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## Adapting typing for national laboratory-based surveillance of methicillin-resistant

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► **To cite this version:**

A. Vainio, S. Koskela, A. Virolainen, J. Vuopio, S. Salmenlinna. Adapting typing for national laboratory-based surveillance of methicillin-resistant. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, pp.789-797. 10.1007/s10096-011-1158-5 . hal-00663400

**HAL Id: hal-00663400**

**<https://hal.science/hal-00663400>**

Submitted on 27 Jan 2012

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1 **Adapting *spa* typing for national laboratory-based surveillance of methicillin-**  
2 **resistant *Staphylococcus aureus***

3

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26 **Abstract**

27 Laboratory-based surveillance of methicillin-resistant *Staphylococcus aureus*  
28 (MRSA) monitors the baseline occurrence of different genotypes, and identifies  
29 strains and transmission chains responsible for outbreaks. The consequences of  
30 substituting pulsed-field gel electrophoresis (PFGE) with *spa* typing as a first-line  
31 typing method were analyzed by typing 589 strains isolated between 1997 and 2006,  
32 with a focus on both short- and long-term correspondence between the PFGE and *spa*  
33 typing results. The study, covering these ten years, included all Finnish MRSA blood  
34 isolates and representatives of the two most prevalent MRSA strains (PFGE types  
35 FIN-4 and FIN-16) in Finland. In addition, all sporadic isolates from 2006 were  
36 included. *spa* typing was more expensive, but approximately four times faster to  
37 perform than PFGE. Nearly 90% of FIN-4 and FIN-16 isolates showed consistent *spa*  
38 types, t172 and t067 respectively. *spa* typing predicted the PFGE result of the blood  
39 isolates by a Wallace coefficient of 0.9009, recognized internationally successful  
40 strains (t041, t067) to be common also in Finland, and identified a separate cluster of  
41 isolates, also related in time and place among the FIN-4 strains. Additional typing by  
42 another method was needed to provide adequate discrimination, or to characterize  
43 isolates with a newly recognized *spa* type in Finland.

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51 **Keywords**

52 MRSA; *spa* typing; MLST; PFGE; laboratory-based surveillance

53 **Introduction**

54 Methicillin-resistant *Staphylococcus aureus* (MRSA) is a persistent public health  
55 problem worldwide. Currently, MRSA infection can be acquired both in healthcare  
56 institutions and in the community. In Finland, the annual number of MRSA cases has  
57 increased from 340 in 2001 to 1473 in 2004 [1] and in the year 2008, MRSA case  
58 numbers reached a level higher than ever before, at 1741 new cases [2].

59 Pulsed-field gel electrophoresis (PFGE) was originally developed for outbreak  
60 investigations, and its use in long-term laboratory-based surveillance can be  
61 misleading [3, 4]. Lately, the interpretation of PFGE typing results has become even  
62 more challenging as the number of profiles in the databases continues to grow.  
63 Concurrently, *spa* typing has become a widely used method to distinguish different  
64 MRSA strains. It allows unambiguous data interpretation, and therefore also  
65 international comparison [5, 6]. However, in some instances, discriminatory power of  
66 *spa* typing is not as high as that of PFGE [7-9].

67 To enable straightforward and comprehensive international comparability and to  
68 avoid excessive work load in the national MRSA surveillance and epidemiological  
69 investigations, we analyzed the consequences of substituting PFGE with *spa* typing as  
70 the primary method for MRSA typing in Finland. We focused both on the short- (year  
71 2006) and long-term (years 1997-2006) correspondence between the PFGE and *spa*  
72 typing results. As study material, we selected MRSA blood isolates, sporadic MRSA  
73 isolates, and two strains, PFGE types FIN-4 and FIN-16, which are currently the most  
74 common community-associated MRSA (CA-MRSA) and healthcare-associated (HA-  
75 MRSA) strains, respectively, in Finland [10].

