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1 # A novel multidrug-resistant PVL-negative CC1-MRSA-IV clone emerging in
2 Ireland and Germany likely originated in South-Eastern Europe

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35 Running title: **Whole-genome sequencing of a novel CC1-MRSA-IV clone**
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46 Abbreviations: Clonal complex, CC; core-genome single nucleotide polymorphism, cgSNP;
47 mobile genetic element, MGE; multidrug-resistant, MDR; neighbour-joining tree, NJT;
48 sequence type, ST; single-molecule real-time, SMRT; staphylococcal chromosomal cassette
49 *mec*, SCC*mec*; Western Australia, WA; whole-genome multilocus sequence typing,
50 wgMLST; whole-genome sequencing, WGS.
51

52 **Abstract**
53
54 This study investigated the recent emergence of multidrug-resistant Panton-Valentine
55 leukocidin (PVL)-negative CC1-MRSA-IV in Ireland and Germany.
56
57 Ten CC1-MSSA and 139 CC1-MRSA isolates recovered in Ireland between 2004 and 2017
58 were investigated. These were compared to 21 German CC1-MRSA, 10 Romanian CC1-
59 MSSA, five Romanian CC1-MRSA and two UAE CC1-MRSA, which were selected from an
60 extensive global database, based on similar DNA microarray profiles to the Irish isolates. All
61 isolates subsequently underwent whole-genome sequencing, core-genome single nucleotide
62 polymorphism (cgSNP) analysis and enhanced SCC*mec* subtyping.
63
64 Two PVL-negative clades (A and B1) were identified among four main clades. Clade A
65 included 20 German isolates, 119 Irish isolates, and all Romanian MRSA and MSSA isolates,
66 the latter of which differed from clade A MRSA by 47-130 cgSNPs. Eighty-six Irish clade A
67 isolates formed a tight subclade (A1) exhibiting 0-49 pairwise cgSNPs, 80 of which
68 harboured a 46 kb conjugative plasmid carrying both *ileS2*, encoding high-level mupirocin
69 resistance, and *qacA,* encoding chlorhexidine resistance. The resistance genes *aadE*, *aphA3*
70 and *sat* were detected in all clade A MRSA and the majority (8/10) of clade A MSSA isolates.
71 None of the clade A isolates harboured any enterotoxin genes other than *seh*, which is
72 universally present in CC1. Clade B1 included the remaining German isolate, 17 Irish isolates
73 and the two UAE isolates, all of which corresponded to the Western Australia MRSA-1 (WA
74 MRSA-1) clone based on genotypic characteristics. MRSA within clades A and B1 differed
75 by 188 cgSNPs and clade-specific SCC*mec* characteristics were identified, indicating
76 independent acquisition of the SCC*mec* element.
77
78 This study demonstrated the existence of a European PVL-negative CC1-MRSA-IV clone
79 that is distinctly different from the well-defined PVL-negative CC1-MRSA-IV clone, WA
80 MRSA-1. Furthermore, cgSNP analysis revealed that this newly defined clone may have
81 originated in South-Eastern Europe, before spreading to both Ireland and Germany.
82
83 **Keywords:** CC1-MRSA-IV; *Staphylococcus aureus*; multidrug-resistant; whole-genome
84 sequencing; emerging MRSA clone; *ileS2*- and *qacA*-encoding conjugative plasmid
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86

87 **1. Introduction**
88
89 *Staphylococcus aureus* is both a major global pathogen and a prevalent commensal inhabitant
90 of the skin and mucosal membranes of humans and animals (Aires-de-Sousa, 2017; Young et
91 al., 2012). Approximately 25% of the *S. aureus* genome is composed of accessory genes,
92 many of which are horizontally transferable between cells on mobile genetic elements
93 (MGEs) such as plasmids, bacteriophages and pathogenicity islands (Carroll et al., 1995;
94 Lindsay and Holden, 2004). The exact composition of the accessory genome is largely
95 influenced by environmental factors and hence, *S. aureus* is capable of adapting to a wide
96 variety of different hosts and stressful conditions (Lindsay and Holden, 2004; Malachowa and
97 Deleo, 2010). Upon acquisition of the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*)
98 MGE, which encodes either *mecA* or *mecC*, *S. aureus* develop into methicillin-resistant *S.*
99 *aureus* (MRSA) (Katayama et al., 2000; Shore et al., 2011). MRSA exhibit resistance to
100 almost all beta-lactam antibiotics and infection with multidrug-resistant strains further limits
101 treatment options (Assis et al., 2017; Hartman and Tomasz, 1984). Preventing the spread of
102 MRSA is therefore of vital importance.
103
104 Global surveillance of MRSA is essential for the identification of international transmission
105 routes and the subsequent development of effective infection prevention and control strategies
106 (World Health Organisation, 2014). Surveillance investigations typically involve the
107 categorisation of MRSA into presumptive clones (i.e. strains that have emerged from a single
108 SCC*mec* acquisition event) and/or sub-clones. Traditionally, putative MRSA clones/sub-
109 clones have been defined using conventional molecular typing techniques such as multilocus
110 sequence typing (MLST), standard SCC*mec* typing, *spa* typing and PCRs for the detection of
111 certain marker genes (Stefani et al., 2012, Coombs et al., 2012, 2014, Grundmann et al., 2014,
112 Shore et al., 2014, Irish NMRSARL, 2016), including those encoding the Panton-Valentine
113 leukocidin (PVL) (Panton and Valentine, 1932). Importantly, however, these methods involve
114 the characterisation of only small subsections of the *S. aureus* genome and thus, their
115 resolution is limited. More recently, whole-genome sequencing (WGS) has become
116 increasingly widespread and the use of techniques such as single nucleotide polymorphism
117 (SNP) analysis has enabled the quantitative comparison of strains with exceptionally high
118 resolution (Earls et al., 2017; Kinnevey et al., 2016; Price et al., 2013). While WGS is
119 generally considered the technical gold-standard during surveillance, a combination of DNA
120 microarray profiling and enhanced SCC*mec* subtyping has also been used to accurately
121 differentiate between clones (Earls et al., 2018; Monecke et al., 2016, 2011). As of 2017,

122 approximately 300 different MRSA clones and 60 different SCC*mec* subtypes had been
123 defined using these techniques (Monecke et al., 2016; *S. aureus* Genotyping Kit 2.0
124 Arraymate database (Abbott [Alere Technologies GmbH], Jena, Germany)). DNA microarray
125 profiling and enhanced SCC*mec* subtyping can therefore be used to select relevant strains for
126 WGS-based investigations.
127
128 To date, only one PVL-negative ST1-MRSA-IV clone has been well-defined in the literature
129 (Monecke et al., 2011). Often referred to as Western Australia (WA) MRSA-1, this CC1
130 clone was first recovered in Australia in the late 1980s (Udo et al., 1993) and isolates
131 indistinguishable from WA MRSA-1 by MLST, *spa* and array profiling have since been
132 detected in the UAE, Egypt and Europe (Monecke et al., 2011). As suggested by its origin in
133 the community, WA MRSA-1 is not typically associated with multidrug resistance.
134 Specifically, this clone is generally associated with the penicillin resistance gene *blaZ*, the
135 macrolide, lincosamide and streptogramin (MLS) resistance gene *erm*(C) and virulence-
136 associated genes *sak*, *scn*, *sea*, *seh*, *sek* and *seq* (Coombs et al., 2011). The fusidic acid
137 resistance gene, *fusC*, which is carried on an SCC element (SCC*fus*), is also common among
138 WA MRSA-1. Furthermore, WA MRSA-57 and WA MRSA-45, the latter of which also
139 harbours SCC*fus*, have been defined as sub-clones of WA MRSA-1 (Coombs et al., 2011).
140 All three of these variants are typically associated with *spa* type t127 (Coombs et al., 2011).
141 Interestingly, PVL-negative ST1-MRSA-IV-t127 have also been recovered from pigs, cattle,
142 horses, rooks, companion animals and wild boars (Cuny et al., 2015; Loncaric et al., 2014,
143 2013; Porrero et al., 2013). Notably, however, these animal strains have not been investigated
144 using WGS and their placement into a global context has not yet been established. A PVL-
145 positive ST1-MRSA-IV clone known as USA400 has also been well-defined (Herold et al.,
146 1998). Like WA MRSA-1/45/57, this clone is not typically associated with multidrug
147 resistance.
148
149 A recent WGS-based study identified multidrug-resistant (MDR) PVL-negative CC1-MRSA-
150 IV as the predominant CC1-MRSA clone among humans in Ireland (Earls et al., 2017). This
151 study determined that a distinct variant of this specific CC1-MRSA-IV clone, which generally
152 exhibited mupirocin resistance conferred by a conjugative *ileS2*-encoding plasmid (p140355),
153 was responsible for a protracted hospital outbreak in Ireland (Earls et al., 2017). These
154 findings were particularly alarming considering both the difficulty associated with treating
155 MDR infections and the importance of mupirocin, which in many countries (including
156 Ireland), is used in combination with chlorhexidine for nasal and body decolonisation,

