r13r23r31r05r17r25r17r25r16r28	TJENCMOMOKR
(n=34)	t020	1	1	r26r23r1r29r17r31r29r17r25r17r25r16r28	TJNF2MNF2MOMOKR
	t022	8	7	r26r23r13r23r31r29r17r31r29r17r25r17r25r16r28	TJEJNF2MNF2MOMOKR
	t032	17	13	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	TJJEJNF2MNF2MOMOKR
	t1465	1	1	r26r23r23r13r23r31r29r17r31r29r17r24r25r17r25r16r28	TJJEJNF2MNF2MQOMOKR
	t2618	1	1	r26r23r13r23r31r05r17r25r17r25r17r25r16r28	TJEJNCMOMOMOKR
	t2818	1	1	r26r23r23r13r23r31r29r17r31r29r17r25r17r24r25r16r28	TJJEJNF2MNF2MOMQOKR
	t515	1	1	r26r23r23r13r23r31r29r17r31r29r17r25r16r16r28	TJJEJNF2MNF2MOKKR
	t578	2	2	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r28	TJJEJNF2MNF2MOMOR
	t891	1	1	r26r23r13r23r31r05r17r25r17r25r28	TJEJNCMOMOR
CMRSA9	t008*	57	35	r11r19r12r21r17r34r24r34r22r25	YHGFMQBLO
(n=58)	t955	1	1	r11r19r12r21r17r34r24r34r22r33r25	YHGFMQBLO
CMRSA10	t008*	186	37	r11r19r12r21r17r34r24r34r22r25	YHGFMQBLO
(n=198)	t024	4	1	r11r12r21r17r34r24r34r22r25	YGFMBQBLO
	t068	1	1	r11r19r19r12r21r17r34r24r34r22r25	YHHGFMQBLO
	t1705	1	1	r11r34r24r34r22r25	YBQBLO
	t1767	1	1	r11r19r12r21r17r34r24r24r34r22r25	YHGFMQBLO
	t2067	1	1	r11r19r12r21r66r34r24r34r22r25	YHGFF4BQBLO
	t3023	2	1	r11r19r12r19r17r34r24r34r22r25r25	YHG??MBQBLOO
	t3135	1	1	r11r19r34r24r34r24r34r22r25	YHBQBQBLO
	t3154	1	1	r11r19r12r21r17r34r24r05r25	YHGFMQBLO
	t351	1	1	r11r19r12r21r22r25	YHGFLO
	t574	2	1	r11r19r12r12r34r24r34r22r25	YHGGBQBLO
	t723	1	1	r11r19r12r34r22r25	YHGBLO
	t818	1	1	r11r19r12r21r17r34	YHGFMB

*Refer to discussion section for assignment of *spa* types t008, t030, t037 and t275 to a CMRSA epidemic type

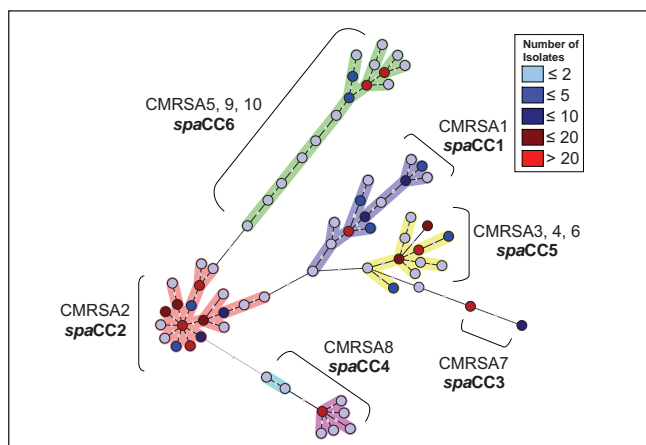


Figure 2) Minimal spanning tree analysis for the *spa* types of 1452 Canadian methicillin-resistant *Staphylococcus aureus* (CMRSA) isolates (types 1 to 10). Clusters of related *spa* types were arbitrarily assigned clonal complexes (*spaCC*1-6) and are indicated in bold. Pulsed-field gel electrophoresis epidemic types (CMRSA1-10) associated with each *spaCC* is also indicated

revealed that *spa* types were not confined to a specific PFGE banding pattern, and that indistinguishable PFGE patterns could contain anywhere from one to at least five different *spa* types. As a result, *spa* typing could not reliably be used as an indicator of a specific PFGE fingerprint type. However, closer examination of the repeat successions of the *spa* region for these isolates were similar (Table 1) and, therefore, a less discriminatory approach involving the clustering of related *spa* and PFGE epidemic types was examined.