76 **Materials and methods**

77 Bacterial isolates

78 In Finland, clinical microbiology laboratories report all MRSA findings from clinical  
79 or screening samples to the national infectious disease register (NIDR) and multiple  
80 findings within 50 years from the same person are combined as one. Corresponding  
81 MRSA isolates are sent to the Staphylococcal Reference Laboratory at the National  
82 Institute for Health and Welfare (THL) for genotyping.

83

84 In this study a total of 589 MRSA isolates (one per person), genotyped previously by  
85 PFGE, was analyzed by *spa* typing. To analyze the short-term correspondence  
86 between the two methods, 108 FIN-4 (typically ST375, *SCCmec* IV) and 116 FIN-16  
87 (typically ST125, *SCCmec* IA) isolates from the year 2006, as well as all 49 sporadic  
88 strain types (as determined by PFGE) from 2006 were chosen. For the FIN-4 and FIN-  
89 16 isolates, the first ten isolates of every month in 2006, if available, were chosen.

90 To study the long-term correspondence, three sets of isolates between the years 1997  
91 and 2006 were chosen: i) all Finnish MRSA blood isolates (N=124), ii) 80 FIN-16  
92 isolates, and iii) 112 FIN-4 isolates (excluding FIN-4 and FIN-16 blood isolates). For  
93 FIN-4 and FIN-16 MRSA isolate sets, the first isolate of every month between the  
94 years 1997 and 2006 (max. 12 isolates per year), if available, were chosen. For 2006,  
95 every eleventh isolate for each month was chosen (to avoid overlapping with the first  
96 ten isolates already included in the short-term analysis).

97

98 Pulsed-field gel electrophoresis

99 The 589 MRSA isolates were previously genotyped by pulsed-field gel  
100 electrophoresis (PFGE) according to the Harmony protocol as described previously

101 [11, 12]. The strain NCTC 8325 was used as a control. PFGE patterns were analysed  
102 by BioNumerics (Applied Maths, Kortrijk, Belgium) by using the Dice coefficient to  
103 analyse the similarity of the banding patterns, and the unweighted pair group method  
104 using arithmetic averages (UPGMA) for cluster analysis. As in previously published  
105 studies, a similarity cut-off of 80% [13] or the criterion of a difference of  $\leq 6$  bands [4]  
106 were used to assign a PFGE type. The PFGE types were considered different if a  
107 seven or more band difference occurred. PFGE subtypes were not assigned. PFGE  
108 profiles differing by more than six bands, compared to any other profile in the local  
109 PFGE database, were regarded as sporadic types.

110

#### 111 *Spa* typing

112 The *spa* typing was done as described previously [5, 14]. Sequences were analyzed by  
113 the Ridom StaphType program (Ridom GmbH, Würzburg, Germany) [15]. Clustering  
114 of clonal complexes of related *spa* types was done by using the repeat pattern (BURP)  
115 algorithm of the Ridom StaphType software [16]. The default parameters of the  
116 software were used for the cluster definition: *spa* types were clustered if the calculated  
117 cost between members of a group was less than or equal to four and *spa* types shorter  
118 than five repeats were excluded. A bionighbourjoining tree of *spa* types was created  
119 by Splits Tree4 (version 4.10) program [17] by using cost values for analysing  
120 distances between *spa* types of blood isolates.

121

#### 122 Multilocus sequence typing

123 Multilocus sequence typing (MLST) was performed as described previously [18].  
124 Eight FIN-4 and 11 FIN-16 representative MRSA strains were selected for MLST,  
125 based on different *spa* typing results compared to the most common *spa* type of FIN-4

126 (t172) and FIN-16 (t067) strains. In addition, to study clonality of the typical FIN-16  
127 MRSA strains with *spa* type t067, between 2002 and 2006, five strains were selected  
128 for MLST. Lineage assignment to clonal complexes (CCs) for each sequence type (ST)  
129 was performed by eBURST analysis using default stringent parameters [19].

130

#### 131 SCC*mec* typing

132 Staphylococcal cassette chromosome *mec* (SCC*mec*) analysis was performed for the  
133 same 19 representative MRSA strains which went through the MLST analysis. The  
134 *ccr* type (*ccrAB1* to *ccrAB4*, or *ccrC*) and *mec* class (A, B, or C) within SCC*mec*  
135 were analyzed by PCR (M-PCR 1 and -2) as described previously by Kondo and co-  
136 workers [20].