157 respectively (Irish Department of Health, 2013; Poovelikunnel et al., 2015). The aims of the
158 present study relate to these recent findings. Firstly, this study investigated whether the MDR
159 CC1-MRSA-IV clone identified in Ireland is present elsewhere. This involved comparing the
160 whole-genome sequences and SCC*mec* elements of Irish MDR CC1-MRSA-IV and
161 international isolates exhibiting similar genotypic characteristics to the Irish isolates, as
162 determined by DNA microarray profiling. Secondly, considering that multidrug resistance is
163 not typically associated with human PVL-negative CC1-MRSA-IV, this study investigated
164 whether the MDR CC1-MRSA-IV clone identified in Ireland constitutes a sub-clone of WA
165 MRSA-1/45/57 or a novel clone yet to be formally characterised. This involved (a) comparing
166 the whole-genome sequences and SCC*mec* elements of MDR CC1-MRSA-IV and WA
167 MRSA-1/45/57 and (b) comparing the whole-genome sequences of MDR CC1-MRSA-IV
168 and MSSA isolates exhibiting similar genotypic characteristics to the MDR clone, as
169 determined by DNA microarray profiling. Finally, considering the crucial role of mupirocin
170 as a decolonising agent, this study aimed to further analyse the *ileS2*-encoding plasmid
171 identified in the Irish outbreak variant in order to elucidate the factors driving its selection. To
172 date, ten different *ileS2*-encoding plasmids have been described in staphylococci, six of which
173 are members of the pSK41/pGO1 plasmid family and eight of which encode additional
174 antimicrobial resistance genes (Ho et al., 2016; Pérez-Roth et al., 2010).
175

176 **2. Materials and Methods**

177

178 **2.1 Isolates**

179 The present study included all 139 CC1-MRSA-IV isolates identified at the Irish National
180 MRSA Reference Laboratory (NMRSARL) between 2007 and 2017 (Table 1). Eighty nine of
181 these isolates (recovered between June 2013 and June 2016) underwent *spa* typing, DNA
182 microarray profiling, WGS and MLST as part of the aforementioned study on a protracted
183 hospital outbreak (Earls et al., 2017). During the present study, the remaining 50/139 isolates
184 were identified as CC1-MRSA-IV based firstly, on their exhibiting *spa* types corresponding
185 to CC1 and secondly, on their assignment to CC1-MRSA-IV following *in silico* DNA
186 microarray profiling (see below). All available CC1 MSSA isolates (10 isolates recovered
187 between 2004 and 2017) identified at the NMRSARL based on *spa* typing, were included for
188 comparison to the Irish CC1-MRSA-IV isolates (Table 1). The Irish *S. aureus* isolates
189 included two PVL-positive MRSA and three PVL-positive MSSA isolates, which were
190 included as potentially useful comparators isolates. Thirty-eight international *S. aureus*
191 isolates were also investigated, including 21 MRSA isolates recovered in Germany between
192 2007 and 2018, five MRSA isolates recovered in Romania between 2010 and 2012, 10 MSSA
193 isolates recovered in Romania between 2009 and 2012 and two MRSA isolates recovered in
194 from the UAE in 2009 (Table 1). These international isolates were selected from a *S. aureus*
195 isolate microarray profile database including approximately 25,000 strains recovered from
196 humans and animals worldwide. Further details relating to this global database and the
197 rationale used to select the international isolates are described below. All available
198 epidemiological data associated with the 187 (149 Irish and 38 international) isolates
199 investigated are detailed in Table S1. Isolates underwent species identification and methicillin
200 resistance detection as previously described (Earls et al., 2018). All isolates were stored at -
201 80°C on individual Protect Bacterial Preservation System cryogenic beads (Technical
202 Services Consultants Ltd., Heywood, United Kingdom).

203

204 **2.2 *spa* typing**

205 For the Irish isolates, *spa* typing was performed with genomic DNA extracted using the
206 InstaGene matrix solution (BioRad, München, Germany), according to the manufacturer's
207 instructions. Amplification of the variable X region in the *spa* gene, PCR clean-up, DNA
208 sequencing and *spa* type assignment were performed as previously described (Earls et al.,
209 2018). All international isolates underwent *spa* typing using Ridom SeqSphere+ version 4.1
210 (Ridom GmbH, Germany) following WGS (see below) (Bletz et al., 2015).

211 **2.3 WGS**

212 All isolates underwent WGS. Genomic DNA was extracted by enzymatic lysis using the *S.*
213 *aureus* Genotyping Kit 2.0 (Abbott [Alere Technologies GmbH], Jena, Germany) and the
214 Qiagen DNeasy blood and tissue kit (Qiagen, West Sussex, United Kingdom). DNA quality
215 was assessed and DNA dilutions were performed, as previously described (Earls et al., 2018).
216 The Nextera XT DNA Library Preparation Kit (Illumina, Eindhoven, The Netherlands) was
217 used according to the manufacturer's instructions and libraries underwent paired-end
218 sequencing using the 500-cycle MiSeq Reagent Kit v2 (Illumina). Libraries were scaled to
219 exhibit at least 50x coverage and the quality of each sequencing run was assured following
220 cluster density and Q30 assessment. All reads were assembled into contigs using SPAdes
221 v3.7.1 (Bankevich et al., 2012).

