Cost-Effectiveness and Efficacy of spa, SCCmec, and PVL Genotyping of Methicillin-Resistant *Staphylococcus aureus* as Compared to Pulsed-Field Gel Electrophoresis

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Abstract

Pulsed-field gel electrophoresis (PFGE) is a valuable molecular typing assay used for methicillin-resistant *Staphylococcus aureus* (MRSA) surveillance and genotyping. However, there are several limitations associated with PFGE. In Alberta, Canada, the significant increase in the number of MRSA isolates submitted to the Provincial Laboratory for Public Health (ProvLab) for PFGE typing led to the need for an alternative genotyping method. In this study, we describe the transition from PFGE to *Staphylococcus* protein A (spa), Staphylococcal cassette chromosome (SCCmec), and Panton-Valentine leukocidin (PVL) typing. A total of 1915 clinical MRSA isolates collected from 2005 to 2009 were used to develop and validate an algorithm for assigning PFGE epidemic types using spa, SCCmec, and PVL typing and the resulting data was used to populate a new Alberta MRSA typing database. An additional 12620 clinical MRSA isolates collected from 2010 to 2012 as part of ongoing routine molecular testing at ProvLab were characterized using the new typing algorithm and the Alberta MRSA typing database. Switching to *spa*, SCCmec, and PVL from PFGE typing substantially reduced hands-on and turn-around times while maintaining historical PFGE epidemic type designations. This led to an approximate $77,000 reduction in costs from 2010 to 2012. PFGE typing is still required for a small subset of MRSA isolates that have *spa* types that are rare, novel, or associated with more than one PFGE epidemic type.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is widespread in hospital and community settings and has become progressively costly to treat and control [1–4]. In Alberta, Canada, MRSA was designated as a “pathogen under surveillance” since June 2005 by Alberta Health, requesting all regional laboratories to submit the first clinical MRSA isolate from each patient within a one year period to the Provincial Laboratory for Public Health (ProvLab) for molecular typing. Routine MRSA genotyping identifies trends in prevalence, distribution, and epidemiology in an effort to enhance patient outcome and reduce transmission. Global dissemination of MRSA is largely attributed to a small number of epidemic MRSA clones that are often predominant in specific geographic regions [5–7]. Ten Canadian pulsed-field gel electrophoresis (PFGE) epidemic types (CMRSA 1 to CMRSA 10) have been identified using PFGE by the Canadian Nosocomial Infection Surveillance Program [8,9].

Although PFGE characterization is useful, it is a labor-intensive and time-consuming technique. Furthermore, PFGE results are prone to subjective interpretation making inter-laboratory comparisons difficult [10]. The number of MRSA isolates submitted annually to ProvLab for genotyping has increased dramatically from 1999 to 2008: with an average of 93 isolates per year in 1999–2004, to an average of 3106 isolates per year in 2005–2008. The difficulties associated with PFGE are enhanced with this increase, making an alternative typing method necessary.

*Staphylococcus* protein A (*spa*) typing is a DNA sequencing assay that assigns *spa* types based on the repeats present in the polymorphic X region of the *Staphylococcus* protein A gene [11]. The National Microbiology Laboratory (NML) in Winnipeg, Canada, uses *spa* typing for MRSA characterization because of the high concordance observed between *spa* types and PFGE epidemic types [12–15]. However, some *spa* types correspond to multiple PFGE epidemic types and require additional molecular typing before they can be grouped into a PFGE epidemic type [12].

Golding et al., [12] propose that a PCR-based assay to detect the presence of Panton-Valentine leukocidin (PVL) [16] helps to differentiate these strains. Staphyloccocal cassette chromosome (SCCmec) typing targeting the *niv* gene complex could also further assist differentiation [17,18].

In this study, we describe the transition from PFGE-based assignment of Canadian PFGE epidemic types to using *spa*, SCCmec, and PVL typing. The cost, turn-around times, and assigned PFGE epidemic types using the two different methodolog-
Table 1. Association of MRSA PFGE epidemic types with spa, SCCmec, and PVL types in Alberta from June 2005 to March 2009.