137

#### 138 Discriminatory power and Wallace's coefficient

139 The discriminatory power (discriminatory index, DI) of different genotyping methods  
140 were calculated using the Ridom StaphType software as described earlier [12, 21, 22].  
141 The quantitative concordance between typing methods was analyzed by Wallace  
142 coefficient (W) [23, 24]. Wallace's coefficient value indicates the probability that two  
143 strains classified as the same type by one method are also classified as the same by  
144 another method. A high value of W coefficient indicates that the result obtained by a  
145 given typing method could have been predicted by the other method.

146

#### 147 Cost and time analysis

148 The mean hands-on time and total time used for processing one set of isolates for *spa*  
149 typing (N=24) and PFGE (N=12) was estimated by stopwatch analysis of each step of  
150 the processes at least three times by three different persons. The time used for

151 analysing the results was included in the hands-on time. The material costs of both  
152 methods were estimated based on material usage per isolate. Sequencing was  
153 provided as a service from another laboratory.

154

155



156 **Results**

157 MRSA blood isolates (years 1997-2006)

158 The 124 MRSA blood isolates belonged to 38 different *spa* types comprising eight  
159 *spa* clusters (*spa*-CC), including one without founder, and 10 singleton *spa* types (12  
160 isolates). The most prevalent *spa* type was t067 (32%), and 33 *spa* types were found  
161 in less than four isolates of each (Table1). By PFGE, 25 different types were  
162 identified; six of these were sporadic.

163

164 In seven of eight *spa*-CCs, one PFGE type comprised the majority of isolates in each  
165 *spa*-CC (Table 1 and Fig. 1). For instance, among the 20 isolates of *spa*-CC2521/041,  
166 18 were of PFGE type FIN-21 whereas one isolate was FIN-24 and another one was  
167 sporadic. Isolates showing other than the typical PFGE type of the particular *spa*-CC  
168 are indicated in Table 1. Isolates within *spa*-CC012 showed the most variable PFGE  
169 types (FIN-2, FIN-5, FIN-9, FIN-13, and two sporadic types). Seven of twelve  
170 isolates with singleton *spa* types had a distinct PFGE type (FIN-1, -12, -20, -14, and  
171 sporadic) from any other isolate and four of which (*spa* types t032, t148, t160, and  
172 t355) were also distant from any *spa*-CCs in the bionighbourjoining tree (Table 1 and  
173 Fig. 1).

174

175 Isolates of nine (FIN-4, -5, -7, -10, -11, -15, -16, -19, and -21) of the 25 PFGE types  
176 showed variation in *spa* types. (Table 1). Within isolates of four PFGE types (FIN-5, -  
177 10, -15, and -19), some *spa* types were not members of the same *spa*-CC, but were  
178 mostly singleton or excluded *spa* types. However, among all but one PFGE type  
179 (FIN-5), a single *spa* type dominated and the rest occurred only once. The three FIN-5  
180 isolates showed each a different *spa* type. FIN-10 was a highly variable PFGE-type;

181 three isolates were t015, and five isolates of each showed a different *spa* type. As a  
182 whole 48% (59/124) of *spa* types of blood isolates were associated with multiple  
183 PFGE types or were recognised in Finland for the first time.

184

185 Among the blood isolates *spa* typing had higher discriminatory power than PFGE  
186 types as calculated by Ridom and PFGE had higher discriminatory power than the  
187 *spa*-CC (Table 2). *spa* type had a higher probability to predict PFGE types  
188 ( $W=0.9009$ ) than PFGE types to predict *spa* types ( $0.7853$ ) as calculated by Wallace  
189 coefficient. PFGE types performed better in predicting *spa*-CC ( $W=0.9870$ ) than *spa*-  
190 CC in predicting PFGE types ( $0.7810$ ).

