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2 Extensive Genetic Diversity Identified Among Sporadic Methicillin-
3 Resistant *Staphylococcus aureus* Isolates Recovered in Irish
4 Hospitals Between 2000-2012

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23 **ABSTRACT**

24 Clonal replacement of predominant nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA)
25 strains has occurred several times in Ireland during the last four decades. However, little is known about
26 sporadically-occurring MRSA in Irish hospitals or in other countries. Eighty-eight representative *pvl*-
27 negative sporadic MRSA isolates recovered in Irish hospitals between 2000-2012 were investigated.
28 These yielded unusual pulsed-field gel electrophoresis and antibiogram-resistogram typing patterns
29 distinct from those of the predominant nosocomial MRSA clone, ST22-MRSA-IV, during the study
30 period. Isolates were characterized by *spa* typing and DNA microarray profiling for multilocus sequence
31 type (MLST) clonal complex (CC) and/or sequence type (ST) and SCC*mec* type assignment, and for
32 detection of virulence and antimicrobial resistance genes. Conventional PCR-based SCC*mec* subtyping
33 was undertaken when necessary.

34 Extensive diversity was detected including 38 *spa* types, 13 MLST-CCs including 18 STs among
35 62 isolates assigned to STs and 25 SCC*mec* types including two possible novel SCC*mec* elements and
36 seven possible novel SCC*mec* subtypes. Fifty-four MLST-*spa*-SCC*mec* type combinations were identifed.
37 Overall 68.5% of isolates were assigned to nosocomial lineages with ST8-t190-MRSA-IID/IIE +/- SCC_{M1}
38 predominating (17.4%) followed by CC779/ST779-t878-MRSA-ψSCC*mec*-SCC-SCC_{CRISPR} (7.6%) and
39 CC22/ST22-t032-MRSA-IVh (5.4%). Community-associated clones including CC1-t127/t386/t2279-
40 MRSA-IV, CC59-t216-MRSA-V, CC8-t008-MRSA-IVa, CC5-t002/t242-MRSA-IV/V and putative
41 animal-associated clones including CC130-t12399-MRSA-XI, ST8-t064-MRSA-IVa, ST398-t011-
42 MRSA-IVa and CC6-t701-MRSA-V were also identified. In total, 53.3% and 47.8% of isolates harbored
43 resistance genes to two or more classes of antimicrobial agents and two or more mobile genetic element-
44 encoded virulence-associated factors, respectively.

45 Effective ongoing surveillance of sporadic nosocomial MRSA is warranted for early detection of
46 emerging clones and reservoirs of virulence, resistance and SCC*mec* genes.

47

47 **INTRODUCTION**

48 *Staphylococcus aureus* colonizes the anterior nares of approximately 30% of the human
49 population and can give rise to a wide range of infections of skin and soft tissues, bones, joints
50 and prosthetic implants and can be responsible for a variety of toxinoses caused by specific toxins
51 such as toxic shock toxin, enterotoxins, exfoliative toxins and Panton-Valentine leukocidin (1).
52 *Staphylococcus aureus* can evolve to methicillin-resistant *S. aureus* (MRSA) upon acquisition of
53 a large staphylococcal chromosomal cassette (SCC) element harboring either the methicillin
54 resistance gene *mecA* or *mecC* (SCC*mec*), both of which encode a modified penicillin-binding
55 protein PBP2a (2-4).

56 Within SCC*mec*, *mecA* or *mecC* form part of the *mec* gene complex, which may also
57 harbor the *mec* regulatory genes *mecI* and *mecR1*, as well as insertion sequences and in some
58 instances, *blaZ* (1-3). Based on various combinations and truncations of the *mec* complex genes
59 five classes of the *mec* gene complex (A, B, C1, C2 and E) have been identified in MRSA (3, 5).
60 In addition, each SCC*mec* element also harbors a chromosome cassette recombinase (*ccr*) gene
61 complex, consisting of *ccrA* and *ccrB* together or *ccrC* which encode polypeptides that catalyze
62 site- and orientation-specific integration and excision of SCC*mec* into *orfX* within the *S. aureus*
63 chromosome (1, 6). Seven types of *ccr* gene complex (1-5, 7 and 8) have been described in
64 MRSA each with a different combination of *ccrA* and *ccrB* or *ccrC* alleles (5, 7). SCC elements
65 that carry *ccr* genes but lack *mec* genes have also been described as well as pseudo (ψ) SCC*mec*
66 and SCC elements that lack *ccr* genes, individual SCC*mec* elements with multiple *ccr* genes and
67 composite islands consisting of two or more elements (5).

68 Eleven SCC*mec* types (I-XI) have been described to date in MRSA, each with a different
69 combination of *mec* class and *ccr* type (3). Numerous SCC*mec* subtypes have also been described

70 in MRSA which differ from SCC*mec* types based on DNA sequence variation or the presence or
71 absence of mobile genetic elements (MGEs) in the joining or "J" regions" which are located
72 outside of the *ccr* and *mec* complexes (7). MRSA often exhibit resistance to a range of
73 antimicrobial agents that can be due to the carriage of multiple antimicrobial resistance genes
74 located on MGEs including transposons, plasmids and SCC/SCC*mec* elements (8, 9).

75 The first report of MRSA appeared in the literature in 1961 shortly after the introduction
76 of methicillin into clinical use and just ten years later, in 1971, MRSA were first reported in Irish
77 hospitals (10, 11). Following a major increase in the prevalence of MRSA in Irish hospitals in the
78 late 1970s and during the 1980s and 1990s, it has now been endemic for more than three decades
79 (12-17). Since 1999 the prevalence rate of MRSA among *S. aureus* causing bloodstream
80 infections (BSIs) in Ireland has been monitored by the European Antimicrobial Resistance
81 Surveillance Network (EARS-Net). Annual rates of MRSA among *S. aureus* from BSIs in Ireland
82 reached 42% (592 MRSA among 1,412 *S. aureus*) in 2006, the highest level reported to date, and
83 declined in recent years with a rate of 22.8% (242 MRSA among 1,060 *S. aureus*) reported for
84 2012 (18, 19).

85 Clonal replacement of predominant nosocomial MRSA strains has occurred several times
86 in Ireland during the last four decades (20). The different MRSA lineages that predominated in
87 Irish hospitals at different time periods have been well characterized, including multilocus
88 sequence type (ST) 250-MRSA-I/I-*pls* in the 1970s and early 1980s, ST239-MRSA-III/III-
89 pI258/Tn*554* in the mid- to late-1980s and early 1990s, ST8-MRSA-IIA-IIE throughout the
90 1990s together with ST36-MRSA-II and ST22-MRSA-IV in the late 1990s, and since 2002 the
91 ST22-MRSA-IV clone has dominated (17, 20). Prior to 1999, ST22-MRSA-IV was only detected
92 sporadically among MRSA in Ireland but by 2003 it accounted for 80% of MRSA BSIs, and

93 despite a decline in the proportion of *S. aureus* infections due to MRSA in recent years, has
94 continued to account for 70-80% of MRSA BSIs each year to the present day (19).

95 While a limited number of sporadically-occurring MRSA clones from patients in Irish
96 hospitals in the 1980s and 1990s have been characterized using multilocus sequence typing
97 (MLST), SCC*mec* typing and DNA microarray analysis e.g. ST5-MRSA-II and ST247-MRSA-
98 Ia, there have been no systematic detailed studies of the genetic diversity of sporadic MRSA
99 strains in Ireland (20, 21). These account for approximately 20-30% of MRSA BSIs in Ireland
100 each year, as well as being identified each year among non-BSI isolates submitted to the Irish
101 National MRSA Reference Laboratory (NMRSARL) from patients in hospitals with a variety of
102 infections and from patient and environmental screening samples (19). In fact, the number of
103 sporadic MRSA identified among BSIs in Ireland increased from 12.1% in 2005 to 23.1% in
104 2011 (19). Numerous studies have shown that many MRSA clones that occur sporadically or not
105 at all in one geographic region are often prevalent in another region and vice versa (20, 22, 23).
106 However, previous studies that have investigated sporadic MRSA populations are limited in
107 terms of sample size and/or depth of analysis (24-27).