| PFGE epidemic type | Ridom spa type | Kreiswirth repeat succession | SCCmec | PVL   | Total |
|--------------------|----------------|-------------------------------|--------|-------|-------|
| CMRSA 1 (USA600)   |                |                               |        |       |       |
| t004               | A2AKEEMBKB     | II                            |        | 2     |       |
| t026               | XKB            | IV                            |        | 2     |       |
| t065               | A2AKBEMBKB     | IV                            |        |       | 9     |
|                   |                | V*                            |        |       | 3     |
|                   |                | VI*                           |        |       | 1     |
| t1081              | XKAX2BMB       | IV                            |        |       | 1     |
|                   |                | V*                            |        |       | 1     |
| t1082              | XKBKAMK        | II                            |        | 1     |       |
| t116               | XKAKEEMBKB     | IV                            |        | 2     |       |
| t1248              | A2AKBEMBKE     | IV                            |        |       | 1     |
|                   |                | V*                            |        |       | 2     |
| t130               | A2EBMKB        | IV                            |        | 1     |       |
| t1768              | XKAX2BMBMB     | IV                            |        | 1     |       |
| t230               | XKAKB          | II                            |        | 1     |       |
| t371               | A2AKBKB        | II                            |        | 1     |       |
|                   |                | IV                            |        |       | 1     |
| t5497              | A2AK           | III                           |        | 1     |       |
| t5980              | A2AKBBBMBBBBBB | IV                            |        | 1     |       |
| t715               | A2AKBEMB       | IV                            |        | 1     |       |
| t779               | X               | IV                            |        | 1     |       |
| t865               | UJGFMBBBB      | IV                            | +      | 1     |       |
| t880               | A2AKB          | IV                            |        | 1     |       |
| CMRSA 1 (USA600) Total |                |                               |        |       | 36    |
| CMRSA 2 (USA100)   |                |                               |        |       |       |
| t002               | TJMBDMGMMK      | II                            |        | 281   |       |
| t003               | TMDMGMK        | II                            |        | 88    |       |
| t010               | TMBDMGMK       | II                            |        | 10    |       |
| t014               | TMDMGMMMMK     | II                            |        | 15    |       |
| t045               | TMDMGMK        | II                            |        | 1     |       |
| t062               | TJMGMK         | II                            |        | 2     |       |
| t105               | TJMBMDMMMK     | II                            |        | 1     |       |
| t111               | TJMK           | II                            |        | 1     |       |
| t1154              | TDMGMK         | V*                            |        | 7     |       |
| t1220              | TJMEMDMGMMK     | II                            |        | 1     |       |
| t1282              | TMDGMMMMK      | II                            |        | 2     |       |
| t179               | TJMBMDMGKK     | II                            |        | 1     |       |
| t2051              | TJMBMDGKM      | II                            |        | 1     |       |
| t242               | TJMEMDMGKK     | II                            |        | 43    |       |
| t2958              | C3MBDMGKK      | V*                            |        | 2     |       |
| t306               | TJMBMDMGMMK    | II                            |        | 7     |       |
| t311               | TJMBDMGMK      | II                            |        | 7     |       |
|                   |                | V*                            |        | 3     |       |
| t3234              | TJMBME         | II                            |        | 1     |       |
| t3786              | TMDMBMK        | II                            |        | 1     |       |
| t3948              | TGGMK          | II                            |        | 1     |       |
| t442               | C3MBDMGMK      | V*                            |        | 2     |       |
| t4695              | TJMEMGMK       | II                            |        | 1     |       |
| t5081              | TJMBGKM        | V*                            |        | 1     |       |
| t548               | TJMBMDGMK      | II                            |        | 2     |       |
| t579               | TJMMDGMK       | II                            |        | 1     |       |
| PFGE epidemic type | Ridom spa type | Kreiswirth repeat succession | SCC\textit{mec} | PVL | Total |
|-------------------|---------------|-------------------------------|----------------|------|-------|
| t5810             | TMDMGMGMGMK   | II                           | -              | 1    |       |
| t586              | TK            | II                           | -              | 1    |       |
| t688              | TJMBMK        | II                           | -              | 2    |       |
|                   |               | V*                           | -              | 2    |       |
| t985              | TJMBMGMGMK    | II                           | -              | 1    |       |
| CMRSA 2 (USA100)   |               |                               |                |      | 490   |
| CMRSA 2 (USA800)   |               |                               |                |      |       |
| t001              | TO2MBMDMGMK   | IV                           | -              | 1    |       |
| t002              | TJMBMDMGMK    | IV                           | -              | 14   |       |
|                   |               |                               |                |      | +     |
| t003              | TMDMGMGMK     | IV                           | +              | 4    |       |
| t088              | TJMBMDMGMK    | IV                           | -              | 3    |       |
| t1154             | TDMGMK        | IV                           | -              | 42   |       |
| t1781             | TK            | IV                           | -              | 2    |       |
| t179              | TJMBMDMGMK    | IV                           | -              | 2    |       |
| t242              | TJMBMDMGMK    | IV                           | -              | 1    |       |
| t306              | TJMBMDMGMK    | IV                           | +              | 1    |       |
| t311              | TJMBMDMGMK    | IV                           | -              | 33   |       |
| t5081             | TJMBGMK       | IV                           | -              | 3    |       |
| t539              | TJMBGMK       | IV                           | -              | 1    |       |
| t548              | TJMBMDMGMK    | IV                           | -              | 3    |       |
|                   |               |                               |                |      | +     |
| t5975             | T?            | IV                           | -              | 1    |       |
| t5987             | TJMBDMGMK     | IV                           | -              | 1    |       |
| t688              | TJMBMK        | IV                           | -              | 5    |       |
| CMRSA 2 (USA800)   |               |                               |                |      | 134   |
| CMRSA 3/6         |               |                               |                |      |       |
| t037              | WGKAOMQ       | III                          | -              | 60   |       |
| t275              | WGKAOMQQ      | III                          | -              | 1    |       |
| CMRSA 3/6 Total   |               |                               |                |      | 61    |
| CMRSA 4 (USA200)   |               |                               |                |      |       |
| t007              | WGKKKAOM      | II                           | -              | 1    |       |
| t012              | WGKKKAOMQQ    | II                           | -              | 4    |       |
|                   |               | IV                           | -              | 3    |       |
| t018              | WGKKKAOMQQ    | II                           | -              | 3    |       |
| t021              | WGKKKAOMQ     | IV                           | -              | 2    |       |
|                   |               | V*                           | +              | 1    |       |
| t233              | WG            | IV                           | -              | 1    |       |
| t318              | WGKKKAOMQ     | IV                           | +              | 1    |       |
|                   |               | V*                           | +              | 1    |       |
| t338              | WFKAOOMQ      | IV                           | -              | 1    |       |
| t3732             | WFKAOOMQQ     | IV                           | -              | 1    |       |
| t5976             | WFKKAOMQQ     | IV                           | -              | 1    |       |
| CMRSA 4 (USA200)   |               |                               |                |      | 20    |
| CMRSA 5 (USA500)   |               |                               |                |      |       |
| t008              | YHGFMQBQLO    | IV                           | *              | +    | 1     |
| t064              | YHGCMQBQLO    | II                           | -              | 1    |       |
|                   |               | IV                           | -              | 1    |       |
| t1677             | YHGGBQLO      | IV                           | -              | 1    |       |
| t451              | YGCBQLO       | IV                           | -              | 1    |       |
| CMRSA 5 (USA500)   |               |                               |                |      | 5     |
| CMRSA 7 (USA400)   |               |                               |                |      |       |
| t127              | UJFKBPE       | II                           | -              | 1    |       |
|                   |               | IV                           | -              | 5    |       |
Table 1. Cont.