191

192 MRSA FIN-4 and FIN-16 epidemic isolates

193 Of the 220 FIN-4 MRSA isolates between the years 1997 and 2006, nine *spa* types  
194 were found. The *spa* types belonged to one *spa* cluster, *spa*-CC 172, and to one  
195 singleton. The majority (87%, 191/220) of the isolates were of *spa* type t172. Three  
196 sequence types (ST) among eight uncommon *spa* types of FIN-4 isolates were  
197 identified by MLST: ST87 (N=1), ST59 (N=1) and ST375 (N=6) (Table 3.). All the  
198 STs belonged to one clonal complex (CC), CC59. Seven of eight isolates possessed  
199 SCC*mec* type IV and one isolate was non-typeable.

200

201 Twelve *spa* types were found among the 196 FIN-16 isolates between the years 1997  
202 and 2006. All belonged to one *spa* cluster, *spa*-CC 002. The most prevalent *spa* type  
203 was t067 (88%). Among 11 *spa* types other than t067, three STs were found by  
204 MLST; ST5 (N=6), ST125 (N=3) and ST146 (N=2) (Table 3). The five FIN-16  
205 isolates with *spa* type t067 were ST125. All the STs belonged to CC5. Among the

206 same 11 isolates, eight possessed SCC*mec* type IV, and the remaining three carried  
207 SCC*mec* type I, V, and a non-typable SCC*mec* type. Because of excluded, singleton  
208 or nontypable *spa* type, 4% (8/220) of FIN-4 and 3% (5/196) of FIN-16 were not  
209 recognized as belonging to the same clonal lineage based on *spa* typing alone.

210

211 Sporadic isolates (year 2006)

212 Among the 49 sporadic isolates 29 *spa* types were found. The most prevalent *spa* type  
213 was t008 (12%). Sporadic isolates were divided into four *spa* clusters (*spa*-CC 002,  
214 *spa*-CC 172, *spa*-CC 008 and a cluster with no founder) and into 11 singleton *spa*  
215 types (14 isolates). Eighteen (18/49, 37%) of the sporadic isolates shared a *spa* type  
216 with the representatives of Finnish epidemic strains defined earlier by PFGE [12].  
217 Forty-five (92 %) of the sporadic isolates associated with *spa* type which related to  
218 multiple PFGE types, previously unknown *spa* type among Finnish isolates, or an NT  
219 *spa* type result.

220

221 Cost and time analysis

222 Total times required to type 24 isolates by *spa* typing was 9 h 15 min, and 12 isolates  
223 by PFGE was 40 h. Hands-on times were 6 h 30 min for both methods. Approximate  
224 material cost for PFGE was 5 € per isolate and for *spa*-typing 9 € per isolates.

225

226

227 **Discussion**

228 Nationwide laboratory-based MRSA surveillance requires accurate typing with a  
229 possibility both to detect local outbreaks and to perform international comparisons.  
230 Ideally, the typing process should also be fast and economic. The aim of this study  
231 was to estimate the consequences of replacing PFGE with *spa* as a primary typing  
232 method in a centralized reference laboratory for the ability of local infection control  
233 bodies to perform surveillance and outbreak detection, which has been based on  
234 PFGE-determined strain nomenclature.

235

236 *spa* typing possessed a high probability to predict PFGE type, and was faster to  
237 perform, but more expensive than PFGE. The total time to perform PFGE was  
238 approximately four times longer than time required for *spa* typing. The hands-on  
239 times used for both methods were equal. However, some inaccuracy exists in  
240 estimating PFGE hands on time. The time required for interpretation of PFGE patterns  
241 was highly variable, and because of practical reasons, the number of isolates  
242 processed at a time was 12 in PFGE, whereas it was 24 in *spa* typing.

243

244 The 124 Finnish MRSA blood isolates from the period 1997 to 2006 were genetically  
245 diverse, comprising 38 *spa* types. According to a recent study of European invasive *S.*  
246 *aureus* covering the period 2006–2007, three of the most frequent *spa* types among  
247 MRSA isolates were t032, t008 and t041. *spa* type t041 was found to be common also  
248 in our study, comprising 13% of all blood isolates during the period 1997–2006 [25].