108 Due to the potential of sporadic MRSA strains to replace currently dominant MRSA
109 clones and because they account for a significant proportion of MRSA infections in Ireland each
110 year, it is essential that populations of new and emerging MRSA strains are monitored. In
111 addition, sporadic MRSA strains may constitute a significant potential reservoir for virulence and
112 resistance genes located on MGEs, in particular SCC*mec* elements. Therefore, the present study
113 investigated the genotypes, SCC*mec* types, virulence and resistance genes within 88 MRSA
114 isolates representative of 1663 *pvl*-negative sporadically-occurring MRSA isolates from patients
115 in Irish hospitals between 2000 and 2012. Isolates were investigated using *spa* typing, MLST,

116 SCC*mec* typing and DNA microarray profiling. The 88 sporadic MRSA isolates were selected at
117 the NMRSARL based on unusual antibiogram-resistogram (AR) and/or pulsed-field gel
118 electrophoresis (PFGE) typing patterns which were different to that of the endemic strain that
119 predominated in Irish hospitals during the study period, i.e. ST22-MRSA-IV. All *pvl*-positive
120 MRSA from Irish hospitals and community sources submitted to NMRSARL for examination
121 during the same time period have been investigated as part of a separate study (31).

122

122 ## MATERIALS AND METHODS

123 **Bacterial isolates.** MRSA isolates identified by the NMRSARL were deemed to be sporadic if
124 they exhibited unusual AR and/or PFGE typing patterns which were different to that of the
125 endemic strain in Irish hospitals during the study period, i.e. ST22-MRSA-IV. Unusual AR type
126 patterns included those that were different to previously described ST22-MRSA-IV AR (AR06)
127 type patterns. Unusual PFGE patterns were identified using the criteria of Tenover et al. (28) and
128 differed from PFGE patterns previously identified among ST22-MRSA-IV isolates by ≥ 7 PFGE
129 bands. Using these criteria a total of 1,663 *pvl*-negative sporadic MRSA isolates were identified
130 by the NMRSARL from patients in Irish hospitals between 2000 and 2012. Of the 1,663 isolates,
131 841 were investigated and determined to be *pvl*-negative either by PCR, as described previously
132 (29), or using an in-house real-time PCR assay. The remaining 822 isolates were not investigated
133 for *pvl* as they yielded AR and/or PFGE typing patterns indicative of strains not previously
134 associated with *pvl* e.g. AR13 and AR14, and therefore a *pvl*-negative status was inferred for
135 these (29, 30). All *pvl*-positive isolates recovered in Ireland during the study period between 2000
136 and 2012 were investigated as part of a separate study (31). Eighty-seven of the 1663 *pvl*-
137 negative sporadic MRSA isolates, representative of approximately 5% of sporadic *pvl*-negative
138 MRSA isolates identified each year from patients in Irish hospitals during the 12-year study
139 period (supplemental Table S1), and representing as diverse a range as possible of AR and/or
140 PFGE typing patterns, were selected for detailed investigation. In addition, one *pvl*-negative
141 MRSA isolate recovered from a patient in the community but who had previously been
142 hospitalized on several occasions was also included for investigation. This isolate harbored *mecC*
143 (SCC*mec* type XI) and was included because this clone was recovered sporadically in two
144 patients in two separate hospitals in Ireland in 2010 (2) and has recently been reported in several
145 other European countries (32). The majority of the MRSA isolates (73.8%, 65/88) selected for

146 study were recovered from infections (89%, 58/65, BSIs), (10.8%, 7/65 SSTIs), 11% (10/88)
147 were colonizing isolates from patient screening and no information was available for the
148 remainder (17%, 13/88). Isolates were identified as *S. aureus* and methicillin resistance was
149 confirmed as described previously (29). Isolates were stored on Protect beads (Technical Service
150 Consultants Limited, Heywood, United Kingdom) at –70°C prior to subsequent detailed analysis.

151 **DNA microarray analysis.** The 88 sporadic MRSA isolates were investigated by DNA
152 microarray profiling using the StaphyType kit (Alere Technologies GmbH, Jena, Germany). The
153 StaphyType kit consists of individual DNA microarrays mounted in 8-well microtiter strips
154 which detect 333 *S. aureus* gene sequences and alleles, including species-specific, antimicrobial-
155 resistance and virulence-associated genes, SCC*mec* genes, typing markers and a staining control
156 (33, 34). ArrayMate software (version 2012-01-18) (Alere Technologies) was used to analyze
157 data generated by the microarray system and to assign isolates to inferred multilocus sequence
158 type (MLST) sequence types STs and/or clonal complexes (CCs) by comparing the DNA
159 microarray profile results of test isolates to microarray profiles of an extensive range of reference
160 strains held in the ArrayMate database that have been previously typed by MLST (33, 34). The
161 DNA array can assign all isolates investigated to the correct MLST clonal complex (CC) with a
162 98% correlation with STs assigned by MLST (21). Genomic DNA for use with the DNA
163 microarray was extracted from all isolates by enzymatic lysis using the buffers and solutions
164 provided with the StaphyType kit and the Qiagen Dneasy Blood and Tissue kit (Qiagen, Crawley,
165 West Sussex, UK). The primers, probes and protocols for this DNA microarray system have been
166 described in detail previously (34).

167 In order to visualize the similarities between the 88 sporadic isolates investigated
168 (although not necessarily true phylogenetic relationships), a network tree was constructed using

169 the complete DNA microarray hybridization profile data of the isolates using the software
170 program SplitsTree version 4.11.3 (35) as described previously (1). Array hybridization profiles
171 of the isolates were converted into a series of strings of letters that can be handled by the software
172 as sequences. For comparison, array profiles of 3139 MRSA isolates representative of MRSA
173 globally that were characterized in a previous study were included for comparison (1).

174 **Molecular typing.** Genomic DNA for *spa* typing, MLST and SCC*mec* typing was extracted from
175 each isolate using enzymatic lysis and the DNeasy blood and tissue kit (Qiagen) according to the
176 manufacturer's instructions. Unless otherwise stated, PCRs were performed using GoTaq Flexi
177 DNA polymerase (Promega Corporation, Madison, Wisconsin, USA) according to the
178 manufacturer's instructions and using the published methods for each PCR protocol as described
179 below. PCR amplifications were performed in a G-storm GS1 thermocycler (Applied Biosystems,
180 Foster City, CA). PCR products were visualized by conventional agarose gel electrophoresis and
181 purified with the GenElute PCR cleanup kit (Sigma-Aldrich Ireland Ltd., Arklow, County
182 Wicklow Ireland).

183 All isolates underwent *spa* typing using the primers and thermal cycling conditions
184 described by the European Network of Laboratories for Sequence Based Typing of Microbial
185 Pathogens (SeqNet [http://www.seqnet.org]). Sequencing was performed commercially by
186 Geneservice Limited (Source Bioscience, Dublin, Ireland) using an ABI 3730xl Sanger
187 sequencing platform. Sequences were analyzed and were assigned to *spa* types using the Ridom
188 StaphType software program, version 1.3 (Ridom GmbH. Wuerzburg, Germany) (36).

189 Although all isolates were assigned to MLST-STs and/or CCs using the DNA microarray,
190 this system only became available during the latter half of the study. Prior to 2006, MLST had

191 been performed on a subset of sporadic MRSA isolates (*n* = 27) representative of different *spa*
192 types (Table 1). MLST was performed as described previously (37), sequences were analysed
193 using BioNumerics software version 7.1 (Applied Maths, Ghent, Belgium) and alleles and STs
194 were assigned using the MLST database (http://www.mlst.net/).