| PFGE epidemic type | Ridom spa type | Kreiswirth repeat succession | SCC\textit{mec} | PVL | Total |
|--------------------|----------------|-----------------------------|-----------------|-----|-------|
| t128               | UJFKBPE        | II                          |                 |     | 3     |
|                    |                | IV                          |     -           | 20  |       |
|                    |                |                             |                 |     | 19    |
| t1508              | WBPE           | IV                          |                 |     | 1     |
| t175               | UJFPPFKPE      | IV                          |                 |     | 2     |
|                    |                |                             |                 |     | 1     |
| t1784              | UBPE           | IV                          | +               | 1   |       |
| t5469              | UJKP           | IV                          |                 |     | 1     |
| t5475              | UMBBPB         | II                          |                 |     | 1     |
| t5977              | UJK            | IV                          | +               | 1   |       |
| t5978              | UJDFKBE        | IV                          |                 |     | 1     |
| t5979              | UJFFFKPKPE     | IV                          | +               | 1   |       |
| CMRSA 7 (USA400) Total |                |                             |                 |     | 58    |
| CMRSA 8 (EMRSA-15) |                |                             |                 |     |       |
| t005               | TJEJCMOMOKR    | V\*                        |                 |     | 1     |
| t022               | TJEJNF2MNFKMOMOKR | IV                    |                 |     | 5     |
| t032               | TJEJNF2MNFKMOMOKR | IV                    |     -           | 11  |       |
|                    |                |                             |                 |     | 1     |
| t2113              | TJEJNF2MNFKMOKR | IV                          |                 |     | 1     |
| t223               | TJEJCMOMOKR    | IV                          | V\*            |     | 1     |
| t515               | TJEJNF2MNFKMOKR | IV                          |                 |     | 1     |
| t578               | TJEJNF2MNFKMOMOKR | IV                   |                 |     | 4     |
| t5982              | UJEJCMOMOKR    | IV                          | +               | 2   |       |
| t5983              | UJEJCMOMOKR    | IV                          | +               | 1   |       |
| t852               | UJEJCMOMOKR    | IV                          | +               | 3   |       |
| CMRSA 8 (EMRSA-15) Total |                |                             |                 |     | 31    |
| CMRSA 10 (USA300) |                |                             |                 |     |       |
| t008               | YHFMBQBLO      | IV                          | IV\*           |     | 1     |
|                    |                |                             | IV             | 20  |       |
|                    |                |                             | IV\*           |     | 206   |
| t024               | YGMBQBLO       | IV                          |                 |     | 1     |
| t059               | YHO            | IV                          |                 |     | 3     |
| t121               | YHFMBQBLO      | IV                          |                 |     | 1     |
| t1578              | YGFMBQBMM      | IV                          |                 |     | 1     |
| t1635              | YHFMBBO        | IV                          |                 |     | 3     |
| t197               | YCBQBLO        | IV                          |                 |     | 1     |
| t211               | YHGFMQBLO      | IV                          |                 |     | 1     |
| t2792              | YHGFBQBLO      | IV                          |                 |     | 2     |
| t451               | YGMBQBLO       | IV                          |                 |     | 1     |
| t530               | YHGBQBK        | IV                          |                 |     | 1     |
| t5989              | YHFMBQBLO      | IV                          |                 |     | 1     |
| t622               | YHGFMBLO       | IV                          |                 |     | 1     |
| t818               | YHGFMB        | IV                          |                 |     | 1     |
|                    |                |                             | +               | 2   |       |
| t919               | YHGFKBQBLO     | IV                          |                 |     | 1     |
| CMRSA 10 (USA300) Total |                |                             |                 |     | 249   |
| European           |                |                             |                 |     |       |
| t044               | UJGBBPB        | IV                          |                 |     | 14    |
| t5984              | UJGBBB         | IV                          |                 |     | 1     |
| t5986              | UJGBPB         | IV                          |                 |     | 1     |
| PFGE epidemic type | Ridom spa type   | Kreiswirth repeat succession | SCCmec | PVL | Total |
|--------------------|------------------|-----------------------------|--------|-----|-------|
| European Total     |                  |                             |        |     | 16    |
| Non-assigned       | t041             | TO2MBMDMBMDMGMK              | IV     | —   | 1     |
|                    | t078             | ZFGU2DMGGM                  | IV     | +   | 1     |
|                    | t084             | UG8BGGJAGJ                  | IV     | +   | 1     |
|                    | t091             | UJFMBGGAGG                  | IV     | —   | 1     |
|                    | t108             | XKAX2BMB                    | IV     | —   | 2     |
|                    | t1379            | ZFGMDMGMK                   | IV     | —   | 1     |
|                    | t149             | TO2MEMDMGAGG                | IV     | —   | 2     |
|                    | t160             | UJFOPLM                     | IV     | —   | 1     |
|                    | t164             | UG2MBBLB                    | IV     | +   | 1     |
|                    | t183             | TJFMBBQPB                   | V*     | +   | 1     |
|                    | t202             | YMJMXXKOO                   | IV     | +   | 2     |
|                    | t209             | UKGB                         | IV     | —   | 1     |
|                    | t293             | XKAOP2P2P22                 | IV     | —   | 1     |
|                    | t298             | XMQ                          | IV     | +   | 1     |
|                    | t332             | TJFMBBQPB                   | V*     | +   | 2     |
|                    | t334             | YGFMBLO                      | V*     | +   | 1     |
|                    | t345             | TJFMBBQPB                   | V*     | +   | 1     |
|                    | t375             | Y2EJCMBPB                   | IV     | —   | 1     |
|                    | t380             | TBPB                         | IV     | —   | 1     |
|                    | t405             | UGBKBE                       | II     | —   | 1     |
|                    | t455             | UGBEBP                       | IV     | +   | 1     |
|                    | t525             | Y2BIJCMBP                   | IV     | —   | 1     |
|                    | t597             | UBEBBP                      | IV     | +   | 1     |
|                    | t598             | TJFBMBBQBP                   | V*     | +   | 1     |
|                    | t657             | TJFMBBP                      | V*     | +   | 2     |
| Non-assigned Total |                  |                             |        |     | 40    |
| ST88               | t1816            | UGFMBBBBPPB                  | IV     | —   | 1     |
|                    | t186             | UGFMBBBBPPB                  | IV     | —   | 2     |
|                    | t6441            | UEGFEBBBPPB                  | IV     | —   | 1     |
|                    | t690             | UGFMBBBBPPB                  | IV     | +   | 1     |
|                    | t692             | UGFMBBBBPPB                  | IV     | +   | 3     |
| ST88 Total         |                  |                             |        |     | 8     |
| ST97               | t044             | UGBBP                        | II     | —   | 1     |
|                    | t131             | UGBBP                        | II     | —   | 1     |
|                    | t2112            | TJFMBBBBPPB                  | II     | —   | 2     |
|                    | t2297            | UJGFBMBBBPPB                 | V*     | —   | 1     |
|                    | t267             | UJGFBMBBBPPB                 | IV     | —   | 4     |
|                    |                  |                             |        |     | 1     |
ologies are compared. This study also evaluates whether SCCmec and PVL data can differentiate MRSA isolates that share the same spa type but are associated with multiple PFGE epidemic types.