249

250 Although several *spa* types among blood isolates showed more than one PFGE type,  
251 in most instances the PFGE type was consistent within the *spa*-CC. Variation in

252 PFGE types occurred especially in *spa*-CC 12. Since PFGE typing had been  
253 performed earlier (between years 1997 and 2006), these results were not interpreted at  
254 the subtype level. This affected the discriminatory power of PFGE, and explains the  
255 discrepancy with several studies that have shown PFGE to be more discriminatory  
256 than *spa* typing [9, 14]. The two genotyping methods measure different markers of  
257 genetic variation in *S. aureus*. PFGE targets the whole genome whereas *spa* typing  
258 targets the polymorphic repeat region of the *spa* gene. The mutation rate of the given  
259 bacterial clone affects the pattern stability of PFGE [3, 26] and epidemiological  
260 interpretations of related PFGE patterns depend on the time and space scale of the  
261 study [26]. The *spa* region has been shown to be fairly stable [27, 28], although Kahl  
262 and co-workers demonstrated a rather high mutation rate for this region among *S.*  
263 *aureus* strains isolated from cystic fibrosis patients with persistent *S. aureus* infections  
264 [29]. However, the relation of the *spa* region variability to the overall evolution of the  
265 *S. aureus* genome is not yet fully understood, and may differ between *S. aureus*  
266 clones.

267

268 At the time of analysis, nearly half of the blood isolates had a *spa* type recognised in  
269 Finland for the first time, or associated with multiple PFGE types. Therefore, PFGE  
270 or another typing method is still required as has also been reported in other studies [7,  
271 8]. Four sporadic isolates by PFGE would have been classified as non-unique by *spa*  
272 typing. However, among the two major MRSA strains (FIN-16 and FIN-21) among  
273 blood isolates, majority (90%) would have been consistently identified as t067 or  
274 t041, respectively, based on *spa* typing alone.

275

276 The FIN-4 isolates during 1997–2006 were largely homogenous (*spa*-CC172,  
277 SCC*mec*IV) and the predominant *spa* type t172 was found throughout these years.  
278 The FIN-4 isolates are known to be community-associated and found throughout  
279 Finland. Between 2004 and 2006 FIN-4 comprised 10% of all MRSA isolates in  
280 Finland [10]. *spa* types provided additional information in one instance where a small  
281 cluster was recognised among the FIN-4 isolates with *spa* type t3627 (one extra repeat  
282 compared to repeats in t172) in 2006. The strains were isolated from the same  
283 healthcare centre during June and July. Three strains with the same *spa* type were also  
284 found in 2002 and 2003 from the same healthcare district.

285

286 According to the content of the Ridom SpaServer, *spa* type t172 has been isolated in  
287 eight European countries, but to the best of our knowledge, it does not belong to the  
288 most prevalent type of strains in clinical settings outside the Nordic countries [19].  
289 Recently, isolates with *spa* t172 were identified as the most commonly found MRSA  
290 in municipal waste water in Sweden [30]. The genotypes recognized in waste water  
291 corresponded to those in clinical settings, and therefore, likely originated from human  
292 carriage.

293

294 The FIN-16 isolates between 1997 and 2006 were genetically close to each other, as  
295 most had *spa* type t067, and all belonged to *spa*-CC002. However, variation on  
296 SCC*mec* types was noticed among FIN-16 isolates, which can be a consequence of  
297 different ancestors or the susceptibility of staphylococcal isolates to receive,  
298 recombine, and/or replace different SCC*mec* elements [31]. During 1997–2000, the  
299 *spa* type t002 was predominant among the FIN-16 isolates but it was replaced by *spa*  
300 type t067 from 2001 onwards. Outbreaks of FIN-16 (t067) seem to be responsible for

301 the increase of reported MRSA cases observed from 1997 to 2004 [1] and between  
302 2004 and 2006 FIN-16 comprised nearly one third of all MRSA isolates in Finland  
303 [32]. The same clone, MRSA-ST125-IV, has also been recognized as the most  
304 prevalent hospital-acquired-MRSA in Spain [8]. A clone closely related to FIN-16 (  
305 ST5, *spa* type t067 and SCC*mec* II) has also been indentified in Portuguese hospitals  
306 [33]. In addition, according to the content of the Ridom SpaServer, *spa* type t067  
307 isolates have been identified in 13 different countries.