195 Fifty-two sporadic MRSA isolates underwent additional PCR-based SCC*mec* typing to
196 distinguish between SCC*mec* types and subtypes when the DNA microarray was unable to further
197 differentiate them. This included (i) SCC*mec* IV subtyping using the method previously
198 described by Milheirico et al. (38), (ii) SCC*mec* IIA and IIB differentiation using a novel primer,
199 Tn*554*r (5′ GATAGCAGTATGCCTTAATG 3′) targeting Tn*554* which is present only in
200 SCC*mec* IIA and a previously described *ccrAB2* forward primer α2 (39), (iii) SCC*mec* IIIA and
201 IIIB differentiation using a multiplex PCR previously described by Oliveira et al. (40) and (iv)
202 *ccrC* allotype identification using a previously described multiplex PCR to differentiate between
203 SCC*mec* type V (*ccrC2*) and V_T (*ccrC2* and *ccrC8*) (41). In addition, two isolates harboring
204 potentially novel SCC*mec* types were also further investigated using two previously described
205 multiplex PCR schemes targeting the *mec* class and the *ccr* gene complexes (39). Finally, one
206 isolate underwent PCR to confirm the presence of *mecC* and long-range PCR to confirm the
207 presence of SCC*mec* XI using previously described primers (2). Long-range PCRs were
208 performed using the Expand Long Template PCR system (Roche Diagnostics GmdH, Lewes,
209 East Sussex, United Kingdom). MRSA control reference strains and clinical isolates were used as
210 positive controls for SCC*mec* typing as follows: AR07.4/0237 (SCC*mec* IIA/B) (20), E0898
211 (SCC*mec* III) (20), CA05 (SCC*mec* IVa) (42), 8/63-P (SCC*mec* IVb) (42), JCSC/4788 (SCC*mec*
212 IVc) (43), JCSC/4469 (SCC*mec* IVd) (44), M04/0177 (SCC*mec* IVg) (17), E1749 (SCC*mec* IVh)
213 (17), WIS (SCC*mec* V) (45), PM1 (SCC*mec* V_T) (41) and M10/0061 (SCC*mec* XI) (2).

214 **RESULTS**

215 **Genotyping.** Fifty-four different combinations of MLST CC/ST, *spa* types and SCC*mec* types
216 were identified among the 88 isolates, 41 of which were each represented by just one isolate
217 (Table 1). The most prevalent type combination was CC8/ST8-t190-MRSA-IID & SCC_{M1}
218 (11.4%, 10/88) followed by CC779/ST779-t878-MRSA-ψSCC*mec*-SCC-SCC_{CRISPR} (6.8%, 6/88)
219 and CC22/ST22-t032-MRSA-IVh (5.7% 5/88). Three type combinations occurred in 4.5% (4/88)
220 of isolates including CC8/ST8-t190-MRSA-IIE & SCC_{M1}, CC30/ST36-t018-MRSA-II and
221 CC45/ST45-t727-MRSA-IVa. Seven type combinations occurred in 2.3% (2/88) of isolates each
222 including CC1-t127-MRSA-IVa & SCC*fus*, ST1-t2279-MRSA-IVa & SCC*fus*, CC1-t2279-
223 MRSA-IVa, ST5-t045-MRSA-II, ST5-t242-MRSA-V_T, CC8/ST8-t008-MRSA-IVa and ST59-
224 t316-MRSA-V (harboring *ccrC8*) (Table 1). A total of 37 *spa* types and 13 MLST-CCs were
225 identified (Table 1). The identification of STs using MLST or the DNA microarray or both was
226 possible for 63/88 isolates resulting in 17 STs, four of which were novel (Table 1). Overall,
227 isolates belonging to CC8 predominated (24/88; 27.3%), followed by isolates belonging to CC5
228 (17/88, 19.3%), CC1 (10/88, 11.4%), CC22 (9/88, 10.2%,), CC45 (9/88, 10.2%), CC779 (6/88,
229 6.8%), CC30 (6/88, 6.8%) and CC59 (2/88, 2.3%). The remaining CCs (CCs 6, 78, 398, 361 and
230 130) were each represented by one isolate (Table 1).

231 A total of 25 SCC*mec* types and subtypes were identified including SCC*mec* IVa (20.5%,
232 18/88), which was the most prevalent followed by SCC*mec* IID & SCC_{M1} (11.4%, 10/88),
233 SCC*mec* II (10.2%, 9/88), SCC*mec* IVh (10.2%, 9/88), ψSCC*mec*-SCC-SCC_{crispr} (6.8%, 6/88),
234 SCC*mec* IIE & SCC_{M1}, SCC*mec* V_T and SCC*mec* IVa & SCC*fus* (4.5%, 4/88), SCC*mec* IVc
235 (3.4%, 3/88), SCC*mec* IIIB and SCC*mec* IVg (2.3%, 2/88) and six SCC*mec* types were detected

236 in just one isolate including SCC*mec* types IID, III & SCC*hg*, IIIA, SCC*mec* IVd, VI and XI
237 (Table 1).

238 Two isolates (CC5/ST100-t002 and CC45/ST45-t747) carried possible novel SCC*mec*
239 elements because *mec*A was identified, but no *ccr* gene could be detected by the DNA microarray
240 or by PCR-based SCC*mec* typing (Table 1). The remaining nine isolates harbored six possible
241 novel SCC*mec* subtypes (10.2%, 9/88). Of these, three isolates harbored SCC*mec* elements
242 assigned to previously described SCC*mec* types but additional *ccr* genes were also identified i.e.
243 SCC*mec* I & *ccrC* (ST5-t109), SCC*mec* II & *ccrC* (ST36-t018) and SCC*mec* IV (non
244 subtypeable) & *ccrB4* (CC5-t067) (Table 1) and two isolates (CC5-t002 and ST930-t002)
245 harbored SCC*mec* IV elements that could not be subtyped (Table 1).

246 Two novel SCC*mec* V or V_T variants were identified in four isolates due to the carriage of
247 a class C *mec* complex but unusual combinations of *ccr* genes. The SCC*mec* V or V_T elements
248 described in the literature to date have been described as harboring class C *mec* and (i) *ccrC* allele
249 *ccrC1* in SCC*mec* V (5C) in MRSA strain WIS (45), (ii) *ccrC8* & *ccrC10* in SCC*mec* V (5C2&5)
250 in MRSA strain UMCG-M4 (46) or (iii) *ccrC2* & *ccrC8* in SCC*mec* V_T (5C2 & 5) in MRSA
251 strain PM1 (41, 47). However, four isolates in the present study carried class C *mec*, but one
252 harbored *ccrC2* only (CC5-t002) and three isolates carried *ccrC8* only (CC5-t442 and two ST59-
253 t316) (Table 1).

254 Overall, SCC*mec* IV types and subtypes predominated and accounted for 45.5% (40/88)
255 of isolates, followed by SCC*mec* II (29.5%, 26/88), SCC*mec* V (9%, 8/88), pseudo element
256 ψSCC*mec*-SCC-SCC_{crispr} (6.8%, 6/88) and SCC*mec* III (3.4%, 3/88) (Table 1). The majority of
257 isolates carried *mecA* with just one isolate (CC130-t12399) carrying *mecC*.

258 **Virulence-associated genes.** Immune evasion cluster (IEC) genes were detected among 84.1% 259 (74/88) of sporadic MRSA isolates and included *scn* (84%, 74/88), *sak* (84%, 74/88), *chp* 260 (44.3%, 39/88), *sea* (34.1%, 30/88), and *sep* (6.8%, 6/88) (Table 1). The most common IEC type 261 as defined by Van Wamel et al. (2006) was IEC type B (34.1%, 30/88) followed by D (28.4%, 262 25/88), E (9.1%, 8/88), A (6.8%, 6/88), F (4.5%, 4/88), and G (1.1%, 1/88) (Table 1) (48). Clonal 263 complex 5 exhibited the most IEC types including IEC types A, B and D-G while clonal 264 complexes CC22, CC45, CC59 and CC779 harbored IEC type B only. The clonal complexes 265 CC1, CC8 and CC30 harbored multiple IEC types and all CC8-MRSA isolates harboring 266 SCC*mec* IID & SCC_{M1} and SCC*mec* IIE & SCC_{M1} elements that harbored IEC genes exhibited 267 IEC type D and this association has been reported previously (Table 1) (21). The accessory gene 268 regulator (*agr*) allele I was the most dominant *agr* type (47.7%, 42/88) followed by *agr* III 269 (27.3%, 24/88), *agr* II (19.3%, 17/88) and *agr* IV (6.8%, 6/88) (Table 1). The capsule gene type 5 270 predominated and was detected in 60.2% (53/88) of isolates (Table 1).