222

223 **2.4 Genotyping and SCC*mec* subtyping using *in silico* DNA microarrays**

224 The sequences of the hybridization probes utilized in *S. aureus* Genotyping Kit 2.0 (Abbott
225 [Alere Technologies GmbH], Jena, Germany) DNA microarray and a previously described
226 SCC*mec* subtyping array (Monecke et al., 2016) were mapped on the assembled genomes to
227 generate hybridisation patterns comparable to real array hybridization experiments, as
228 previously described (Monecke et al., 2016). To compare the complement of antimicrobial
229 and virulence-associated genes between groups of isolates, the two-tailed Fisher's exact test
230 was performed using the GraphPad QuickCalcs website:
231 https://www.graphpad.com/quickcalcs/contingency1/ (accessed July 2018). Results were
232 considered statistically significant if $p < 0.05$. Specific SCC*mec*-related alleles of interest
233 were compared using Clustal Omega (Sievers et al., 2011), while regions of interest within
234 the SCC*mec* element were investigated using the publicly available National Centre for
235 Biotechnology Information (NCBI) BLAST database
236 (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

237

238 **2.5 Details relating to the global *S. aureus* database**

239 The database was generated using *in vitro* and/or *in silico* versions of the *S. aureus*
240 Genotyping Kit 2.0 (Abbott [Alere Technologies GmbH], Jena, Germany), comprising
241 experimental datasets for ca. 25,000 MRSA and MSSA isolates, as well as *in silico* re-
242 analyses of ca. 3,000 published genome sequences. In addition, the aforementioned SCC*mec*
243 subtyping array (Monecke et al., 2016) was applied experimentally to a subset of ca. 2,000
244 MRSA isolates and *in silico*, to all the genome sequences analysed. The global database
245 includes 385 CC1-MRSA-IV isolates recovered in Europe, Australia, the Middle East, the

246 USA and New Zealand. The vast majority (382/385; 99.2%) of these CC1-MRSA-IV isolates
247 exhibit one of four main genotypic patterns, while those remaining exhibit unusual
248 characteristics (such as SCC pseudoelements or composite SCC elements) and possibly
249 represent sporadic strains or variants/mutants. Three of the four main CC1-MRSA-IV patterns
250 each match the description of previously defined clones (Monecke et al., 2011). These clones
251 are (i) PVL-negative strains with SCC*mec* IV or SCC*mec* IV+SCC*fus* elements, referred to as
252 WA MRSA-1/45/57, (ii) the PVL-positive "USA400" strain and (iii) a PVL-positive strain
253 with a SCC*mec* IV+SCC*fus* composite element that appears to be restricted to Australia and
254 New Zealand. A fourth pattern can be defined that differs from any well-defined CC1-MRSA-
255 IV clones in several markers (see Results). This pattern corresponds to CC1-MRSA-IV
256 previously identified in Ireland (Earls et al., 2017) and Romania (Monecke et al., 2014).
257 Hereafter, this fourth genotypic pattern is referred to as the "undefined" pattern or clone.
258

259 **2.6 Selection of international isolates**

260 Following *in silico* genotyping, the Irish isolates could be compared to those represented in
261 the global strain collection. The vast majority of Irish MRSA isolates investigated exhibited
262 either the WA MRSA-1/45/57 or the undefined genotypic pattern. Representative (based on
263 country/location of isolation, genotypic variations and/or date of recovery) selections of
264 international MRSA isolates exhibiting each of these two genotypic patterns were selected
265 from the global strain collection for further analysis using WGS. Specifically, one German
266 and two UAE CC1-MRSA-IV isolates exhibiting the WA MRSA-1/45/57 genotypic pattern,
267 and a further nineteen German and five Romanian CC1-MRSA-IV isolates exhibiting the
268 undefined genotypic pattern were selected. A representative selection of international MSSA
269 isolates exhibiting the undefined genotypic pattern (minus the SCC*mec* genes) was also
270 selected from the global strain collection for further analysis using WGS. This included 10
271 Romanian CC1-MSSA isolates. Importantly, it was noted that under-resourced countries were,
272 despite all efforts, often poorly represented in the strain collection.
273

274 **2.7 Neighbour-joining tree analysis**

275 The WGS data were analysed using BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem,
276 Belgium). All isolate genomes underwent whole-genome MLST (wgMLST) using both
277 assembly-free and assembly-based algorithms, as previously described (Earls et al., 2018).
278 This wgMLST scheme consists of 3,904 wgMLST loci (Roisin et al., 2016), 1,861 of which
279 are core genes (Leopold et al., 2014). A multiple sequence alignment of the concatenated core
280 genes was generated and a core-genome (cg) SNP analysis was performed. cgSNPs were

281 called exclusively in positions shared by all samples. Only cgSNPs with at least 5x read
282 coverage (including 1x coverage in each direction) were considered. Potentially indel-related
283 cgSNPs, occurring within 12 bp of each other, were removed. Positions with ambiguous base
284 calls and cgSNPs in repetitive regions were excluded. Two separate neighbour-joining trees
285 (NJTs) were generated with permutation resampling (1000 replicates) based on the cgSNP
286 analysis. The first NJT included all MRSA isolates only (Fig. S1). This tree was used to
287 confirm the clonality of the isolates exhibiting the undefined genotypic pattern (Fig. S1). The
288 second NJT included all MRSA and MSSA isolates (Fig. 1). This tree was used to determine
289 whether any of the MSSA isolates investigated were closely related to the undefined clone. A
290 distance matrix based on cgSNP differences was also generated. The quality statistics window
291 in BioNumerics was used to assess the quality of the sequence read sets, *de novo* assemblies,
292 and cgMLST allele calls. Traditional STs were assigned following MLST analysis using
293 Ridom SeqSphere+ version 4.1 (Ridom GmbH).
294

295 **2.8 Plasmid sequence analysis**

296 A conjugative *ileS2*-encoding plasmid, p140355 (GenBank accession number: KY465818)
297 was previously detected in 50 mupirocin-resistant CC1-MRSA-IV isolates from Ireland, all of
298 which were also included in the present study (Earls et al., 2017). In this previous study, a
299 single mupirocin-resistant CC1-MRSA-IV isolate underwent single-molecule real-time
300 (SMRT) sequencing and the entire *ileS2*-encoding plasmid (p140355) was obtained on a
301 single contig. The sequence reads of the remaining 49 mupirocin-resistant CC1-MRSA-IV
302 isolates from that study were then aligned to the SMRT sequence. In the present study, the
303 sequence reads of an additional 32 *ileS2*-encoding CC1-MRSA-IV isolates were mapped
304 against the p140355 sequence using the Burrows-Wheeler aligner (BWA-mem;
305 http://arxiv.org/abs/1303.3997). The Artemis sequence viewer
306 (https://www.sanger.ac.uk/science/tools/artemis) was used to visually assess the mapping of
307 reads. The genetic organisation of the SMRT-derived p140355 sequence from was confirmed
308 by PCR using either Go Taq DNA polymerase (Promega, Madison, Wisconsin, USA) or the
309 Expand Long Template PCR system (Roche Products Ireland Limited, Dublin, Ireland),
310 according to the manufacturers' instructions. The primers used are detailed in Table S2.
311 SnapGene v4.1.9 was used to construct a genetic map of the plasmid (Fig. 2). All open
312 reading frames (ORFs) comprising at least 30 codons were identified and annotated, if
313 possible, using the NCBI BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This
314 database was also used to compare the p140355 sequence with other plasmid sequences of
315 relevance.

316 **2.9 Accession numbers**

317 The contigs of a representative isolate (clade A; A_01; undefined CC1-MRSA-IV genotypic
318 pattern) were submitted to GenBank (accession number: RBVO00000000.1). The sequence
319 read sets of all isolates investigated were submitted to the NCBI Sequence Read Archive
320 database (accession number: PRJNA494507).
321

322 **3. Results**

323

324 **3.1 Identification of four distinct clades using cgSNP analysis**

325 A total of 2,891 core-genome sequence positions exhibited polymorphisms that fulfilled all
326 filtering criteria. Neighbour-joining tree analysis based on these 2,891 positions revealed that
327 the 187 isolates investigated grouped into one of four main clades (A, B, C and D; Fig. 1).
328 Clade A included 143 MRSA isolates, 86 of which formed a tight subclade termed subclade
329 A1, and 10 MSSA isolates. Clade B included 23 MRSA isolates, 20 of which formed a loose
330 subclade termed subclade B1, and six MSSA isolates. Clade C included one MRSA and two
331 MSSA isolates, while clade D included two MSSA isolates only.