**Materials and Methods**

**Bacterial Strains and DNA Extraction**

There were three sets of MRSA isolates characterized in this study. The first set includes a selection of 1269 isolates from samples submitted to ProvLab for routine molecular typing between June 2005 and March 2009. These isolates were previously genotyped using PFGE, SCCmec, and PVL typing and were selected because they had a unique combination of PFGE, SCCmec, and PVL types. The 1269 isolates comprised the validation panel and were spa typed and used to populate the Alberta MRSA spa typing database. The second set consists of an additional 646 consecutive post-validation clinical isolates received by ProvLab between August 2009 and November 2009. These isolates were spa, SCCmec, and PVL typed and used for the preliminary evaluation of the developed MRSA typing algorithm. Lastly, ongoing routine MRSA molecular typing continued from January 2010 to December 2012 and included 12620 isolates that were used for the final evaluation of the developed MRSA typing algorithm.

**Table 1. Cont.**

| PFGE epidemic type | Ridom spa type | Kreiswirth repeat succession | SCCmec | PVL | Total |
|--------------------|----------------|------------------------------|--------|-----|-------|
| t359               | UJGFMBBBPB     | IV                           |        | -   | 1     |
| t521               | UJGFMBBBBPB    | IV                           | -      | 4   | 18    |
| t527               | UJGFMBBBBBPB   | IV                           | -      | 2   | 2     |
|                    |                | IV                           | -      | 1   | 1     |
| ST97 Total         |                |                              |        |     | 39    |
| USA1000, China/Taiwan |           |                              |        |     | 43    |
| t163               | ZDMDMA3KB      | II                           |        | -   | 1     |
| t1751              | ZDMDOE         | IV                           |        | -   | 1     |
| t216               | ZDMO        | IV                           | -      | 10  | 10    |
|                    |                | V*                           |        | +   | 2     |
|                    |                |                               |        |     | 2     |
|                    |                |                               |        |     | 1     |
|                    |                |                               |        |     | 3     |
|                    |                |                               |        |     | 2     |
|                    |                |                               |        |     | 7     |
|                    |                |                               |        |     | 1     |
|                    |                |                               |        |     | 1     |
|                    |                |                               |        |     | 2     |
| USA1000, SWP/Oceania |            |                              |        |     | 16    |
| t019               | XKAKAOMQ       | IV                           | +      | 13  |       |
| t1133              | XKAKAOAMQ     | IV                           | +      | 1   |       |
| t4341              | XKAMQ          | IV                           | +      | 1   |       |
| t5447              | UAKAOMQ        | IV                           | +      | 1   |       |
| USA1000, SWP/Oceania Total |         |                              |        |     | 16    |
| USA700             |                 |                              |        |     | 23    |
| t126               | UJGFMGGM       | IV                           | -      | 1   |       |
| t1346              | UJGFMGGGM     | IV                           | -      | 4   |       |
|                    |                | V*                           |        | -   | 1     |
|                    |                |                               |        |     | 2     |
|                    |                |                               |        |     | 1     |
|                    |                |                               |        |     | 3     |
| USA700 Total       |                |                              |        |     | 23    |
| Grand Total        |                |                              |        |     | 1269  |

Isolates marked with an * were SCCmec typed using primers from Kondo et al. (18); all other isolates were SCCmec typed using primers from Oliveira et al. (17).

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were used to determine the efficacy of the typing method as well as the associated time and cost requirements. MRSA isolates were inoculated onto sheep blood agar plates (BAPs; Dalynn Biologicals, Calgary, Alberta, Canada) from frozen cultures for overnight growth at 37°C. DNA was extracted using a modified method described by Holland et al. [19]. A single colony was picked from the BAP and suspended in 200 μL of rapid lysis buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.3, 1 mM EDTA pH 9.0, 1% Triton-X), kept frozen at −80°C for 15 minutes, and boiled for 15 minutes. Following cooling at room temperature and centrifugation at 13,000 × g for five minutes, supernatant was removed and used as DNA template for PCR.

**spa Typing**

PCR was performed using primers targeting the spa gene as described by Golding et al. [12]. PCR products from isolates in the validation panel (n = 1269) and post-validation isolates (n = 646) were sent to the Genomic Core DNA facility at the National Microbiology Laboratory (NML) in Winnipeg, Canada, for PCR product cleanup and sequencing. PCR products of isolates that were part of ongoing routine molecular typing (n = 12620) were cleaned and sequenced at ProvLab.

**PFGE, SCCmec, and PVL Typing**

PFGE was performed as previously described by Mulvey et al. [20] using the restriction endonuclease Smal. PFGE results were analyzed using BioNumerics (version 5.1; Applied Maths, USA) and PFGE epidemic type designation was completed based on guidelines detailed by NML and using PFGE fingerprint profiles of reference strains provided by NML [8,9]. Briefly, isolates were assigned to a PFGE epidemic type if there was a difference of less than seven bands between the PFGE fingerprint pattern of the isolate and a provided reference strain. Isolates were designated as “non-assigned” if they differed by more than seven bands from all reference strains. All isolates were grouped into one of the following PFGE epidemic types: Canadian community-associated MRSA (CA-MRSA) epidemic clones CMRSA 2 (USA800; ST5), CMRSA 7 (USA400; ST1), and CMRSA 10 (USA300; ST8); Canadian hospital-associated MRSA (HA-MRSA) epidemic clones CMRSA 1 (USA600; ST45), CMRSA 2 (USA100, ST5), CMRSA 3/6 (“Punjabi” clone; ST239), CMRSA 4 (USA200; ST36), CMRSA 5 (USA500; ST8), CMRSA 8 (EMRSA-15; ST-22), and CMRSA 9 (no USA equivalent); European epidemic clones (ST88, ST97, and ST80); epidemic clones from the United States USA700 (ST72), USA1000 (China/Taiwan; ST59), and USA1100 (Southwest Pacific/SWP/Oceania; ST30); SCCmec typing was performed using the primers and methods described by Oliveira et al. [17]. Epidemic types USA100 and USA800, both characterized as Canadian epidemic type CMRSA 2, were differentiated using SCCmec data as only USA800 is SCCmec type IV [12,21]. Isolates that could not be typed using the Oliveira et al. protocol [17] were SCCmec typed using the method outlined in Kondo et al. [18]. There were no isolates in this study that were not typeable by both methods. PVL characterization [16] was performed using previously described methods.