308

309 Using *spa* typing instead of PFGE would not have affected interpretation of the  
310 majority of the two most prevalent MRSA strains in Finland, FIN-4 and FIN-16.  
311 Nearly ninety percent of the *spa* types of FIN-4 and FIN-16 were consistent. For the  
312 rest, PFGE was additionally required because the isolate was non-typeable, the *spa*  
313 type (t002) obtained was known to be associated with multiple PFGE types, or it was  
314 previously unknown among Finnish isolates.

315

316 Using *spa* typing as the primary genotyping method will influence on the amount of  
317 isolates interpreted as sporadic types, since 37% defined as unique by PFGE shared a  
318 *spa* type with an epidemic strain. Most of the sporadic isolates would have required  
319 PFGE due to previously unknown *spa* type among Finnish isolates, an NT *spa* type  
320 result, or *spa* type associated with multiple PFGE types. However, the need for  
321 additional typing by PFGE will decrease when the knowledge on less common *spa*  
322 types and their associated PFGE types accumulates through adoption of the new  
323 typing scheme in Finland.

324

325 In our revised typing process, PFGE will follow *spa* typing if a *spa* type is recognised  
326 in Finland for the first time, a new *spa* type according to *spa* server is encountered, on  
327 occasions where a *spa* type is known to be associated with multiple PFGE types, or if  
328 the isolate is not typable by *spa* typing. In 2009, additional typing by PFGE was  
329 needed for 22% of isolates in Finland. *spa* typing qualifies as a first-line typing  
330 method, and can be adapted for laboratory-based MRSA surveillance in countries  
331 with a long history of band pattern –based strain nomenclature.

332

### 333 **Acknowledgement**

334 This work was supported by The Ministry of Social Affairs and Health and the  
335 Finnish Cultural Foundation.

336 We thank Mari Hyvönen, Saija Perovuo and Terhi Vesa for skilled technical  
337 assistance. We also thank Tiina Hannunen from Molecular Medicine Sequencing  
338 Laboratory (Biomedicum, Helsinki) for offering information of the time required to  
339 perform sequencing. This data was partly presented at the 8th International Meeting  
340 on Epidemiological Markers, May 2008, Zakopane, Poland, Poster no. A2.

341

### 342 **Conflict of interest**

343 The authors declare that they have no conflict of interest.



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## Figure caption

**Fig 1** Bionighbourjoining tree of *spa* types of the blood isolates (N=124) excluding those (N=2) with less than five repeat units. Spa-CCs are shown by gray circles and the most prevalent PFGE-type of each spa-CC is indicated. S indicates a singleton *spa* type. Asterisk indicates the most prevalent *spa* type within spa-CC.

Table 1. MRSA blood isolates between 1997 and 2006 in Finland.

<i>spa</i> -CC <sup>a</sup>	<i>spa</i> type	<i>spa</i> repeat	No. of isolates (N=124)	PFGE type	Isolation year/years
002	t002	26-23-17-34-17-20-17-12-17-16	2	FIN-3	2005, 2006
008	<b>t008</b>	11-19-12-21-17-34-24-34-22-25	3	FIN-7	2001, 2005, 2006
	<b>t008</b>	11-19-12-21-17-34-24-34-22-25	2	FIN-15	2001, 2004
	<b>t008</b>	11-19-12-21-17-34-24-34-22-25	1	FIN-25	2006
	t024	11-12-21-17-34-24-34-22-25	1	FIN-7	2005
	t068	11-19-19-12-21-17-34-24-34-22-25	2	FIN-7	2003, 2004
	t121	11-19-21-17-34-24-34-22-25	1	FIN-7	2006
	<b>t596</b>	11-19-12-21-17-34-34-24-34-22-25	1	FIN-7	2006
	<b>t596</b>	11-19-12-21-17-34-34-24-34-22-25	1	FIN-10	2002
012	t012	15-12-16-02-16-02-25-17-24-24	1	FIN-5	2006
	t018	15-12-16-02-16-02-25-17-24-24-24	1	FIN-5	1997
	t030	15-12-16-02-24-24	1	FIN-9	2005
	<b>t037</b>	15-12-16-02-25-17-24	4	FIN-2	1998, 1999, 2004
	<b>t037</b>	15-12-16-02-25-17-24	2	FIN-13	1998
	<b>t037</b>	15-12-16-02-25-17-24	2	sporadic	2002, 2004