271 The virulence-associated genes detected among the isolates belonging to the different CCs 272 are shown in Table 1. The most common toxin genes detected were the enterotoxin gene cluster 273 (*egc*) which was detected in 48.9% (43/88) of sporadic isolates belonging to six CCs and the 274 enterotoxin A gene *sea* which was detected in 34.1% (30/88) belonging to four CCs (CCs 1, 5, 8 275 and 30). The enterotoxin genes *sek* and *seq* were harbored by 16% (14/88) of isolates (CCs 1, 8 276 and 59) and 11.4% (10/88) of isolates (all CC1) harbored the enterotoxin gene *seh*. The toxic 277 shock toxin gene, *tst* was detected in 9% (8/88) of isolates all of which belonged to CC30 278 (83.3%, 5/6), or CC5 (15.8%, 3/19). The enterotoxin genes *sec* and *sel* were harbored by 7.9% 279 (7/88) of isolates from four CCs (CCs 22, 45 and 78) and *seb* was detected in 4.5% (4/88) of 280 isolates from four CCs (CCs 1, 8 and 59). Various combinations of the enterotoxin genes *sed, sej*

281 and *ser* were detected in 11.4% (10/88) of isolates (CC5, ST30, CC22 and-CC779). The ACME-
282 *arc* genes were detected in 2.3% (2/88) of isolates (both ST8-MRSA-IVa). The exfoliative toxin
283 gene *etD* and the epidermal cell differentiation inhibitor gene, *edinB* were detected among all
284 ST779-MRSA isolates (6.8%, 6/88) and the *sep* enterotoxin gene was detected in 6.8% (6/88) of
285 isolates in one CC (CC5).

286 **Antimicrobial-resistance genes.** The antimicrobial-resistance genes detected among the isolates
287 belonging to the different CCs are shown in Table 1. The most prevalent antimicrobial-resistance
288 genes detected among the 88 sporadic MRSA isolates other than *mec* were the beta-lactamase
289 resistance gene *blaZ* (96.6%, 84/88 isolates) and *sdrM*, encoding an unspecific efflux pump
290 (89.8%, 79/88). The *erm*(A) gene (encoding resistance to macrolides, lincosamides and
291 streptogramin B compounds) was detected in 40.9% of isolates (36/88) belonging to 6/13 CCs
292 (CCs 1, 5, 8, 30, 45 and ST88). The aminoglycoside resistance gene *aacA-aphD* (encoding
293 resistance to amikacin, gentamicin, kanamycin and tobramycin) was detected in 39.8% of isolates
294 (35/88) in 6/13 CCs (CCs 5, 8, 22, 30, ST361 and ST398). Other significant antimicrobial
295 resistance genes detected included the fusidic acid resistant genes *fusB* and *fusC*, which were
296 detected in 7.9% (7/88) and 11.4% (11/88) of isolates, respectively. The *fusB* gene was detected
297 in three CCs (CCs 8, 45 and 59) and the *fusC* gene was detected in two CCs (CCs 1 and 779).
298 The mupirocin resistance gene, *mupA* was present in 10.2% of isolates (9/88) belonging to seven
299 different CCs (CCs 5, 8, 22, 30, 45, 59 and 779).

300 Two or more resistance genes that encoded resistance to commonly used antimicrobial
301 agents including aminoglycosides, macrolides-lincosamides, tetracycline, fusidic acid and
302 mupirocin were detected among 55.7% (49/88) of isolates and included isolates from all CCs
303 except for the CCs represented by one isolate only (CCs 6, 78, 398, 361 and 130) (Table 1).

304 **Similarities between sporadic and global isolates based on microarray data.** Fig. 1, panel (a)
305 shows a graphic representation of the diversity detected among the 88 sporadic MRSA isolates
306 based on DNA microarray profiles. SplitsTree analysis using the transformed microarray profile
307 data separated all 88 isolates into their MLST CCs and within each CC, similar isolates were
308 grouped closely together. For example, two closely related ACME-*arc* positive ST8-MRSA-IVa
309 isolates (isolates 39 and 53, Table 1) clustered together and were separate from a more distantly
310 related ACME-*arc* negative ST8-MRSA-IVd isolate (isolate 35, Table 1). Fig. 1, panel (b) shows
311 a graphic representation of the relationships between the 88 sporadic MRSA isolates based on
312 array profile data relative to a very large population of global MRSA isolates (n = 3139). Within
313 the 88 sporadic MRSA isolate population, isolates with specific CCs distributed among global
314 isolates with the same CC in each case (Fig. 1, panel (b)).

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324 **DISCUSSION**

325 Many detailed investigations of the predominant nosocomial MRSA clones prevalent in
326 different regions of the world have been reported, whereas in depth systematic investigations of
327 sporadically-occurring MRSA clones are scarce. The highly clonal ST22-MRSA-IV strain
328 continues to predominate in Irish hospitals, but the prevalence of sporadically-occurring MRSA
329 from BSIs increased two-fold between 2005 and 2011 (19). This study is the first to investigate in
330 detail the molecular epidemiology of sporadic MRSA isolates in Irish hospitals, and has revealed
331 extensive diversity in genetic backgrounds, SCC*mec* elements, virulence and resistance genes.
332 Comparative analysis of DNA microarray data from the 88 sporadic isolates investigated and the
333 corresponding data from 3139 global MRSA isolates revealed that the relationships between the
334 sporadic MRSA isolates from patients in Irish hospitals reflects the relationships between global
335 MRSA (Fig. 1, panels (a) & (b)). An apparently reduced diversity of SCC*mec* elements in the 88
336 sporadic MRSA isolates compared to the global MRSA population likely reflects the reduced
337 biodiversity associated with a restricted/insular geographic location (Fig. 1).

338 A total of 54 MLST, *spa* and SCC*mec* type combinations were identified among the 88
339 sporadic MRSA isolates investigated with 49 isolates (55.7%) carrying genes encoding resistance
340 to two or more commonly used antimicrobial agents and 40 (38.6%) harboring two or more
341 virulence-associated genes previously reported to be located on MGEs. Isolates belonging to
342 CC8/ST8-t190-IID/IIE +/- SCC_{M1} predominated. Previous studies have demonstrated a reduced
343 fitness associated with larger SCC*mec* elements (49, 50) and we previously speculated that the
344 potential fitness cost associated with carrying a large SCC*mec*-SCC composite island (CI) may
345 have contributed to the decline of ST8-MRSA-IIA-IIE &SCC_{M1} (21) in Irish hospitals. However,

346 the many resistance genes detected among isolates of this clone may also have contributed to its
347 decline due to reduced fitness, but other lineage specific factors may also have contributed (51).

348 The second most prevalent clone, ST779-t878-MRSA-ψSCC*mec*-SCC-SCC_{crispr}, also
349 carried multiple resistance genes and a large SCC-CI element that may have originated in
350 coagulase-negative staphylococci (CoNS). We recently reported the emergence of this clone in
351 Irish hospitals (6) and ST779-MRSA has also been reported sporadically in Australia, Canada,
352 Germany, Malaysia, Thailand, the United Arab Emirates and the United Kingdom
353 (http://saureus.mlst.net/) (2, 6, 24, 52). Fitness costs associated with the carriage of a large SCC-
354 CI and multiple MGE-located resistance genes may curtail the widespread emergence of this
355 clone in the absence of selective pressure.

356 Extensive diversity was detected among the SCC*mec* elements harbored by the sporadic
357 MRSA isolates and included 25 different SCC*mec* types and subtypes encompassing types and/or
358 subtypes of SCC*mec* types I-VI, SCC*mec* type XI, two possible novel SCC*mec* elements and six
359 possible novel SCC*mec* subtypes. SCC*mec* type IV predominated accounting for almost half of
360 the isolates. Since SCC*mec* IV is also the SCC*mec* type of the ST22-MRSA-IV clone endemic in
361 Irish hospitals for the last decade (17) it is clear that SCC*mec* IV is the dominant SCC*mec*
362 element among all nosocomial MRSA in Ireland. However, eight different subtypes were
363 identified among the sporadic isolates with SCC*mec* IVa being the most common (20.5%, 18/40).
364 In contrast, SCC*mec* IVh predominates among isolates of the endemic ST22-MRSA-IV clone
365 (17). It is not possible to discriminate between most SCC*mec* IV subtypes using the DNA
366 microarray and considering that these are associated with particular pandemic MRSA clones e.g.
367 SCC*mec* IVh in ST22 and SCC*mec* IVa in ST8/USA300, it is essential that detailed SCC*mec* IV
368 subtyping is performed to ensure effective tracking and typing of these clones.