**Data Analysis**

Sequencing results for validation and post-validation isolates were obtained in-house. All sequences were analyzed in BioNumerics, and submitted to the online Ridom spa server (http://www.ridom.de/spaserver), developed by Ridom GmbH and curated by SeqNet.org (http://www.SeqNet.org), for Ridom spa type designation [22]. For the validation isolates (n = 1269) that were genotyped using PFGE, SCCmec, and PVL typing prior to this study, spa-based assignment of PFGE epidemic types was done using databases from NML [12] and Sunnybrook Health Sciences Centre (unpublished data) in Toronto, Canada, that correlate spa types with PFGE epidemic types. PFGE epidemic type designations based on spa data and PFGE data were compared. Post-validation (n = 646) and ongoing routine molecular typing (n = 12620) isolates were assigned PFGE epidemic types using the Alberta MRSA spa typing database and the typing algorithm outlined in this study. Simpson’s Index of Diversity [23] was calculated using the online tool hosted at the Comparing Partitions website (http://darwin.phyloviz.net/ComparingPartitions/).

**Alberta MRSA Typing Database**

The genotyping results and PFGE epidemic type designations from the isolates in the validation panel (n = 1269) were used as the initial dataset to build the Alberta MRSA typing database. An algorithm to assign PFGE epidemic types based on the association between spa, SCCmec, and PVL types with PFGE epidemic types was developed. The database and the algorithm were evaluated using the post-validation subset of isolates (n = 646). PFGE was then performed on a random selection of the post-validation samples to determine if there is a consensus in PFGE epidemic type designation between the different typing methods. Genotyping data from the post-validation isolates and the isolates that were part of routine testing at ProvLab was added to the Alberta MRSA typing database.

**Cost and Time Analysis**

The cost and time analysis was based on routine testing of 12620 MRSA isolates submitted to ProvLab for genotyping between January 2010 and December 2012. Values were calculated by averaging the time required for genotyping batches of 20 MRSA isolates during the study period and were rounded to the nearest half hour. Turn-around time calculations began from the isolation of single colonies from overnight cultures and ended when data analysis was complete. Hands-on time was expressed as the sum of the average labor and analysis times required from laboratory technologists.

**Results**

**Building the Alberta MRSA Typing Database**

The association of spa, SCCmec, and PVL types with PFGE epidemic types for the validation panel of isolates (n = 1269) that were spa typed is shown in Table 1. This genotyping data was used as the foundation for the Alberta MRSA typing database. A total of 160 spa types were identified and four of these spa types- t008, t044, t11081, and t451 (n = 253; 20% of the genotyped isolates) corresponded to more than one epidemic type. SCCmec typing was needed to assign PFGE epidemic types to t008 (n = 228), which is one of the most common spa types observed, because of its association with PFGE epidemic types CMRSA5, CMRSA9, and CMRSA10. The majority of the t008 isolates (n = 226; 99.1%) were characterized as SCCmec type IV using primers from Oliveira et al. [17] and classified as CMRSA 10. A small number (n = 2) of t008 isolates were not typeable using these primers and could only be SCCmec typed using primers from Kondo et al. [18]. Both t008 isolates were SCCmec type IV and PVL positive and PFGE data was needed to resolve the PFGE epidemic types (one was CMRSA 5; the other was CMRSA 10). PFGE epidemic type classification for spa types t044 (n = 17), t11081 (n = 8), and t451
(n = 2) also required PFGE. In total, only 29 of 1269 isolates (2.3%) needed PFGE data for PFGE epidemic type assignment.

**PFGE Epidemic Type Assignment using spa, SCCmec, and PVL Data**

An algorithm for PFGE epidemic type designation was developed (Figure 1) based on the data generated for the new Alberta MRSA typing database. Uncharacterized MRSA isolates are spa, SCCmec, and PVL typed. The typing data is then compared to combinations in the new Alberta MRSA typing database: if a match is found and present in sufficient numbers (at ProvLab the arbitrary minimum count is ten isolates) then the PFGE epidemic type is assigned and the database is updated. PFGE epidemic type assignment using PFGE is required for isolates under the following criteria such as 1) no match in the MRSA typing database; 2) rare spa types; or 3) typing data combinations that correspond to multiple PFGE epidemic types (Figure 1). PFGE epidemic type designations are then reported as they have been done historically.

A total of 646 consecutive post-validation MRSA isolates, collected from August 2009 to November 2009, were used to evaluate the described MRSA typing algorithm and were assigned PFGE epidemic types using the Alberta MRSA typing database (Table 2). There were 30 spa types identified, and two of these spa types- t008 (n = 361) and t044 (n = 1) – were associated with multiple epidemic types. The majority (n = 567; 87.8%) of the isolates could be grouped into a PFGE epidemic type using spa, SCCmec, and PVL data. In particular, SCCmec typing data resolved the PFGE epidemic types for 359 of the 361 (99.4%) t008 isolates. PFGE epidemic type designation using PFGE was only needed for 78 isolates (12.1%; Table 2). This group includes isolates with spa types corresponding to more than one PFGE epidemic type that cannot be resolved with SCCmec and PVL data (n = 3); isolates that have rare spa types or ones not previously observed in Alberta (n = 74); and isolates that have novel spa types not present in the online Ridom spa server (n = 1). One additional isolate with spa type t034 was not typeable using PFGE and a PFGE epidemic type was assigned based on existing data in the NML MRSA typing database.