015	t015	08-16-02-16-34-13-17-34-16-34	3	FIN-10	2005, 2006
	t583	08-16-02-16-34-13-16-34	1	sporadic	2002
	t630	08-16-02-16-34-17-34-16-34	1	FIN-10	2006
067	<b>t067</b>	26-23-17-34-17-20-17-12-17	39	FIN-16	2002-2006
	<b>t067</b>	26-23-17-34-17-20-17-12-17	1	sporadic	2002
	t1084	26-23-17-34-17-20-17-12	1	FIN-16	2005
	t3148	26-23-17-34-17-13-17-12-17	1	FIN-16	2005
	t442	35-17-34-17-20-17-12-17-16	1	FIN-16	2006
376	t044	07-23-12-34-34-33-34	2	FIN-11	1999, 2005
	t267	07-23-12-21-17-34-34-33-34	1	FIN-19	2006
	t376	07-23-12-34-34-34-33-34	1	FIN-11	2006
2521/041	<b>t041</b>	26-30-17-34-17-20-17-34-17-20-17-12-17-16	14	FIN-21	2004-2006
	<b>t041</b>	26-30-17-34-17-20-17-34-17-20-17-12-17-16	1	FIN-24	2005
	<b>t041</b>	26-30-17-34-17-20-17-34-17-20-17-12-17-16	1	sporadic	2004
	t1628	26-30-17-34-17-20-17-34-17-20-17-12-17-16-16	2	FIN-21	2006
	t2521	26-30-17-13-17-20-17-34-17-20-17-12-17-16	1	FIN-21	2004
	t3181	26-30-17-13-17-20-17-13-17-20-17-12-17-16	1	FIN-21	2004
no founder	t172	04-20-17-45-16-34	12	FIN-4	1999, 2001-2004, 2006
	t976	04-20-17-20-31-16-34	1	FIN-4	2005
singleton	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	2	FIN-12	2000, 2006
	t127	07-23-21-16-34-33-13	2	FIN-19	2002, 2004
	t148	07-23-12-21-12-17-20-17-12-12-17	1	FIN-20	2005
	t160	07-23-21-24-33-22-17	1	FIN-14	1999
	t355	07-56-12-17-16-16-33-31-57-12	1	sporadic	2006
	t550	08-17-34-16-34	1	FIN-10	2005
	t562	11-19-21-12-21-17-34-22-25	1	FIN-1	2000
	t689	11-19-12-22-25	1	FIN-15	2004

	t2099	08-16-16-34-34	1	sporadic	2006
	t2163	01-12-16-16-16-02-16-02-25-24-24	1	FIN-5	2003
excluded	t1644	08-13-16-34	1	FIN-10	2004
	t3020	08-17-13-34	1	FIN-10	2004

<sup>a</sup>*spa* types were clustered if the calculated cost between members of a group was less than or equal to four and *spa* types shorter than five repeats were excluded.

*spa* types marked in bold font showed multiple PFGE types.

Table 2. Discriminatory power of different genotyping methods among blood isolates.

Method	No. of different types	of DI	95% CI
<i>spa</i>	38	0.866	[0.819-0.914]
<b>spa-CC</b>	17	0.811	[0.761-0.861]
<b>PFGE</b>	26	0.847	[0.798-0.895]

Table 3. *spa* typing, MLST and SCC*mec* results of FIN-4 and FIN-16 MRSA strains in Finland. *spa* types shorter than five repeats were excluded.