332

333 The clade A MRSA isolates exhibited the undefined CC1-MRSA-IV genotypic pattern, while
334 the subclade B1 isolates exhibited the WA MRSA-1/45/57 genotypic pattern. The clade A
335 MRSA isolates were therefore characterised in detail and compared to the subclade B1
336 isolates. A single centrally-located subclade A1 isolate was selected as a subclade
337 representative for all analyses involving clade A, while subclade A1 was examined
338 independently. Hereafter, clade A is therefore described as including 58 MRSA isolates and
339 10 MSSA isolates. The remaining isolates in clade B and those in clades C and D were
340 examined separately.

341

342 **3.2 Identification of clade specific SCC*mec* characteristics**

343 Clades A and B1 were differentiated by 171 cgSNPs, while clade A MRSA specifically were
344 differentiated from clade B1 by 188 cgSNPs. All clade A MRSA isolates harboured a
345 SCC*mec* type IVa element similar to that identified in the MW2 (USA400) MRSA strain
346 (GenBank accession number: BA000033.2). In contrast to MW2-like SCC*mec* IVa, however,
347 the clade A SCC*mec* element harboured a 4710 nucleotide insertion in the downstream
348 constant segment (*dcs*) adjacent to *orfX*. This sequence encodes five different hypothetical
349 proteins and corresponds to nucleotide positions 280690-285400 of GenBank entry
350 RBVO000005.1. This gene cluster has also been detected in the SCC*mec* elements of several
351 *S. aureus* (e.g. GenBank accession number: CP007672.1) and *Staphylococcus epidermidis*
352 (e.g. LT571449.1) strains and one *Staphylococcus capitis* strain (CP007601.1). The subclade
353 B1 isolates harboured MW2-like SCC*mec* IVa (without the *dcs* insertion). Half (10/20) of the
354 subclade B1 isolates also carried an SCC*fus* element that included the *tirS* marker gene, *ccrA1*
355 and *ccrB1* (i.e. SCCfus_{476}, as described in GenBank entry BX571857.1).

356

357 All clade A MRSA isolates harboured the same allelic variants of the cassette chromosome
358 recombinase genes, *ccrA2* (1350 bp) and *ccrB2* (1629 bp). All subclade B1 isolates harboured
359 the same *ccrA2* allele and the vast majority (16/20) harboured the same *ccrB2* allele, while
360 those remaining harboured an allele which differed from the predominant *ccrB2* allele by 1
361 SNP. The *ccrA2* alleles in the SCC*mec* elements of clades A and B1 differed by 23 SNPs. The
362 *ccrB2* allele in the clade A SCC*mec* element differed from the predominant *ccrB2* allele in the
363 subclade B1 SCC*mec* element by 51 SNPs.
364
365 **3.3 Clade A MRSA**
366 The 58 clade A MRSA isolates were recovered in Ireland (*n* = 33), Germany (*n* = 20) and
367 Romania (*n* = 5), and exhibited 0-109 (average 62.6, standard deviation [SD] 13.8) pairwise
368 cgSNPs (Fig. 1; Table 1). All isolates were identified as ST1-MRSA-IVa-t127, with the
369 exception of a single ST4911-MRSA-IVa-t127 isolate from Germany (Table 1). The 32 Irish
370 MRSA isolates were recovered in 11 different hospitals/healthcare facilities (HCFs) and the
371 community between 2007 and 2017 (Table 1) (Earls et al., 2017). Notably, the first and
372 second clade A MRSA isolates identified in Ireland (which differed by 52 cgSNPs) were
373 recovered in the community in 2007 and 2008, respectively, while the third such isolate was
374 recovered in a hospital in 2012 (Table S1). The German isolates included 1/5 isolates (i.e. one
375 of five isolates in the global database exhibiting the undefined genotypic pattern) recovered in
376 a hospital in the Saxon city of Dresden in 2016, and 19/37 isolates recovered between 2016
377 and 2018 in two different hospitals and the community in the Bavarian city of Regensburg.
378 The Romanian isolates included 5/40 isolates recovered in a hospital in the north-eastern city
379 of Iaşi between 2008 and 2012. Interestingly, no phylogenetic subgrouping of isolates
380 recovered in the same country was observed (Fig. 1). Indeed, the Irish isolates exhibited 1-
381 102 (average 69.0, SD 14.0) pairwise cgSNPs, the German isolates exhibited 12-115 (average
382 79.0, SD 16.2) pairwise cgSNPs and the Romanian isolates exhibited 53-71 (average 59.0, SD
383 6.8) pairwise cgSNPs.
384
385 The MRSA isolates within clade A harboured a broader range of antimicrobial resistance
386 genes than those in clade B (Table 2). Specifically, *aphA3*, encoding neomycin and
387 kanamycin resistance, *aadE*, encoding aminoglycoside resistance and *sat*, encoding
388 streptothricin resistance, were detected in all clade A MRSA but were universally absent from
389 clade B. Furthermore, resistance genes *tet*(K), encoding tetracycline resistance and *erm*(C)
390 were more common in MRSA in clade A than in clade B1 (Table 2). The majority (47/58) of
391 clade A MRSA isolates harboured the immune evasion cluster (IEC) type E (*sak* and *scn*),

392 while those remaining harboured undisrupted *hlb*. None of the clade A MRSA isolates
393 harboured any enterotoxin genes other than *seh*, which is universally present in CC1.
394

395 **3.4 Subclade A1**

396 The 86 Irish MRSA isolates within subclade A1 exhibited 0-49 (average 17.4, SD 8.6)
397 pairwise cgSNPs, and were recovered in ten different hospitals/HCFs and the community,
398 between 2013 and 2017 (Table 1) (Earls et al., 2017). The vast majority (84/86) of these
399 isolates were characterised as ST1-MRSA-IVa-t127, while those remaining were identified as
400 ST1-MRSA-IVa-t922. The majority (60/86; 70%) of subclade A1 isolates were recovered in a
401 single hospital (Table S1).

402 The *ileS2* gene was markedly prevalent among subclade A1 isolates, but absent from (non-
403 subclade A1) clade A MRSA (Table 2). Almost all (80/82) *ileS2*-encoding isolates also
404 harboured the general efflux pump-encoding gene, *qacA*, which confers resistance to
405 chlorhexidine amongst other compounds (Table 2). A ~7 kb insertion encoding *qacA*, was
406 identified upstream of the *ileS2* region (Fig. 2). The only two other *ileS2* and *qacA*-encoding
407 plasmid sequences (GenBank accessions numbers: KU882683 and KU882684) in the NCBI
408 database correspond to plasmids unrelated to p140355 (50% and 24% query cover,
409 respectively) that were previously recovered from *Staphylococcus lugdunensis*. The p140355
410 plasmid was characterised as a 45924 bp circular plasmid of the pSK41/pGO1 family,
411 exhibiting a GC content of 28.6% (Fig. 2). The plasmid backbone accounted for
412 approximately 27.5 kb of the p140355 sequence, while the remaining 18.5 kb comprised the
413 accessory region (Fig. 2). A total of 54 ORFs were identified, 29 of which encode known
414 genes, 10 of which have undergone NCBI prediction and 15 of which encode hypothetical
415 proteins (Fig. 2). The sequence reads of the 80 *ileS2* and *qacA*-encoding isolates mapped well
416 to the p140355 sequence (Earls et al., 2017). Correspondingly, the sequence reads of the two
417 *ileS2*-encoding isolates which lacked *qacA* (A1_01 and A1_61) failed to map to the *qacA*
418 region but mapped well to the remainder of the plasmid sequence. The *tet*(K) gene and IEC
419 genes, *sak* and *scn*, were also more common in subclade A1 than in clade A MRSA (Table 2).