Analysis of the clustering of related *spa* types revealed that two of the *spa* clonal clusters contained multiple PFGE epidemic types and, therefore, could not be used for the specific classification of PFGE epidemic types. The clustering of PFGE epidemic types CMRSA5, 9 and 10 in *spaCC*6 (Figure 2) was not surprising because a dendrogram generated from the PFGE fingerprint types of these strains indicated that they were closely related (Figure 1). Secondly, all three PFGE epidemic types, CMRSA5, 9 and 10, have the same multilocus sequence typing (MLST) designation (ST8) (Table 2). However, the clustering of PFGE epidemic types CMRSA4 with CMRSA3 and CMRSA6 in *spaCC*5 was initially surprising (Figure 2).

TABLE 2
International comparison of *spa* types commonly associated with Canadian methicillin-resistant *Staphylococcus aureus* strains 1-10 (CMRSA1-10)

Ridom <i>spa</i> type	Canadian epidemic type	MLST (CC)	Other associated MRSA clones*
t002	CMRSA2	ST5 (CC5)	CC5, Rhine Hesse MRSA (prototype), EMRSA-3, New York clone, Japan clone, Pediatric, USA100 ORSA II, USA800 ORSA IV, ST 5 ORSA I
t004	CMRSA1	ST45 (CC45)	CC45, Berlin MRSA (prototype), USA600 ORSA II, USA600 ORSA IV
t008	CMRSA9, CMRSA10	ST8 (CC8)	CC8, Northern German MRSA (subclone), USA300 ORSA IV (cMRSA in the United States), Archaic/Iberian, ST250 ORSA I
t018	CMRSA4	ST36 (CC30)	CC30, prototype of ST-36, EMRSA-16, USA200 ORSA II
t032	CMRSA8	ST22 (CC22)	Barnim MRSA (prototype & subclone), EMRSA-15, prototype of ST-22, CC22
t037	CMRSA3	ST241 (CC8)	CC8/239, Vienna MRSA, Brazilian/Hungarian, ST239 ORSA III, ST240 ORSA III, EMRSA-1, -4, -7, -9, -11
t064	CMRSA5	ST8 (CC8)	CC8, Archaic/Iberian, USA500 ORSA IV, USA500 ORSA II, ST8 ORSA I, ST8 ORSA IV, ST8 ORSA III
t128	CMRSA7	ST1 (CC1)	ST1 (related to USA400/MW2)

*Obtained from the Ridom Web site <<http://www.spaserver.ridom.de>> (10). CC Clonal complex; cMRSA Community-acquired MRSA; MLST Multilocus sequence typing

CMRSA3 and CMRSA6 are genetically similar by both MLST (Table 2) and PFGE (Figure 1), and would therefore be expected to be closely related. However, CMRSA4 is distantly related to both CMRSA3 and CMRSA6 when comparing these epidemic types in the PFGE dendrogram (Figure 1). In addition, CMRSA4 belongs to a different MLST CC in comparison with CMRSA3 and CMRSA6 (Table 2). Previously observed 'violations' of MLST ST assignment by *spa* typing have been attributed to intergenomic recombination involving the small region of the chromosome that *spa* typing focuses on (13). This was previously shown for lineages belonging to MLST CC8 and CC30 (14), and likely explains the clustering of *spa* types obtained from CMRSA4 with CMRSA3 and CMRSA6. As a result, isolates identified as unrelated by MLST, could be seen as related by *spa* typing because they would both share the same repeats in the polymorphic X region of the protein A gene. It is also interesting to note that CMRSA3 and CMRSA6 do not cluster with any of the MLST CC8 and CC30 lineages, thereby suggesting that PFGE is also unable to correctly classify recombinants from multiple genetic backgrounds. These violations cannot be resolved by *spa* typing alone, but are observed when both *spa* typing and MLST analyses are used.

Despite the inability to assign epidemic types on the basis of *spa* clustering analysis, small genetic differences within the *spa* region still enabled us to differentiate a CMRSA4 isolate from a CMRSA3 or a CMRSA6 isolate, which could be the result of micro- and/or macroevolution of this small chromosomal region over time following the recombination event (15).

Assignment of Canadian epidemic types using *spa* typing

In the present study, and many others (13,15,16), *spa* typing displayed excellent concordance with PFGE fingerprint clusters. For the four *spa* types, t008, t030, t037 and t275, where multiple PFGE epidemic types were found, additional molecular typing would be required for classification. For instance, *spa* type t008 was associated with three different PFGE epidemic types of CMRSA (CMRSA5, CMRSA9 and CMRSA10). However, of these three epidemic types, only CMRSA10 is known to carry PVL. Therefore, for isolates with *spa* type t008, an additional PCR reaction is proposed to detect the presence or absence of PVL. A PVL-positive result for a *spa* type t008

isolate would likely differentiate CMRSA10 from CMRSA5 and CMRSA9 isolates and could, therefore, be reported as CMRSA10. For PVL-negative strains, a PFGE is proposed for identification of CMRSA5, CMRSA9 or a PVL-negative CMRSA10 isolate. To date, CMRSA5 and CMRSA9 strains containing PVL have not been reported, and PVL-negative CMRSA10 isolates in Canada appear to be rare and should, therefore, not result in a large number of samples requiring PFGE.