**Validation of PFGE Epidemic Types Assigned using spa, SCCmec, and PVL Typing**

The accuracy of PFGE epidemic type assignment using the Alberta MRSA typing database and spa, SCCmec, and PVL data was assessed by performing PFGE on 49 isolates randomly selected from the post-validation subset of samples. Isolates were then grouped into PFGE epidemic types based on the PFGE fingerprint patterns. A comparison of spa-, SCCmec-, and PVL-based and PFGE-based epidemic type designation showed that all 49 isolates, representing 10 spa types and 20 PFGE fingerprint profiles, shared the same PFGE epidemic type designation regardless of typing method. Simpson’s index of diversity for spa typing was 0.853 (95% CI, 0.810–0.896) and increased to 0.859 (95% CI, 0.813–0.905) with the addition of SCCmec typing alone or in conjunction with PVL characterization. These values overlapped with Simpson’s index of diversity for PFGE typing (0.916; 95% CI, 0.875–0.960), suggesting the two typing algorithms had similar discriminatory power in this study.

**Distribution of SCCmec and PVL Types in Alberta**

The distribution of SCCmec and PVL types in the set of validation and post-validation isolates is shown in Table 3. Most of
### Table 2. Association of MRSA PFGE epidemic types with spa, SCCmec, and PVL types in Alberta from August 2009 to November 2009.

| PFGE epidemic type       | Ridom spa type | Kreiswirth repeat succession | SCCmec | PVL | Total |
|--------------------------|----------------|------------------------------|--------|-----|-------|
| CMRSA 1 (USA600)         | t065           | A2AKBEMBKB                   | IV     |     | 2b    |
| CMRSA 1 (USA600) Total   |                |                              |        |     | 4     |
| CMRSA 2 (USA100)         | t003           | TMDDMGMMK                    | II     |     | 24    |
| CMRSA 2 (USA100) Total   |                |                              |        |     | 121   |
| CMRSA 2 (USA800)         | t1154          | TDMGMMK                      | IV     |     | 2     |
| CMRSA 2 (USA800) Total   |                |                              |        |     | 5     |
| CMRSA 3/6                | t037           | WGKAOMQ                      | III    |     | 13    |
| CMRSA 3/6 Total          |                |                              |        |     | 13    |
| CMRSA 4 (USA200)         | t012           | WGKAKAOMQQ                   | II     |     | 1b    |
| CMRSA 4 (USA200) Total   |                |                              |        |     | 6     |
| CMRSA 7 (USA400)         | t127           | UJFKBPE                      | IV     |     | 1b    |
| CMRSA 7 (USA400) Total   |                |                              |        |     | 6     |
| CMRSA 8 (EMRSA-15)       | t022           | TJEJNF2MNFM2MOMOKR           | IV     |     | 8b    |
| CMRSA 8 (EMRSA-15) Total |                |                              |        |     | 11    |
| CMRSA 10 (USA300)        | t008           | YHGFMBQBLO                   | IV     |     | 11    |
| CMRSA 10 (USA300) Total  |                |                              |        |     | 11    |
| CMRSA 11 (USA500)        | t024           | YGFMBQBLO                    | IV     |     | 1b    |
| CMRSA 11 (USA500) Total  |                |                              |        |     | 1b    |
| CMRSA 12 (USA600)        | t051           | YHGFMBQBLO                   | IV     |     | 1b    |
| CMRSA 12 (USA600) Total  |                |                              |        |     | 1b    |
| CMRSA 13 (USA700)        | t068           | YHGFMBQBLO                   | IV     |     | 3b    |
| CMRSA 13 (USA700) Total  |                |                              |        |     | 3b    |
| CMRSA 14 (USA800)        | t1610          | YHGFMBQBLO                   | IV     |     | 1b    |
| CMRSA 14 (USA800) Total  |                |                              |        |     | 1b    |
| CMRSA 15 (USA900)        | t1883          | YHGFMBQO                     | IV     |     | 1b    |
| CMRSA 15 (USA900) Total  |                |                              |        |     | 1b    |
| CMRSA 16 (USA1000)       | t2054          | YHGFMBQBLO                   | IV     |     | 1b    |
| CMRSA 16 (USA1000) Total |                |                              |        |     | 1b    |
| CMRSA 17 (USA1100)       | t211           | YHGFMBQBLO                   | IV     |     | 2b    |
| CMRSA 17 (USA1100) Total |                |                              |        |     | 2b    |
| CMRSA 18 (USA1200)       | t3081          | YHGFMBPO                     | IV     |     | 2b    |
| CMRSA 18 (USA1200) Total |                |                              |        |     | 2b    |
the isolates were SCCmec typed using primers from Oliveira et al., [17] but SCCmec typing for some (69 of 1915 isolates; 3.6%) required additional primers [18]. SCCmec type IV (n = 1129; 59.0%) was observed most frequently, followed by II (n = 644; 33.6%) then III (n = 76; 4.0%). The majority of these isolates were PVL negative (n = 1101; 57.5%) rather than PVL positive (n = 814; 42.5%). Isolates with Canadian community-associated PFGE epidemic types were predominantly SCCmec type IV (902 of 907; 99.4%) and PVL positive (699 of 907; 77.1%). In contrast, isolates with Canadian hospital-associated PFGE epidemic types were mostly SCCmec type II (608 of 797; 76.3%) and PVL negative (779 of 797; 97.7%).

**PFGE Epidemic Type Assignment using the Alberta MRSA Typing Database**

From January 2010 to December 2012, a total of 12620 first clinical MRSA isolates were submitted to ProvLab for molecular typing and characterized using the Alberta MRSA typing database and the described typing algorithm (Figure 1). The percentage of isolates requiring PFGE for PFGE epidemic type assignment from 2010 to 2012 is shown in Table 4 and decreased from 15.1% to 9.5% during this time period. SCCmec typing resolved the PFGE epidemic types for over 99% (n = 5923) of the t008 isolates genotyped (Table 4).