420

421 **3.5 Clade A MSSA**

422 Considering that clade A included MRSA isolates recovered in Ireland and Romania as early
423 as 2007 and 2009, respectively (Fig. 1; Table 1), CC1 MSSA from both of these countries
424 were considered potential precursors to clade A MRSA. Forty Romanian CC1-MSSA isolates

425 exhibiting the undefined CC1-MRSA-IV genotypic pattern (excluding genes typically located
426 in SCC*mec*) were identified in the global database. Ten of these isolates were selected for
427 WGS and subsequent cgSNP analysis. They grouped into clade A, differing from the MRSA
428 isolates within this clade by 47-130 cgSNPs (Fig. 1). The Romanian MSSA isolates were
429 recovered in the same hospital as the Romanian ST1-MRSA-IV isolates. The majority (8/10)
430 of Romanian MSSA isolates were characterised as ST1 MSSA, while two isolates were
431 identified as ST4910 and ST4912 (Table 1). Similarly, the majority (8/10) of Romanian
432 MSSA isolates were identified as *spa* type t127, while the ST4910 isolate was identified as
433 *spa* type t5633 and one ST1 isolate was assigned *spa* type t18248 (Table 1). Similar to clade
434 A MRSA, 8/10 clade A MSSA isolates carried *aphA3*, *aadE* and *sat*. The *erm*(C) gene was
435 also detected in 8/10 isolates, while 9/10 isolates harboured *tet*(K). Furthermore, clade A
436 MSSA did not carry any enterotoxin genes other than *seh*, and either harboured IEC type E
437 (8/10) or lacked IEC associated genes (2/10). Importantly, none of the 10 Irish MSSA isolates
438 investigated grouped in close proximity to the clade A MRSA isolates (Fig. 1).
439
440 **3.6 Subclade B1 (MRSA only)**
441 The subclade B1 isolates were recovered in Ireland (*n* = 17), the UAE (*n* = 2) and Germany (*n*
442 = 1), and differed by 0-116 (average 77.9, SD 17.0) pairwise cgSNPs. All subclade B1
443 isolates were identified as t127 (Table 1). The Irish isolates were recovered in nine different
444 hospitals and the community between 2012 and 2017 (Table 1). Eight of the Irish isolates
445 were identified as ST1-MRSA-IVa/SCC*fus*$_{476}$, while seven were identified as ST1-MRSA-
446 IVa and the remaining two were characterised as ST4913-MRSA-IVa and ST4914-MRSA-
447 IVa. The UAE isolates included 2/4 isolates recovered in a hospital in Abu Dhabi in 2009,
448 both of which were characterised as ST1-MRSA-IVa/SCC*fus*$_{476}$. The German isolate
449 represented a group of four isolates recovered in the aforementioned Dresden hospital in 2007
450 and was identified as ST1-MRSA-IVa. A large group of Australian isolates (*n* = 46; recovered
451 between 2001 and 2009) exhibiting WA MRSA-1/45/57 genotypic patterns were also
452 identified in the global database. However, these Australian isolates did not undergo WGS as
453 part of this study.
454
455 Apart from *fusC*, *aadD* (encoding aminoglycoside resistance) and *erm*(A) (encoding MLS
456 resistance) were the only resistance genes that were more common in subclade B1 than in
457 clade A MRSA (Table 2). However, the subclade B1 isolates harboured a wider range of
458 virulence-associated genes than clade A MRSA (Table 2). Specifically, in addition to the
459 CC1-associated *seh* gene, the enterotoxin genes *sea*, *sek* and *seq* were significantly common

460 in clade B (Table 2). The majority (19/20) of subclade B1 isolates harboured IEC type D (*sea*,
461 *sak* and *scn*), while IEC type E (*sak* and *scn*) was detected in a single instance.
462
463 **3.7 Remaining isolates**
464 All remaining isolates were recovered in Ireland (Fig. 1). The nine remaining (non-subclade
465 B1) clade B isolates differed from those in subclade B1 by 83-158 cgSNPs. These isolates
466 included two closely related (10 cgSNPs) PVL-positive ST1-MRSA-IVa-t127 isolates, one of
467 which was identified as USA400 based on its genotypic pattern and the other of which
468 harboured SCC*fus*$_{476}$ (Fig. 1). Clade B also included six ST1-MSSA-t127 isolates, all of
469 which harboured SCC*fus*$_{476}$ and half (3/6) of which were PVL-positive. Clade C included one
470 ST1-MRSA-IVa-t127 and two ST1-MSSA-t127 isolates, all three of which were PVL-
471 negative and harboured SCC*fus*$_{476}$. The clade C MRSA isolate was relatively closely related
472 (38 cgSNPs) to one of the MSSA isolates, while the remaining clade C MSSA isolate differed
473 from its closest neighbour by 119 cgSNPs. Clade D included two PVL- and SCC-negative
474 ST1-MSSA-t127 isolates which were indistinguishable using cgSNP analysis.
475

476 **4. Discussion**

477 This study confirmed the existence of a previously undefined PVL-negative CC1-MRSA-IV
478 clone that may have emerged in Romania or neighbouring regions and has become prevalent
479 in both Ireland and Bavaria, Germany. This clone is distinctly different to the well-
480 characterised PVL-negative CC1-MRSA-IV clone known as WA MRSA-1 (and closely
481 related WA MRSA-57 and WA MRSA-45), which was first identified in Australia. Although
482 indistinguishable using a combination of MLST, SCC*mec* typing and *spa* typing, cgSNP and
483 SCC*mec* analysis revealed a clear distinction between these two clones. Both clones were
484 identified among PVL-negative CC1-MRSA-IV recovered in Ireland. A mupirocin-resistant
485 variant of the newly defined European CC1-MRSA-IV clone harbouring a conjugative *ileS2*-
486 encoding plasmid first came to notice following a protracted hospital outbreak in Ireland
487 (Earls et al., 2017). In the present study, this plasmid was shown to harbour both *ileS2* and
488 *qacA*, encoding resistance to mupirocin and chlorhexidine, respectively, which are commonly
489 recommended as a treatment combination for MRSA decolonisation (Poovelikunnel et al.,
490 2015).

491

492 Analysis of CC1-MRSA-IV isolates from Ireland, Germany, Romania and the UAE identified
493 two main PVL-negative CC1-MRSA-IV clades (A and B1) which were differentiated by 188
494 cgSNPs (Fig. 1). Clade-specific SCC*mec* characteristics identified in the *ccr* genes and *dcs*
495 indicated that the clones represented by these clades likely evolved from MSSA following
496 separate SCC*mec* acquisition events. Furthermore, a potential marker sequence of the clone
497 represented by clade A was identified as an insertion within *dcs*. Genotypic evidence strongly
498 suggests that clade B1 represents the WA MRSA-1/45/57 clone. Indeed, clade B1 isolates
499 generally harboured *blaZ*, *sak*, *scn*, *sea*, *seh*, *sek* and *seq* (Table 2), all of which are
500 characteristic features associated with WA MRSA-1/57/45 (Coombs et al., 2011).
501 Interestingly, the clade B1 isolates which carried SCC*fus*476 (i.e. the isolates matching the
502 WA-45 description) were interspersed between those which did not harbour SCC*fus*476 (i.e.
503 those matching the WA-57 description). Furthermore, a high degree of relatedness (10
504 cgSNPs) was noted between two PVL-positive MRSA isolates in clade B, only one of which
505 carried SCC*fus*476. These observations bring into question the stability of the SCC*fus*476
506 element, although further studies are warranted. There is limited information on fusidic acid
507 consumption in Ireland and Germany. However, it is often used in the community in Europe
508 (Dobie and Gray, 2004; Mason and Howard, 2004). In contrast to clade B1, clade A isolates
509 exhibited genotypic characteristics which differed substantially from those associated with