Although not examined in the present study, additional PCR-based methods for delineating different strains of the same *spa* type could also include the use of staphylococcal cassette chromosome *mec* typing (17) or the arginine catabolic mobile element (18), alone or in combination with PVL, as an indicator of CMRSA10 strains.

spa type t037 was associated with three different PFGE epidemic types of CMRSA (CMRSA3, CMRSA4 and CMRSA6); however, the occurrence of *spa* type t037 in CMRSA4 was only seen once and might represent a rare recombination event. Both CMRSA3 and CMRSA6 are staphylococcal cassette chromosome *mec* type III (3), but MLST could be useful in differentiating *spa* type t037 isolates. However, MLST requires DNA sequencing of seven loci, which is an expensive and time consuming method. PFGE is, therefore, recommended to differentiate MRSA isolates of *spa* type t037. It should be noted that CMRSA3 has dramatically declined in Canada, and it is, therefore, expected that the majority of t037 isolates would be CMRSA6 (3). PFGE would also be recommended for *spa* types t030 and t275, which were both seen in two different epidemic PFGE types. To date, these *spa* types appear to be rare in Canada, having only been seen in four of the 1488 isolates tested, and would, therefore, not be expected to result in large numbers of samples requiring PFGE for identification.

The assignment of CMRSA epidemic types using the provided *spa* guideline (Table 1) was highly successful for the molecular classification of the first 300 MRSA isolates received in 2007 through the CNISP. The high discriminatory ability of *spa* typing has been attributed to the small size of repeats, which are more prone to duplication and deletion via slip-strand mispairing, and a high number of synonymous substitutions per synonymous site (dS=0.72) (15). These slow point mutations and fast-occurring changes in the number of repeats enables *spa*

typing to be effectively used in both long- and short-term epidemiologic studies (15). It should be noted that the use of *spa* typing in an outbreak setting was not assessed in the present study, but has previously been shown to be successful in outbreak investigations (8,10) and early warning systems for the detection of outbreaks (19). It is, therefore, suggested that the combination of PFGE and *spa* typing would be useful for obtaining additional comparative data in such settings and will be important for the continued national surveillance and reporting of MRSA in Canada. For instance, *spa* typing of the first 300 CNISP MRSA isolates received in 2007 revealed that performing PFGE alone potentially misclassified 11 isolates (3.6%) as nonepidemic (Table S2). This has identified a potential limitation of the past definition of epidemic CMRSA isolates in Canada, which is the assignment of epidemic types based primarily on comparisons of PFGE banding patterns with one representative epidemic type strain. *spa* typing of additional nonepidemic-assigned isolates is, therefore, warranted to examine possible alternative definitions of epidemic MRSA types in Canada.

International comparison of *spa* types

spa types are freely comparable using international databases such as Ridom (<http://www.spaserver.ridom.de>) (10), which currently contains over 3300 *spa* types that were obtained from the submission of over 47,000 isolates from 45 different countries. Briefly, *spa* types of MRSA strains defined as epidemic in Canada were also prevalent around the world (Table 2). Comparison of *spa* types obtained from the present study to the Ridom database also revealed two *spa* types, t044 and t437, associated with two pandemic CA-MRSA strains (ST80 'European' and ST59 clones) that have not previously been reported in Canada. The potential emergence of these two CA-MRSA clones in Canada should continue to be monitored.

SUMMARY

The high discriminatory power of PFGE provides an excellent tool for studying short-term outbreaks, and was supported in the present study and others as a useful method for long-term epidemiological surveillance studies. However, PFGE is labour-intensive and technically demanding. The dependence on strict adherence to standardization protocols between laboratories and the subjective interpretation of PFGE results has led to an inability to easily develop MRSA typing databases on a large scale. In comparison with PFGE, *spa* typing was capable of yielding results faster, enabled the processing of more isolates, did not require any type of subjective interpretation and was comparable in cost with PFGE. The *spa* data were also easily exportable, and could be uploaded rapidly into databases for 'real-time' surveillance of MRSA on a national or international scale. The provided guideline for the assignment of Canadian epidemic PFGE types using *spa* typing is preliminary and, therefore, a periodically updated table, including newly defined *spa* types, will be made accessible on-line <www.nml.ca> or available on request.

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