**Comparing the Cost and Time Associated with PFGE and spa, SCCmec, and PVL Typing**

The total hands-on time required for PFGE typing (7.5 hours) is greater than the hands-on time required for spa, SCCmec, and PVL typing (3.5 hours) for 20 strains of MRSA. This is attributed to longer labor and data analysis times (Table 5). In addition, turn-around times are improved for spa, SCCmec, and PVL typing (10.5 hours) compared to PFGE typing (27.5 hours) as the experiment run times are significantly reduced. Of the 12620 MRSA isolates genotyped between January 2010 and December 2012, 11099 isolates did not require PFGE typing. This reduced the required hands-on time by approximately 2220 hours. Assuming the average salary of a laboratory technologist is $35 per hour, ProvLab saved $77,700 in labor costs which offsets the slightly higher cost of materials associated with spa, SCCmec, and PVL typing.

### Table 2. Cont.

| PFGE epidemic type | Ridom spa type | Kreiswirth repeat succession | SCCmec | PVL | Total |
|--------------------|----------------|-----------------------------|--------|-----|-------|
| t5989              | YHQFMB8QBlO    | IV                          | +      | 2   |
| t6442              | YHGFMB8BL0     | IV                          | +      | 1   |
| t723               | YHGBLO         | IV                          | +      | 2   |
| t818               | YHGFMB         | IV                          | +      | 4   |
| CMRSA 10 (USA300) Total |                |                             |        | 388 |
| European           | t044           | UJGBBPB                     | IV     | +   | 1    |
| European Total     |                |                             |        | 1   |
| ST398             | t034           | XKAOAOBQO                   | V*     | +   | 1    |
| ST398 Total        |                |                             |        | 1   |
| ST97               | t521           | UJGFMB8BBVPB                | IV     |     | 1    |
| ST97 Total         |                |                             |        | 1   |
| USA1000, China/Taiwan | t1894         | ZDMDMNNOB                   | V*     | +   | 1    |
| USA1000, China/Taiwan Total |            |                             |        | 1   |
| USA700             | t148           | UJGFGMDGGMGM                | IV     | +   | 1    |
| USA700 Total       |                |                             |        | 1   |
| Non-assigned       | t3320          | TJEFMBBBQPB                 | V*     | +   | 1    |
| Non-assigned Total |                |                             |        | 1   |
| Grand Total        |                |                             |        | 646 |

Isolates with spa types that correspond to more than one epidemic type and require PFGE for PFGE epidemic type assignment, are rare or have not been previously observed in Alberta, are novel spa types, or could not be genotyped using PFGE are shown. Isolates marked with an * were SCCmec typed with primers from Kondo et al. (18); all other isolates were SCCmec typed using primers from Oliveira et al. (17).

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MRSA spa, SCCmec, and PVL Typing Compared to PFGE
Although PFGE is a powerful MRSA genotyping technique, it has many limitations. As the number of MRSA isolates submitted to ProvLab increased, PFGE characterization became much less viable. In this study, we describe the transition from using PFGE to assign MRSA PFGE epidemic types, to using spa, SCCmec, and PVL typing. With the new Alberta MRSA typing database, most (n = 11666; 87.9%) of the 13266 isolates genotyped after the validation of the typing algorithm were grouped into PFGE epidemic types using spa, SCCmec, and PVL data. A total of 1600 isolates (12.1%) still needed PFGE for PFGE epidemic type assignment. The number of first clinical MRSA isolates requiring PFGE for PFGE epidemic type assignment steadily declined from 15.1% in 2010 to 9.5% in 2012, further reducing the time and cost associated with MRSA genotyping at ProvLab. As the new Alberta MRSA typing database becomes more populated, isolates with rare or novel spa types will become less frequent leading to additional reductions in the PFGE workload.

In this study, isolates with community-associated epidemic types were predominantly SCCmec type IV and PVL positive, while those with hospital-associated epidemic types were mostly SCCmec type II and PVL negative. These findings are consistent with global trends (as reviewed in [24]). An isolate with spa type t034 was observed in this study and it could not be genotyped using PFGE or be assigned to a PFGE epidemic type using the new Alberta MRSA typing database. This isolate was grouped into the PFGE epidemic type ST398 based on past designations made by NML, is known to be resistant to SmaI digestion, and is associated with livestock [25,26]. Characterization of similar isolates in the future will need to be done using only spa, SCCmec, and PVL data.

PFGE epidemic type designations were consistent regardless of typing method for selected isolates, a result seen in other studies [12,14,27,28], allowing for an easy transition between the two methods.

### Table 3. Distribution of SCCmec and PVL types in validation and post-validation isolates.

| SCCmec/PVL | II | III | IV | V | VI |
|------------|----|-----|----|---|----|
| PFGE epidemic type | Number of isolates | | | | |
| CMRSA 1 (USA600) | 40 | 5 (12.5) | | 24 (60.0) | | 1 (2.5) | 8 (20.0) | 0 | 1 (2.5) |
| CMRSA 2 (USA100) | 610 | 593 (97.2) | 0 | 0 | 0 | 17 (2.8) | 0 | 0 |
| CMRSA 2 (USA800) | 140 | 0 | 0 | 117 (83.6) | 23 (16.4) | 0 | 0 | 0 |
| CMRSA 3/6 | 74 | 0 | 73 (98.6) | 1 (1.4) | 0 | 0 | 0 | 0 |
| CMRSA 4 (USA200) | 26 | 9 (34.6) | 0 | 0 | 11 (42.3) | 2 (7.7) | 0 | 0 |
| CMRSA 5 (USA500) | 5 | 1 (20.0) | 0 | 0 | 3 (60.0) | 1 (20.0) | 0 | 0 |
| CMRSA 7 (USA400) | 130 | 5 (3.8) | 0 | 0 | 48 (36.9) | 77 (59.2) | 0 | 0 |
| CMRSA 8 (E-MRSA15) | 42 | 0 | 0 | 0 | 33 (78.6) | 8 (19.0) | 0 | 1 |
| CMRSA 10 (USA300) | 637 | 0 | 0 | 0 | 38 (6.0) | 599 (94.0) | 0 | 0 |
| European | 17 | 0 | 0 | 0 | 3 (17.6) | 17 (100.0) | 0 | 0 |
| ST88 | 8 | 0 | 0 | 0 | 4 (50.0) | 4 (50.0) | 0 | 0 |
| ST97 | 40 | 24 (60.0) | 0 | 0 | 12 (30.0) | 1 (2.5) | 3 (7.5) | 0 |
| ST398 | 1 | 0 | 0 | 0 | 0 | 0 | 1 (100.0) | 0 |
| USA700 | 25 | 1 (4.0) | 0 | 0 | 17 (68.0) | 5 (20.0) | 2 (8.0) | 0 |
| USA1000, China/Taiwan | 47 | 1 (2.1) | 0 | 0 | 25 (53.2) | 9 (19.1) | 4 (8.5) | 8 (17.0) |
| USA1100, SWP/Oceania | 30 | 0 | 0 | 0 | 30 (100.0) | 0 | 0 | 0 |
| Non-assigned | 43 | 5 (11.6) | 1 (2.3) | 0 | 10 (23.3) | 10 (23.3) | 5 (11.6) | 12 (27.9) |
| Total | 1915 | 644 (33.6) | 75 (3.9) | 1 (0.1) | 342 (17.9) | 787 (41.1) | 39 (2.0) | 26 (1.4) |