369 SCC*mec* V and V_T subtyping identified novel SCC*mec* V subtypes and provided further
370 evidence of the diversity present in SCC*mec* V elements (46), including *ccrC* alleles. The CC59-
371 MRSA-V clone usually harbors two *ccrC1* complexes (*ccrC1* allele 2 and *ccrC* allele 8) (47).
372 However, the two CC59-MRSA-V isolates and a CC5-t442-MRSA isolate identified in the study
373 only harbored the *ccrC1* allele 8. Additionally, a CC5-t002-MRSA isolate harbored a SCC*mec* V
374 element with just one *ccrC* allotype, *ccrC2*. These may represent possible SCC*mec* V variants or
375 precursors in two separate CCs, CC5 and CC59.

376 The majority of isolates investigated had genotypes generally considered to be healthcare-
377 associated including ST8-MRSA-IID/IIE +/- SCC_{M1}, ST239-MRSA-III, ST36-MRSA-II, ST22-
378 MRSA-IV, ST45-MRSA-IV, ST5-MRSA-II and ST361-t315-MRSA-IVg (1, 53) each of which,
379 apart from the latter, was previously identified in Ireland either as predominant or sporadic strains
380 (2, 20). Many of these clones predominate or have predominated in hospitals in other countries
381 and no major differences were noted between these isolates and those reported previously (1). A
382 number of isolates with CC/ST and SCC*mec* type combinations commonly associated with *pvl*-
383 positive community-associated (CA)-MRSA clones were also detected including CC1-MRSA-
384 IV, (1, 54), CC59-MRSA-V (47, 55), ST8-t008-MRSA-IVa (1), CC5-MRSA-IV (1), CC5-
385 MRSA-V (56) and CC88/ST88-t186-MRSA-IVa (1, 57). It should be noted that potential CA-
386 MRSA-associated clones may be underrepresented in the present study due to the exclusion of
387 *pvl*-positive sporadic MRSA isolates. The prevalence of CA-MRSA (both *pvl*-positive and-
388 negative) among patients in Irish hospitals remains to be determined.

389 This study also found further evidence of the possible zoonotic spread of MRSA in
390 Ireland. Firstly, a CC130-MRSA-XI isolate recovered in 2007 was identified. We previously
391 reported the recovery in 2010 of two sporadic CC130-MRSA-XI isolates from separate hospitals

392 (2). The newly identified isolate exhibited a previously unreported *spa* type (t12399) harboring
393 two additional *spa* repeats compared to *spa* type t843 exhibited by the CC130 MRSA isolates
394 recovered in 2010 (2). The isolate was recovered from an elderly patient in the community who
395 had previously been hospitalized on several occasions and who lived adjacent to a farm. Since its
396 first detection, SCC*mec* XI has been reported sporadically among MRSA isolates belonging to a
397 number of animal-associated MRSA lineages (predominantly CC130) in many different
398 European countries from human and animal sources (32) and several studies have provided
399 evidence for the zoonotic spread of these strains (58, 59). Other clones of possible animal origin
400 were also identified, all recovered between 2007 and 2011, including the equine-associated ST8-
401 t064-MRSA-IVa clone (60, 61), as well as the livestock-associated clones ST398-t011-MRSA-
402 IVa and CC6-MRSA-IVh which has been linked with camels (1, 62, 63). These findings
403 highlight the importance of animals as a reservoir for MRSA and for effective surveillance to
404 minimize the spread of these clones in hospitals.

405 The prevalence and diversity of resistance and virulence genes identified among the
406 sporadic MRSA isolates also highlights the extensive reservoir of these genes that exist within
407 the population of Irish MRSA. This, coupled with the range of genetic backgrounds of the
408 isolates highlights the potential for spread of these resistance genes and thus our ability to treat
409 MRSA colonization and infection. For example, a high rate of the carriage of macrolide (57.9%)
410 and aminoglycoside (43.4%) resistance genes was observed among isolates belonging to an
411 extensive range of genetic backgrounds. Additionally, the high-level mupirocin resistance gene
412 *mupA*, known to be encoded on conjugative plasmids (64), was identified in 9/88 (10.2%) isolates
413 belonging to seven different genetic backgrounds. Mupirocin is commonly used for MRSA nasal
414 decolonization and previous reports from Ireland have reported high-level mupirocin resistance

415 rates among MRSA from BSIs ranging from 1.4% between 1999-2005 to 3.1% in 2011,
416 predominantly among ST22-MRSA-IV and ST8-MRSA-IIA-IIE isolates (19). Lastly, in Ireland
417 the rate of phenotypic fusidic acid resistance among MRSA from BSIs increased from <10% to
418 34% between 1999 and 2011 (19). In the present study 18/88 (20.5%) sporadic isolates harbored
419 either the plasmid located *fusB* gene or the SCC-associated *fusC* gene. More stringent use of
420 these antimicrobial agents is warranted so that resistance does not become more widespread.

421 Few studies focused primarily on the detailed characterization of sporadic MRSA isolates.
422 The main emphasis of most studies that reported such isolates concentrated on identifying the
423 main MRSA lineages present in large populations of MRSA from particular countries or from
424 several hospitals (20, 65, 66). For example, while reporting the clonal replacement of
425 CC5/ST228-MRSA-I and CC5-MRSA-II by the emerging CC22-MRSA-IV and CC45-MRSA-
426 IV clones as the predominant nosocomial strains over an 11-year period in a German tertiary care
427 hospital, Albrecht et al. (2011) also identified 17 *pvl*-negative sporadic MRSA among 778
428 isolates investigated including CC7-MRSA-IV, CC97-MRSA-IV, CC88-MRSA-IV and
429 CC30/ST36-MRSA-II (67), the former two of which were also identified in the present study.
430 The diversity identified among the Irish sporadic MRSA isolates investigated here spans most of
431 the lineages seen at the global level (Fig. 1). This may be because the strains, or at least some of
432 them, have at some stage been endemic in Ireland since their evolutionary origin. However, it is
433 important to emphasize that the orign of some MRSA strains can be polyphyletic resulting from
434 multiple transmissions of identical or similiar SCC*mec* elements from MRSA or CoNS into
435 methicillin-susceptible *S. aureus* (MSSA) of one clonal lineage (1). Recurrent importation of
436 MRSA strains from other countries is also likely to have been another significant contributory
437 factory to the diversity found among the sporadic MRSA. This latter suggestion is reflected by

438 the findings of a recent study from this laboratory on *pvl*-positive MRSA recovered in Ireland
439 over the last decade that revealed frequent importation of MRSA strains, particularly in recent
440 years (31). While the increasing prevalence of sporadic MRSA strains in Ireland may be due to
441 an increase in their importation or to the local emergence of strains, the decreasing prevalence of
442 ST22-MRSA-IV in Irish hospitals may also have contributed allowing for the emergence of these
443 sporadic MRSA with enhanced virulence and resistance potential. However, further studies of
444 both sporadic and endemic MRSA as well as MSSA are required in order to determine this.

445 In conclusion, the diversity detected among the 88 representative sporadic MRSA isolates
446 including SCC*mec* and SCC associated elements, virulence-associated and antimicrobial
447 resistance genes and the number of different genetic lineages identified by MLST, *spa* typing and
448 DNA microarray analysis provides further evidence of the need for effective surveillance of this
449 genetically diverse reservoir. Exchange of genetic material between these and other more
450 prevalent MRSA strains may contribute to the emergence of successful MRSA strains in the
451 future. Shore et al. (2005) previously demonstrated that there is a history of strain replacement
452 approximately once per decade in Ireland and therefore it is important that emerging MRSA
453 strains are detected early (20). The ST22-MRSA-IV clone has predominated for over a decade in
454 Irish hospitals and its recent decline in prevalence suggests that a novel strain(s) may emerge in
455 the near future.