510 WA MRSA-1/45/57. Specifically, clade A isolates generally harboured *aphA3*, *aadE*, *sat* and
511 *tet*(K), none of which are associated with WA MRSA-1/45/57, and lacked *sea*, *sek* and *seq*,
512 all of which are associated with WA MRSA-1/45/57 (Coombs et al., 2011) (Table 2). Notably,
513 the genotypic characteristics exhibited by clade A isolates did not correspond with USA400
514 either (Côrtes et al., 2017), suggesting it unlikely that the clade A clone derived from USA400
515 by loss of the genes encoding PVL. Indeed, microarray genotyping identified one of the clade
516 B isolates as USA400 (Fig. 1).
517
518 A 2014 study used microarray genotyping to identify PVL-negative CC1-MRSA-IV as the
519 predominant MRSA clonal group in a Romanian hospital between 2008 and 2012 (Monecke
520 et al., 2014). Distinct genotypic differences were noted between this CC1-MRSA-IV clone
521 and WA MRSA-1/45/57 (Monecke et al., 2014). It was hypothesised in the Romanian study
522 that this highly prevalent CC1-MRSA-IV clone may have emerged locally due to marked
523 similarities between the microarray profiles of CC1-MRSA-IV and CC1-MSSA isolates
524 recovered in the same region (Monecke et al., 2014). Significantly, the Romanian isolates
525 investigated in the present study were also included in the 2014 study. The results of the
526 present study strongly support the conclusions of the 2014 Romanian study. Firstly, the
527 existence of a PVL-negative CC1-MRSA-IV clone in Romania that is distinct from WA
528 MRSA-1/45/57 was confirmed and secondly, the close relatedness of the Romanian CC1-
529 MRSA-IV and Romanian CC1 MSSA (which differed by as few as 47 cgSNPs) was verified
530 (Fig. 1). The available evidence suggests it is likely that this novel CC1-MRSA-IV clone
531 originated in South-Eastern Europe based on the following facts: (i) CC1 MSSA are common
532 in Iaşi, Romania (Monecke et al., 2014), (ii) the CC1-MRSA-IV clone was predominant in
533 Iaşi by 2008 (Monecke et al., 2014), at which time it had been detected in only two patients in
534 Ireland and (iii) none of the Irish CC1-MSSA isolates investigated grouped in close proximity
535 to clade A. However, the lack of published studies on MRSA and/or MSSA from
536 neighbouring countries means the possibility of this clone having originated elsewhere in
537 South-Eastern Europe cannot currently be ruled out.
538
539 Following its emergence in South-Eastern Europe, putatively in Romania, the European CC1-
540 MRSA-IV clone spread to Ireland (Fig. 1; Table 1). While this clone was present in the Irish
541 community by 2007, it was not detected in the Irish healthcare system until 2012 and did not
542 become prevalent in Ireland until 2013 (Table S1). These findings support the previously
543 outlined suggestion that consideration of risk-factors relating to the acquisition of MRSA in
544 the community (and not only in healthcare settings e.g. previous hospitalisation, non-intact

545 skin) may be appropriate during targeted MRSA screening in Irish hospitals (Earls et al.,
546 2017; Irish Department of Health, 2013). Interestingly, the Irish clade A isolates (which were
547 recovered between 2007-2017) exhibited a relatively high level of genotypic diversity (1-102
548 cgSNPs), suggesting that the European CC1-MRSA-IV clone may have been introduced into
549 Ireland on several occasions. Indeed, the first two European CC1-MRSA-IV isolates
550 identified in Ireland were recovered just 26 days apart (Table S1) and differed by 52 cgSNPs.
551 Correspondingly, the rate of migration from Romania to Ireland rose dramatically in 2007 and
552 was consistently high until 2017 (Irish National Central Statistics Office, 2017, 2009).
553 Phylogenetic analysis also indicated that the European CC1-MRSA-IV clone spread to
554 Germany (Fig. 1; Table 1). Although only sporadically encountered in Dresden, this clone
555 became prevalent in Regensburg between 2016-2018, as demonstrated by its regular detection
556 in the community and in two different hospitals. Remarkably, considering their recovery
557 during a period of approximately two years, the German clade A isolates exhibited a
558 particularly high level of genotypic diversity (12-115 cgSNPs), indicating that the European
559 CC1-MRSA-IV clone may have been introduced into Germany on multiple occasions. Indeed,
560 a 2014 German study demonstrated that 7/51 (14%) Romanian healthcare workers recently
561 employed in a hospital in Aachen were colonised with an unusual MRSA-t127 strain
562 (Scheithauer et al., 2014). In 2015, Romanians represented the second largest group (213,000
563 people) of foreign nationals living in Germany (Federal Office for Migration and Refugees,
564 Germany, 2016), with a particularly large community living in the Regensburg region.
565
566 As previously described, a largely mupirocin-resistant variant of the European CC1-MRSA-
567 IV clone (subclade A1) was responsible for a protracted outbreak in an Irish hospital between
568 2013 and 2016 (Earls et al., 2017). Isolates of this variant were also recovered from several
569 other hospitals and the community (Earls et al., 2017). As part of the present study, cgSNP
570 analysis revealed that the outbreak continued into 2017, and spread to three additional
571 hospitals (Fig. 1; Table 1). Importantly, this study also revealed that *ileS2*-encoding plasmid
572 (p140355) previously identified among the outbreak isolates also encodes *qacA* and therefore
573 constitutes the first reported plasmid in *S. aureus* to encode resistance to the two antimicrobial
574 agents (i.e. mupirocin and chlorhexidine) commonly used for *S. aureus* decolonisation
575 (Poovelikunnel et al., 2015). While successful read alignment of the 80 *ileS2* and *qacA*-
576 encoding outbreak isolates against the p140355 sequence suggested that all such isolates
577 harboured this plasmid, two outbreak isolates appeared to harbour a variant of this plasmid
578 which did not encode *qacA*. Notably, however, this *qacA*-negative plasmid did not
579 disseminate with the same success as p140355. Therefore, despite the general lack of

580 emphasis on antiseptic-resistance in the literature and public domain, it is possible that the
581 combination of mupirocin and chlorhexidine resistance may have driven the selection of
582 p140355-harbouring MRSA in Ireland.
583
584 It is highly likely that the CC1-MRSA-IV clone defined in this study is present in other
585 European countries in addition to Romania, Ireland and Germany. Indeed, PVL-negative ST1-
586 MRSA-IV-t127 have been recovered from humans, cattle, pigs, cow's milk and goat's milk in
587 Italy, and from rooks in Austria (Alba et al., 2015; Basanisi et al., 2017; Cortimiglia et al.,
588 2015; Loncaric et al., 2013; Monaco et al., 2013; Normanno et al., 2015). Furthermore, where
589 detailed, the genotypic characteristics of these PVL-negative ST1-MRSA-IV-t127 correspond
590 to those associated with the European CC1-MRSA-IV clone defined in this study. For
591 example, between 2009 and 2011, PVL-negative ST1-MRSA-IV-t127 which generally
592 harboured *aphA3*, *blaZ*, *sat* and *tet*(K), while exhibiting variable *erm*(C), *sak* and *scn* carriage,
593 were isolated from dairy cows, humans and pigs in Italy (Alba et al., 2015). Indeed, 19% of
594 the non-outbreak (i.e. non-subclade A1) European CC1-MRSA-IV isolates identified during
595 this study did not harbour any human immune evasion genes (i.e. *chp*, *sak* or *scn*), indicating
596 that this clone may be also be prevalent in animals (Table 2). Furthermore, while this study
597 was under review, an Italian study (which included one of the Irish clade A1 isolates
598 investigated in the present study) confirmed the presence of the European CC1-MRSA-IV
599 clone in a paediatric hospital in Florence (Manara et al., 2018). Moreover, in line with the
600 findings of the present investigation, this Italian study estimated that the novel CC1-MRSA-
601 IV clone diverged 6-28 years ago.
602
603 The present study has demonstrated the existence of a European PVL-negative CC1-MRSA-
604 IV clone that is distinctly different from the previously characterised and well-defined PVL-
605 negative CC1-MRSA-IV clone, WA MRSA-1/45/57. Furthermore, cgSNP analysis revealed
606 that this MDR clone may have originated in South-Eastern Europe, before spreading to both
607 Ireland and Germany. Finally, the *ileS2*-encoding plasmid which facilitated the spread of this
608 clone in Ireland was determined to also encode *qacA*, highlighting the increasing importance
609 of effective mupirocin and chlorhexidine alternatives for *S. aureus* decolonisation.