Cell percent values relative to the row total are given in brackets.
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### Table 4. Routine molecular testing of MRSA isolates using the Alberta MRSA typing database from 2010 to 2012.

| Year | Total # of MRSA isolates genotyped | # of MRSA isolates genotyped by PFGE | Total # of t008 isolates | # of t008 isolates resolved by SCCmec typing |
|------|-----------------------------------|-------------------------------------|-------------------------|---------------------------------------------|
| 2010 | 3829 | 578 (15.1%) | 1889 | 1880 (99.5%) |
| 2011 | 4306 | 516 (12.0%) | 2021 | 2012 (99.6%) |
| 2012 | 4485 | 427 (9.5%) | 2013 | 2009 (99.8%) |

Percentages of total isolates are given in brackets.
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**Discussion**

Although PFGE is a powerful MRSA genotyping technique, it has many limitations. As the number of MRSA isolates submitted to ProvLab increased, PFGE characterization became much less viable. In this study, we describe the transition from using PFGE to assign MRSA PFGE epidemic types, to using spa, SCCmec, and PVL typing. With the new Alberta MRSA typing database, most (n = 11666; 87.9%) of the 13266 isolates genotyped after the validation of the typing algorithm were grouped into PFGE epidemic types using spa, SCCmec, and PVL data. A total of 1600 isolates (12.1%) still needed PFGE for PFGE epidemic type assignment. The number of first clinical MRSA isolates requiring PFGE for PFGE epidemic type assignment steadily declined from 15.1% in 2010 to 9.5% in 2012, further reducing the time and cost associated with MRSA genotyping at ProvLab. As the new Alberta MRSA typing database becomes more populated, isolates with rare or novel spa types will become less frequent leading to additional reductions in the PFGE workload.

In this study, isolates with community-associated epidemic types were predominantly SCCmec type IV and PVL positive, while those with hospital-associated epidemic types were mostly SCCmec type II and PVL negative. These findings are consistent with global trends (as reviewed in [24]). An isolate with spa type t034 was observed in this study and it could not be genotyped using PFGE or be assigned to a PFGE epidemic type using the new Alberta MRSA typing database. This isolate was grouped into the PFGE epidemic type ST398 based on past designations made by NML, is known to be resistant to Smal digestion, and is associated with livestock [25,26]. Characterization of similar isolates in the future will need to be done using only spa, SCCmec, and PVL data.

PFGE epidemic type designations were consistent regardless of typing method for selected isolates, a result seen in other studies [12,14,27,28], allowing for an easy transition between the two methods.
typing methodologies. Although the cost of laboratory supplies for PFGE typing at ProvLab are lower, the cost differential is offset by the significant time and subsequent labor cost savings gained from using spa, SCCmec, and PVL typing. The turn-around and hands-on times required for spa, SCCmec, and PVL typing is reduced by more than half compared to PFGE. This is due in part to the computer automation of spa typing analyses. At ProvLab, the spa typing module accompanying the BioNumerics software is used to automatically analyze spa data and assign PFGE epidemic types. In contrast, PFGE data must be manually interpreted and PFGE epidemic types are assigned after careful comparison with representative epidemic strains. As a result, analysis of PFGE data is much more subjective and susceptible to interpretation errors.

SCCmec typing was needed to resolve MRSA isolates associated with more than one PFGE epidemic type and was particularly useful for differentiating t008 isolates, as well as PFGE epidemic types CMRSA 2 (USA100) and CMRSA 2 (USA800) which otherwise could not be resolved using PFGE. PVL characterization was not able to differentiate isolates associated with multiple PFGE epidemic types, although it could be used as a general indicator of PFGE epidemic type for select spa types (t044). Although SCCmec and PVL results are also manually interpreted, these assays produce data that is much faster to analyze.

To conclude, we created a new MRSA typing database in Alberta and validated and used an algorithm for genotyping MRSA using spa, SCCmec, and PVL typing. The shift away from PFGE typing provides significant time and cost savings, and enables high throughput processing while maintaining historical PFGE epidemic type assignments and reporting. Although PFGE typing is still required at ProvLab, its role is limited and will be further reduced as the Alberta MRSA typing database becomes more populated. A limitation of this study is that it was done at a reference provincial public health laboratory; thus the findings may not be applicable to all settings and other typing methods may still have an important role to play. Additionally, the time and cost savings will vary significantly between different laboratories because of differences in equipment, salary, cost of materials, and technical expertise.

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Author Contributions

Conceived and designed the experiments: LL. Performed the experiments: LL. Analyzed the data: VL. Contributed reagents/materials/analysis tools: LC. Drafted the paper: VL. LC.

Table 5. Comparison of time associated with MRSA PFGE and spa, SCCmec, and PVL typing with times calculated in hours.

|                      | PFGE typing (per 20 isolates) | spa/SCCmec/PVL typing (per 20 isolates) |
|----------------------|-------------------------------|----------------------------------------|
| Labor time           | 5.0                           | 3.0                                    |
| Data analysis time   | 2.5                           | 0.5                                    |
| Total hands-on time  | 7.5                           | 3.5                                    |
| Total turn-around time | 27.5                          | 10.5                                   |

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