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739 **Figure legend.**

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741 Figure 1. Network trees generated using the SplitsTree version 4.11.3 software program (39) and
742 the StaphyType DNA microarray profiles as described previously (1) to visualise the similarities
743 and relationships between clonal complexes (CCs) and mobile genetic elements including
744 SCC*mec* for the 88 sporadic MRSA isolates investigated and a global population of MRSA.
745 Panel (a), network tree showing the relationships between the 88 sporadic MRSA isolates
746 investigated. Panel (b), network tree showing the relationships between the 88 sporadic MRSA
747 isolates investigated in the present study and a previously described global collection of MRSA
748 isolates (*n*=3139) (1). Each sporadic MRSA isolate investigated in the present study is indicated
749 with a number in red font (numbers 1-88) and the details of isolates represented by each number
750 are listed in Table 1. The major CCs identified among isolates in the current study and the
751 previous global study are circled in red and Roman numerals indicate SCC*mec* types. In panel (b)
752 CCs and STs that were exhibited by MRSA strains from this study are shown in red font and if a
753 CC or ST was not exhibited by any of the 88 sporadic MRSA strains it is shown in black font.
754 MRSA strains from the previously described global population (1) that lacked the Panton-
755 Valentine leukocidin toxin genes *lukF/S-PV* (*pvl*) are indicated using black letters on a white
756 background and *pvl*-positive MRSA strains are indicated using white letters on a black
757 background. The scale bar in panels (a) and (b) shows how the length of a branch translates in
758 sequence divergence. The unit is divergent nucleotides divided by the length of the sequence
759 analyzed. Abbreviations: A; ACME; F, SCC*fus;* M, SCC*hg*; IRR, irregular SCC*mec* elements;
760 COM, composite or multiple SCC*mec* elements, CI; composite island.

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767 TABLE 1. Molecular characteristics of 88 sporadic MRSA isolates recovered from patients in Irish hospitals between 2000-2012

Isolate reference numbers[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Reports of similar isolates in other locations
52, 88	CC/ST1-t2279	IVa (2)	III	8	D (1), E (1)	*blaZ* (2), *fusC* (1), *sdrM* (2)	*sea* (1), *sek* (2), *seq* (2), *seh* (2)	Western Australia (1)
62, 86	CC1-t2279[c]	IVa & SCC*fus* (2)	III	8	D	*blaZ* (2), *fusC* (2), *sdrM* (2)	*sea* (2), *sek* (2), *seq* (2), *seh* (2)	Malta (68)
48	CC1-t386	IVa (1)	III	8	E	*blaZ*, *erm*(C), *aphA3*, *sat*, *sdrM*	*seh*	Germany (1)
7	CC1-t127	IVa (1)	III	8	D	*blaZ*, *sdrM*,	*sea* (1), *sek*, *seq*, *seh*	Western Australia (1)
38, 41	CC1-t127	IVa & SCC*fus* (2)	III	8	D	*blaZ* (2), *erm*(A) (2), *fusC* (2), *sdrM* (2)	*sea* (2), *sek* (2), *seq* (2), *seh* (2)	Malta (68)
29	CC1/ST1336-t127[c]	IVc (1)	III	8	D	*blaZ, tet*(K), *sdrM*	*sea, seb, sek*, *seq, seh*	None
66	CC1/ST1115-t127[c]	IVa (1)	III	8	E	*blaZ, erm*(C), *aphA3, sat, tet*(K), *sdrM*	*seh*	None
8, 19	CC/ST5-t045	II (2)	II	5	D (1), neg (1)	*blaZ* (2), *erm*(A) (2), *aadD* (2), *sdrM* (2), *fosB* (2), *qac*(A) (1)	*tst* (2), *sea* (1), *egc* (2), *sed* (1), *sej* (1), *ser* (1)	None
32, 68	CC/ST5-t242[c]	V_T (2)	II	5	B	*blaZ* (2), *aacA-aphD* (2), *sdrM* (2), *fosB* (2)	*egc* (2)	USA (69)
85	CC5-t002	V_T (harboring *ccrC2*) (1)	II	5	G	*blaZ, aacA-aphD, sdrM, fosB*	*egc, sep, sed, sej, ser*	None
75	CC5-t002	II (1)	II	5	F	*erm*(A), *aacA-aphD, aadD, mupA, sdrM, fosB, qac*(C)	*egc, sep*	Pandemic (1)
72	CC5-t002	IVc (1)	II	5	F	*blaZ, sdrM, fosB*	*egc, sep, sed, sej, ser*	Denmark (38)
79	CC5-t002	IV (non-subtypeable) (1)	II	5	F	*blaZ, sdrM, fosB*	*egc, sep*	Pandemic (1)

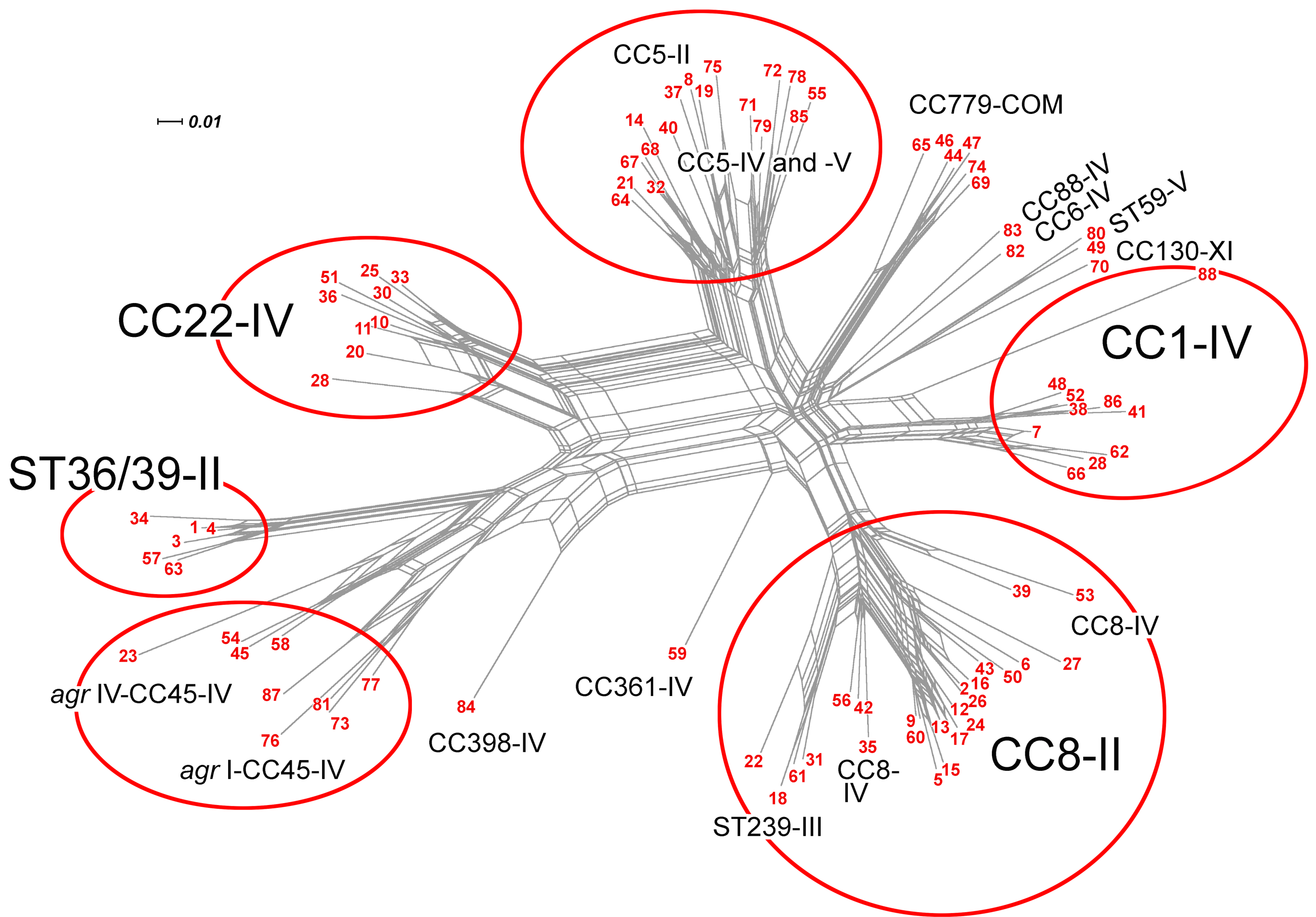
Isolate reference numbers[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Reports of similar isolates in other locations
64	CC5/ST930[cd]-t002[b]	IV (non-subtypeable) (1)	II	5	B	*blaZ, erm*(C), *aacA-aphD, sdrM, fosB*	*egc*	None
21	CC5/ST100-t002[cd]	Novel 1 (*mecA* only detected) (1)	II	5	E	*blaZ, aacA-aphD, sdrM, fosB*	*egc*	None
71	CC5-t067	IV (non-subtypeable) & *ccrB4* (1)	II	5	F	*blaZ, msrA, mph*(C), *aacA-aphD, aadD, aphA3, sat, mupA, sdrM, fosB, qac*(C)	*egc, sep*	Spain (70)
55	CC5-t088	V_T (1)	II	5	D	*erm*(C), *sdrM, fosB*	*sea, egc, sed, sej, ser*	None
14	CC/ST5-t109[cd]	I & *ccrC* (1)	II	5	B	*blaZ, erm*(A),*aacA-aphD, aphA3, sat, tet*(K), *sdr*(M), *fosB, qac*(A)	*egc*	None
67	CC5/ST1435[d]-t242[c]	V_T (1)	II	5	B	*blaZ, aacA-aphD, sdrM,fosB*	*egc*	None
78	CC5-t442	V (harboring *ccrC8*) (1)	II	5	E	*blaZ, aacA-aphD, sdrM, fosB*	*egc, sed, sej, ser*	Australia (71)
37	CC/ST5-t463	II (1)	II	5	A	*blaZ, erm*(A), *aadD, sdrM, fosB*	*tst, sea, egc, sed, sej, ser*	None
40	CC5-t1781	IVa (1)	II	5	G	*blaZ, msrA, mph*(C), *aphA3, sat, sdrM, fosB, qac*(C)	*egc, sep, sej, ser*	Germany, Canada (spa.ridom.de)
82	CC6-t701	IVh (1)	I	8	E	*blaZ, sdrM, fosB*	neg	Australia, Abu Dhabi, Hong Kong (72)
2, 6, 9, 13, 17, 24, 27, 50, 15, 16	CC/ST8-t190	IID & SCC_{M1} (10)	I	5	D (8), neg (2)	*blaZ* (10), *erm*(A) (10), *aacA-aphD* (10), *aadD* (1), *aphA3* (4), *sat* (4), *fusB* (1), *tet*(K) (1), *sdrM* (10), *cat* (1), *fosB* (10), *qacA* (9)	*sea* (8), neg (2)	None