610 **5. Declaration of interest**

611 SM, RE and PS are employees of Abbott (Alere Technologies GmbH). The other authors
612 declare no conflicts of interest.

613 **6. Author Contributions**

614 MRE conceived the study, performed the WGS data analysis and drafted the manuscript. ACS
615 conceived the study and assisted with WGS data analysis and writing the manuscript. DCC
616 conceived the study, purchased the required materials, assisted with data analysis and drafted
617 the manuscript. GB conceived the study assisted with data analysis and drafted the manuscript.
618 AS and WS-B conceived the study, assisted with data analysis and drafted the manuscript. TS
619 and OD conceived the study assisted with data analysis and drafted the manuscript. PS, RE
620 and SM conceived the study, assisted with bioinformatics analysis, microarray analysis data
621 analysis and drafted the manuscript. All authors read and approved the final manuscript.

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633

634 **9. Figure legends**

635

636 **Figure 1.** A neighbour-joining tree based on a cgSNP analysis of 167 CC1-MRSA-IV and 20
637 CC1-MSSA isolates. The countries in which the isolates were recovered are indicated in the
638 colour legend. Subclade A1 is shaded in grey. All branches yielded 100% permutation
639 resampling support. The WA MRSA-1/45/57 isolates and the Irish clade B isolate marked as
640 USA400 were identified based on genotypic characteristics. Abbreviations: cgSNP, core-
641 genome single nucleotide polymorphism.
642

643 **Figure 2.** Genetic map of *S. aureus* multi-resistance plasmid, p140355 (GenBank accession
644 number: KY465818.1). The plasmid backbone is shown in green, while the accessory region
645 is depicted in red. All known genes are shown in black except for the antimicrobial resistance
646 genes, which are highlighted in yellow. Genes which have undergone National Centre for
647 Biotechnology Information prediction are shown in blue: 1, MobA/MobL family protein; 4,
648 Fst family toxin; 5, quinone reductase; 6, quinone reductase; 7, MarR family transcriptional
649 regulator; 13, haloacid dehalogenase-like hydrolase family protein; 16, fructosamine kinase
650 family protein; 17, major facilitator superfamily protein; 18, DDE transposase superfamily
651 protein; 34, XRE family transcriptional regulator. Genes encoding hypothetical proteins are
652 shown in grey.

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667 **10. References**

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Table 1. Epidemiological and genotypic data associated with the 167 CC1-MRSA-IV isolates and 20 CC1-MSSA isolates investigated in the present study.

NJT position[a]	Country	MRSA/ MSSA	No. of isolates	Recovery period	Source(s)	Sequence type(s)[b]	*spa* type(s)[c]	SCC element subtypes
Subclade A1	Ireland	MRSA	86	2013-2017	10 hospitals/HCFs Community	ST1	t127 (*n* = 84) t922 (*n* = 2)	MW2-like SCC*mec* IVa with *dcs* insertion
Remaining clade A	Ireland	MRSA	32	2007-2017	11 hospitals/HCFs Community	ST1	t127	MW2-like SCC*mec* IVa with *dcs* insertion
	Germany	MRSA	20	2016-2018	3 hospitals Community	ST1(*n* = 19) ST4911 (*n* = 1)	t127	MW2-like SCC*mec* IVa with *dcs* insertion
	Romania	MRSA	5	2010-2012	1 hospital	ST1	t127	MW2-like SCC*mec* IVa with *dcs* insertion
		MSSA	10	2009-2012	1 hospital	ST1 (*n* = 8) ST4910 (*n* = 1) ST4912 (*n* = 1)	t127 (*n* = 8) t5633 (*n* = 1) t18248 (*n* = 1)	SCC-negative
Subclade B1	Ireland	MRSA	17	2012-2017	9 hospitals Community	ST1 (*n* = 15) ST4913 (*n* = 1) ST4914 (*n* = 1)	t127	MW2-like SCC*mec* IVa (*n* = 9) SCC*mec* IVa/SCC*fus*$_{476}$ (*n* = 8)
	UAE	MRSA	2	2009	1 hospital	ST1	t127	SCC*mec* IVa/SCC*fus*$_{476}$
	Germany	MRSA	1	2007	1 hospital	ST1	t127	MW2-like SCC*mec*
Remaining clade B	Ireland	MRSA	3	2014-2016	3 hospitals	ST1	t127	MW2-like SCC*mec* IVa (*n* = 1) SCC*mec* IVa/SCC*fus*$_{476}$ (*n* = 2)
		MSSA	6	2004-2017	4 hospitals	ST1	t127	SCC*fus*$_{476}$
Clade C	Ireland	MRSA	1	2016	1 hospital	ST1	t127	SCC*mec* IVa/SCC*fus*$_{476}$
	Ireland	MSSA	2	2013	2 hospitals	ST1	t127	SCC*fus*$_{476}$
Clade D	Ireland	MSSA	2	2017	1 hospital	ST1	t127	SCC-negative

[a]Based on core-genome single nucleotide polymorphism analysis.

865 [b]Multilocus sequence typing was performed using Ridom SeqSphere+ version 4.1 (Ridom GmbH). Allelic profiles: ST1, 1-1-1-1-1-1-1; ST4910, 1-1-
866 1-1-40-1-1; ST4911, 1-1-1-1-1-1-649; ST4912, 1-731-1-1-1-1-1; ST4913, 1-1-1-1-1-1-648; ST4914, 1-1-663-1-1-1-1.
867 [c]*spa* typing was performed either using Ridom SeqSphere+ version 4.1, or as previously described (Earls et al., 2018). *spa* repeat successions: t127, 07-
868 23-21-16-34-33-13; t922, 07-23-21-16-33-13; t5633, 15-13; t18248, 07-23-21-16-34-33-20.
869 Abbreviations: HCFs, healthcare facilities; NJT, neighbour-joining tree; ST, sequence type.

Table 2. Differences in resistance and virulence-associated gene carriage between CC1-MRSA-IV in clade A and subclade B1, and in subclade A1 and the remainder of clade A.

Gene	Clade A[a]		Subclade B1[a]			Subclade A1[a]		Remaining clade A		
	n	%	*n*	%	*p* value[a]	*n*	%	*n*	%	*p* value[a]
Resistance										
aadD	0	0	**3**	**15**	**0.02**	1	1	0	0	1
aadE	58	100	0	0	**<0.0001**	86	100	57	100	1
aacA-aphD	2	3	0	0	1	0	0	2	4	0.16
aphA3	**58**	**100**	0	0	**<0.0001**	86	100	57	100	1
blaZ	58	100	20	100	1	86	100	57	100	1
erm(A)	0	0	**4**	**20**	**0.0034**	0	0	0	0	NA
erm(C)	**57**	**98**	3	15	**<0.0001**	86	100	57	98	1
fusB	0	0	0	0	NA	5	6	0	0	0.16
fusC	0	0	**10**	**50**	**<0.0001**	0	0	0	0	NA
ileS2	1	2	0	0	1	**82**	**95**	0	0	**<0.0001**
lnu(A)	0	0	0	0	NA	2	2	0	0	0.52
mecA	58	100	20	100	1	86	100	57	100	1
qacA	2	3	0	0	1	**80**	**93**	1	2	**<0.0001**
sat	**58**	**100**	0	0	**<0.0001**	86	100	57	100	1
tet(K)	**53**	**91**	0	0	**<0.0001**	**85**	**99**	52	91	**0.04**
Virulence										
hlb[b]	11	19	0	0	0.06	2	2	**11**	**19**	**0.002**
sak	47	81	20	100	0.06	**84**	**98**	46	81	**0.002**
scn	47	81	20	100	0.06	**84**	**98**	46	81	**0.002**
sea	0	0	**19**	**95**	**<0.0001**	0	0	0	0	NA
seh	58	100	20	100	1	86	100	57	100	1
sek	0	0	**19**	**95**	**<0.0001**	0	0	0	0	NA
seq	0	0	**19**	**95**	**<0.0001**	1	1	0	0	1