PFGE type	Year	No. of isolates studied <sup>a</sup>	<i>spa</i> type <sup>b</sup> (No. of isolates)	<i>spa</i> repeat	<i>spa</i> -CC	MLST ST	MLST CC	SCC <i>mec</i>
FIN-4	1997	8	t172	04-20-17-45-16-34	172			
	1998	11	t172	04-20-17-45-16-34	172			
	1999	12	t172	04-20-17-45-16-34	172			
	2000	12	t172	04-20-17-45-16-34	172			

	2001	12	t172	04-20-17-45-16-34	172				
	2002	12	t172 (9)	04-20-17-45-16-34	172				
			t216 (1)	04-20-17-20-17-31-16-34	172	87	59		IV
			t3627 (2)	04-20-17-24-45-16-34	172	375	59		IV
	2003	12	t172 (8)	04-20-17-45-16-34	172				
			t529 (1)	04-34	Excluded	375	59		IV
			t2380 (1)	04-02-17-45-16-34	172	375	59		IV
			t3627 (2)	04-20-17-24-45-16-34	172				
	2004	12	t172 (12)	04-20-17-45-16-34	172				
	2005	12	t172 (12)	04-20-17-45-16-34	172				
	2006	117	t172 (95)	04-20-17-45-16-34	172				
			t458 (1)	26	Excluded	375	59		IV
			t529 (2)	04-34	Excluded				
			t976 (1)	04-20-17-20-31-16-34	172	59	59		NT
			t2380 (3)	04-02-17-45-16-34	172				
			t3424 (2)	04-13	Excluded	375	59		IV
			t3627 (11)	04-20-17-24-45-16-34	172				
			t3718 (1)	04-12-45-16-34	Singleton	375	59		IV
			NT (1)	-	-				
FIN-16	1997	1	t002	26-23-17-34-17-20-17-12-17-16	002	5	5		IV
	1998	2	t002	26-23-17-34-17-20-17-12-17-16	002				
	1999	6	t002 (2)	26-23-17-34-17-20-17-12-17-16	002				
			t067 (1)	26-23-17-34-17-20-17-12-17	002				
			t214 (3)	26-23-17-34-17-20-17-12-17-16-16	002	5	5		IV
	2000	3	t002 (1)	26-23-17-34-17-20-17-12-17-16	002				
			t067 (1)	26-23-17-34-17-20-17-12-17	002				
			NT (1)	-	-				
	2001	8	t002 (2)	26-23-17-34-17-20-17-12-17-16	002				
			t067 (5)	26-23-17-34-17-20-17-12-17	002				
			t242 (1)	26-23-17-13-17-20-17-12-17-16	002	5	5		V
	2002	12	t067 (12)	26-23-17-34-17-20-17-12-17	002	125	5		

2003	12	t067 (11) NT (1)	26-23-17-34-17-20-17-12-17 -	002 -	125	5	
2004	12	t067 (11) t535 (1)	26-23-17-34-17-20-17-12-17 26-17-16	002 Excluded	125 5	5	NT
2005	12	t067 (12)	26-23-17-34-17-20-17-12-17	002	125	5	
2006	128	t067 (119) t442 (3) t548 (1) t777 (1) t952 (1) t1094 (1) t1991 (1) t3109 (1)	26-23-17-34-17-20-17-12-17 35-17-34-17-20-17-12-17-16 26-23-17-34-17-20-17-12-16 26-23-17 26-23-17-34-17-20-12-12-17-16 23-17-34-17-20-17-12-17-16 08-17 26-23-17-34-17-17-12-17	002 002 002 Excluded 002 002 Excluded 002	125 146 146 125 5 5 125 125	5 5 5 5 5 5 5 5	IV IV IV I IV IV IV

<sup>a</sup> 1997-2005: the first FIN-4 and FIN-16 strain of each month, if available.

2006: every eleventh FIN-4 and FIN-16 strain of each month, if available.

<sup>b</sup>NT, non-typeable