Isolate reference numbers[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Reports of similar isolates in other locations
5, 12, 60, 43	CC/ST8-t190	IIE & SCC_{M1} (4)	I	5	D (3), neg (1)	*blaZ* (4), *erm*(A) (4), *aacA-aphD* (4), *aadD* (1), *aphA3* (3), *sat* (3), *mupA* (1), *sdrM* (4), *fosB* (4), *qacA* (4)	*sea* (3), neg (1)	None
26	CC/ST8-t190[c]	IID (1)	I	5	A	*erm*(A), *aacA-aphD, aphA3, sat, sdrM, fosB*	*tst, sea*	None
56	CC/ST8-t190[c]	VI (1)	I	5	E	*blaZ, erm*(A), *aphA3, sat, sdrM, fosB*	neg (1)	None
39, 53	CC8-t008	IVa (2)	I	5	B (1), neg (1)	*blaZ* (2), *erm*(A) (1), *msrA* (1), *mph*(C) (1), *aphA3* (1), *sat* (1), *sdrM* (2), *fosB* (2)	ACME-*arc* (2)	USA (1)
42	CC/ST8-t064[c]	IVa (1)	Ì	5	E	*blaZ, erm*(C), *sdrM, fosB*	*seb, sek, seq*	USA, Switzerland (73, 74)
35	CC/ST8-t4268	IVd (1)	I	5	D	*blaZ, erm*(C), *aacA-aphD, dfrS1, tet*(M), *sdrM, fosB, qac*(A), *qac*(C)	*sea,* neg	None
61	CC/ST8-t1209[c]	IIIB (1)	I	8	neg	*blaZ, erm*(A), *aacA-aphD, aadD, tet*(M), *sdrM, fosB, qac*(A)	*sek, seq*	None
31	CC8/ST239-t030[c]	IIIB (1)	I	8	neg	*blaZ, erm*(A), *tet*(M), *sdrM, fosB, qac*(A)	*sek, seq*	Pandemic (75)
22	CC8/ST239-t037	IIIA (1)	I	8	D	*blaZ, erm*(A), *aacA-aphD, aphA3, sat, tet*(M), *sdrM, fosB, qac*(A)	*sea, sek, seq*	Pandemic (1)

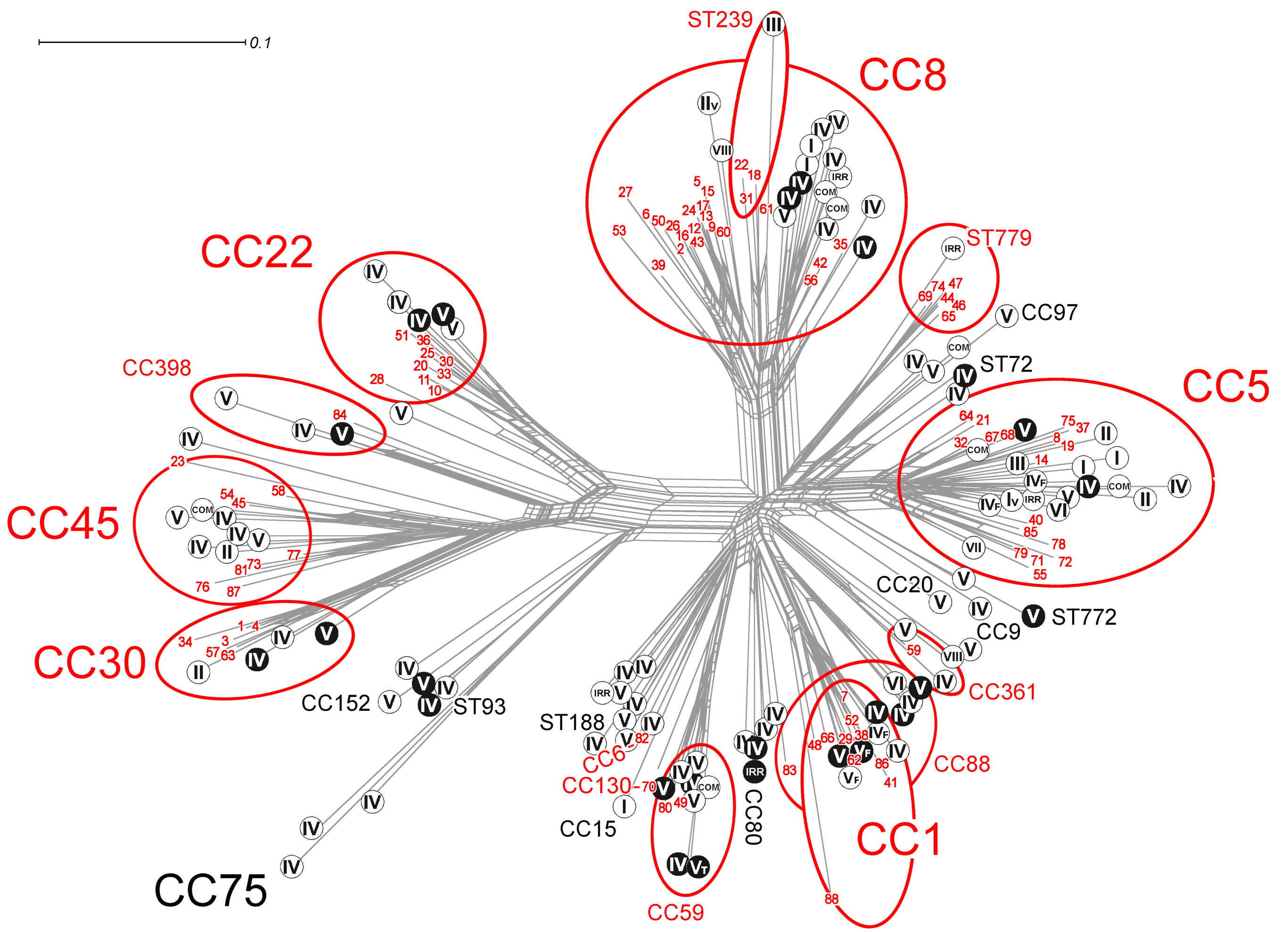
Isolate reference numbers[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Reports of similar isolates in other locations
18	CC8/ST239-t037[c]	III & SCC*hg* (1)	I	8	D	*blaZ, erm*(A), *aacA-aphD, tet*(K), *tet*(M), *sdrM, aphA3, sat, fosB, qac*(A)	*sea, sek, seq*	Pandemic (1)
11, 20, 25, 10, 28	CC/ST22-t032[c]	IVh (5)	I	5	B (3), neg (2)	*blaZ* (5), *erm*(C) (4), *aphA3* (1), *sat* (1), *qac*(A) (1)	*egc* (4), *sec* (2), *sel* (2)	Pandemic (1)
33	CC/ST22-t032[c]	IVg (1)	I	5	B	*blaZ*, *erm*(C)	*egc, sec, sel*	Pandemic (1)
30	CC/ST22-t022[c]	IVh (1)	I	5	B	*blaZ*, *erm*(C)	*egc, sec, sel*	Pandemic (1)
36	CC/ST22-t2951	IVh (1)	I	5	B	*blaZ*, *erm*(C), *lnu* (A), *aacA-aphD, aadD, mupA, cat, fosB, qac*(C)	*egc*	None
51	CC22-t1802	IVh (1)	I	5	B	*blaZ*, *erm*(C), *fosB*	*egc, sed*	None
57, 1, 3, 4	CC/ST36-t018[c]	II (4)	III	8	A (4)	*blaZ* (4), *erm*(A) (4), *aadD* (2), *sdrM* (4), *fosB* (4)	*tst* (3), *sea* (4), *sed* (1), *egc* (4)	UK (76)
63	CC/ST36-t018[c]	II & *ccrC* (1)	III	5	A	*blaZ, erm*(A), *aacA-aphD, aadD, sdrM, fosB*	*tst, sea, egc*	None
34	CC30/ST36-t012	II (1)	III	5	B	*blaZ, erm*(A), *aacA-aphD, aadD, mupA, sdrM, fosB*	*tst, egc*	UK (76)
23, 45, 54, 87	CC/ST45-t727	IVa (4)	IV	8	B (2), neg (2)	*blaZ* (4), *erm*(C) (2), *fusB* (4), *mupA* (1), *tet*(M) (2), *sdrM* (4), *fosB* (1)	*egc* (4)	Hong Kong, Australia (1)
58	CC/ST45-t727[c]	Novel 2 (*mecA* only detected) (1)	IV	8	neg	*blaZ, sdrM*	*egc*	None
77	CC/ST45-t132	IVa (1)	I	8	B	*blaZ, sdrM*	*egc*	Germany, Belgium (1)

Isolate reference numbers[a]	CC/ST-*spa* type	SCC*mec* type/description (*n*)	*agr* type	capsule type	IEC type (*n*)[b]	Antimicrobial resistance genes (*n*)	Virulence genes (*n*)	Reports of similar isolates in other locations
73	CC/ST45-t026	IVa (1)	I	8	B	*blaZ, erm*(C), *sdrM*	*egc, sec, sel*	Germany, Belgium (1)
81	CC/ST45-t065	IVa (1)	I	8	B	*blaZ, erm*(A), *sdrM*	*egc*	Germany, Belgium (1)
76	CC/ST45-t015	IVc (1)	I	8	B	*blaZ, aadD, sdrM*	*egc, sec, sel*	Germany, Belgium (1)
43, 80	CC/ST59-t316[c]	V (harboring *ccrC8*) (2)	I	8	B	*blaZ* (2), *msrA* (2), *fusB* (2), *mupA* (2), *sdrM* (2)	*seb* (2), *sek* (2), *seq* (2)	Australia ,Taiwan (1)
83	CC88/ST88-t186	IVa (1)	III	8	E	*blaZ, erm*(A), *sdrM*	*sec, sel*	Australia, Japan (1, 57)
70	CC130-t12399	XI (1)	III	8	neg	*sdrM*	neg	Europe, UK (32)
59	CC/ST361-t315[c]	IVg (1)	I	8	B	*blaZ, aacA-aphD, aphA3, sat, tet*(K), *sdrM, fosB*	*egc*	Western Australia (32)
84	CC/ST398-t011	IVa (1)	I	5	neg	*blaZ, aacA-aphD, tet*(M), *sdrM*	neg	Hong Kong, Belgium, Germany (1)
65, 69, 74, 44, 46, 47	CC/ST779-t878	ψSCC*mec*-SCC-SCC$_{CRISPR}$ (6)	III	5	B	*blaZ* (6), *aadD* (1), *fusC* (6), *mupA* (1), *sdrM* (6)	*etD* (6), *edinB* (6), *seb* (1), *sed* (1), *sej* (1), *ser* (1)	Uk, Ireland, France, Australia (1, 6)

768 [a] Isolate reference numbers were assigned to each individual sporadic MRSA isolate for inclusion in network trees constructed using
769 complete DNA microarray profile data for all 88 sporadic MRSA isolates using the SplitsTree software package (39) (Fig. 1 a & b).
770 [b]Immune evasion complex (IEC) types were assigned as described previously; IEC type A = *sea, sak, chp* & *scn*, B = *sak, chp* & *scn*,
771 C = *chp* & *scn*, D = *sea, sak & scn*, E = *sak* & *scn*, F = *sep, sak, chp* & *scn*, G = *sep, sak, scn* (48). The number of isolates with each
772 IEC type are only indicated when more than one IEC type was identified within a given type combination.
773 [c]Multilocus-sequencing typing (MLST) was performed on the isolates indicated before the DNA microarray became available. Isolates
774 were selected for MLST based upon *spa* typing results.
775 [d]Novel MLST sequence types detected.
776 Abbreviation: *n*, number of isolates; neg, negative.
777



0.01
CC5-II
CC5-IV and -V
CC779-COM
CC88-IV
CC6-IV
ST59-V
CC130-XI
CC22-IV
CC1-IV
ST36/39-II
CC8-IV
CC8-II
CC8-IV
ST239-III
CC361-IV
CC398-IV
agr IV-CC45-IV
agr I-CC45-IV



0.1
ST239
CC8
CC22
ST779
CC97
ST72
CC398
CC5
CC45
CC20
ST772
CC9
CC30
CC361
CC152
ST93
ST188
CC88
CC6
CC130
CC80
CC1
CC15
CC75
CC59

TABLE S1. Numbers of *pvl*-negative sporadic MRSA isolates recovered from patients in Irish hospitals and identified by the NMRSARL each year between 2000 and 2012

Year	**Total no. of *pvl*-negative sporadic MRSA isolates identified by the NMRSARL[a]**	**Total number of *pvl*-negative sporadic MRSA isolates investigated in the present study**	**Recovered from infection[d] or colonization [*n*]**
2000	195	10	Infection [8], colonized [1], unknown [1]
2001	165	7	Infection [7]
2002	141	6	Infection [6]
2003	114	6	Infection [4], unknown [2]
2004	133	8	Infection [5], unknown [3]
2005	96	5	Infection [5],
2006	107	8	Infection [4], unknown [3], colonized [1]
2007	125	8[c]	Infection [7], colonized [1]
2008	113	5	Infection [5]
2009	129	6	Infection [3], unknown [1], colonized [2]
2010	101	5	Infection [5]
2011	116	9	Infection [4], unknown [1], colonized [4]
2012	128	5	Infection [2], unknown [2], colonized [1]
Totals	**1663**	**88**	**88**

[a]The *pvl*-negative status of isolates was determined either by (i) PCR as described previously (1), (ii) an in-house real-time PCR assay designed to detect *mecA*, *nuc* and *pvl* or (iii) was inferred as they yielded AR and/or PFGE typing patterns indicative of strains not previously associated with *pvl*.

[b]Represents approximately 5% of *pvl*-negative sporadic MRSA isolates identified each year by the NMSRSARL among patients in Irish hospitals.

[c]One additional isolate recovered from an elderly man in the community was also included as he had been hospitalized previously on several occasions.

[d]Information pertaining to whether the isolate was recovered from an infection or colonization was available for 74 isolates. Ten isolates were from colonized patients and 64 were from infections and this included bloodstream infections (65.9%, 58/88) and skin and soft tissue infections (7.9%, 7/88).

(1). Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S, Ehricht R. 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PloS one **6:**e17936.