[a]Statistically significant results are shown in bold.
[b]Undisrupted-*hlb*

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Figure 1

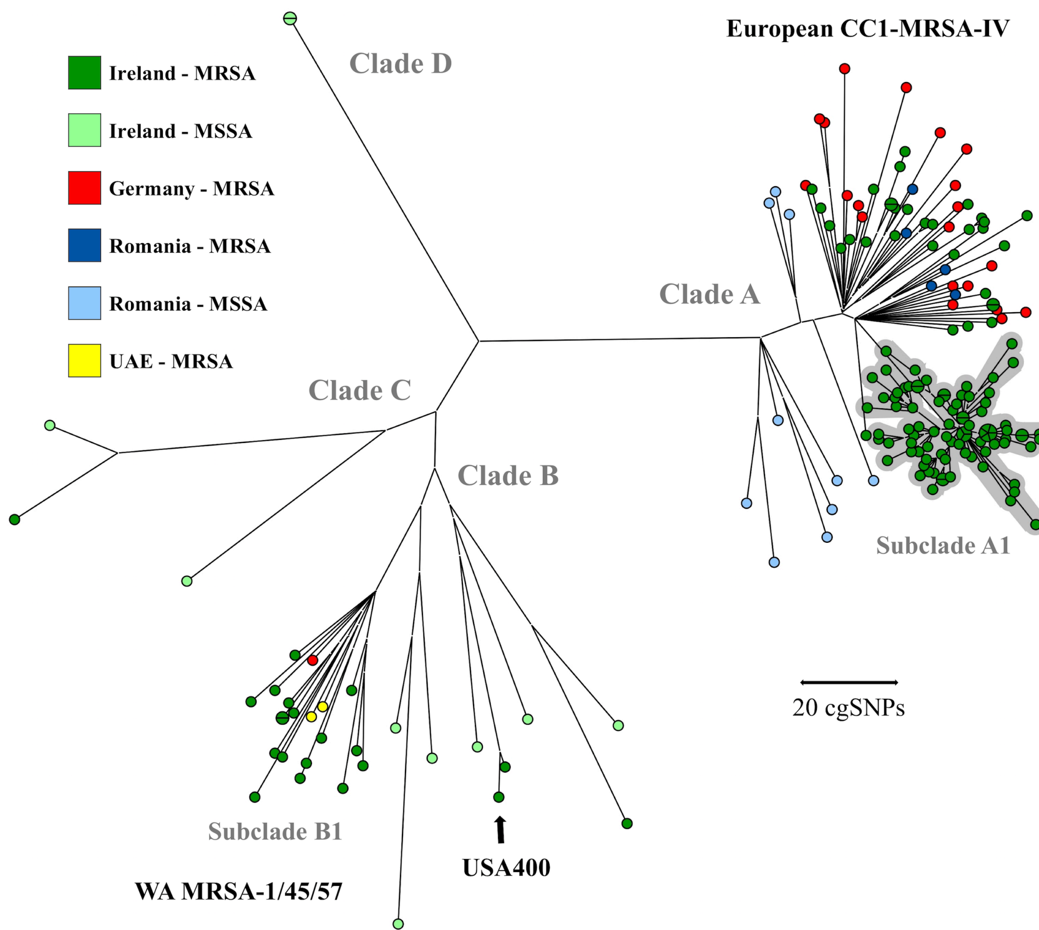
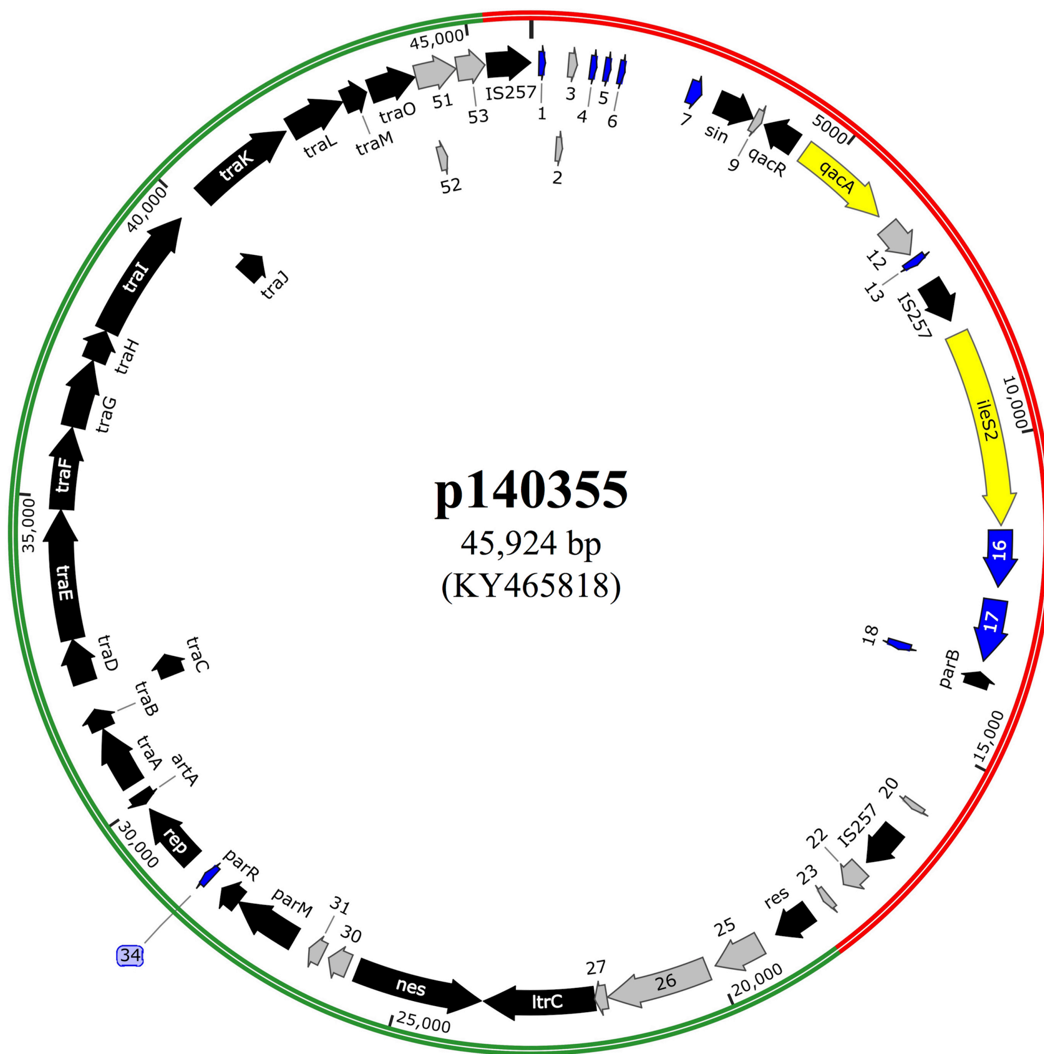


Figure 2



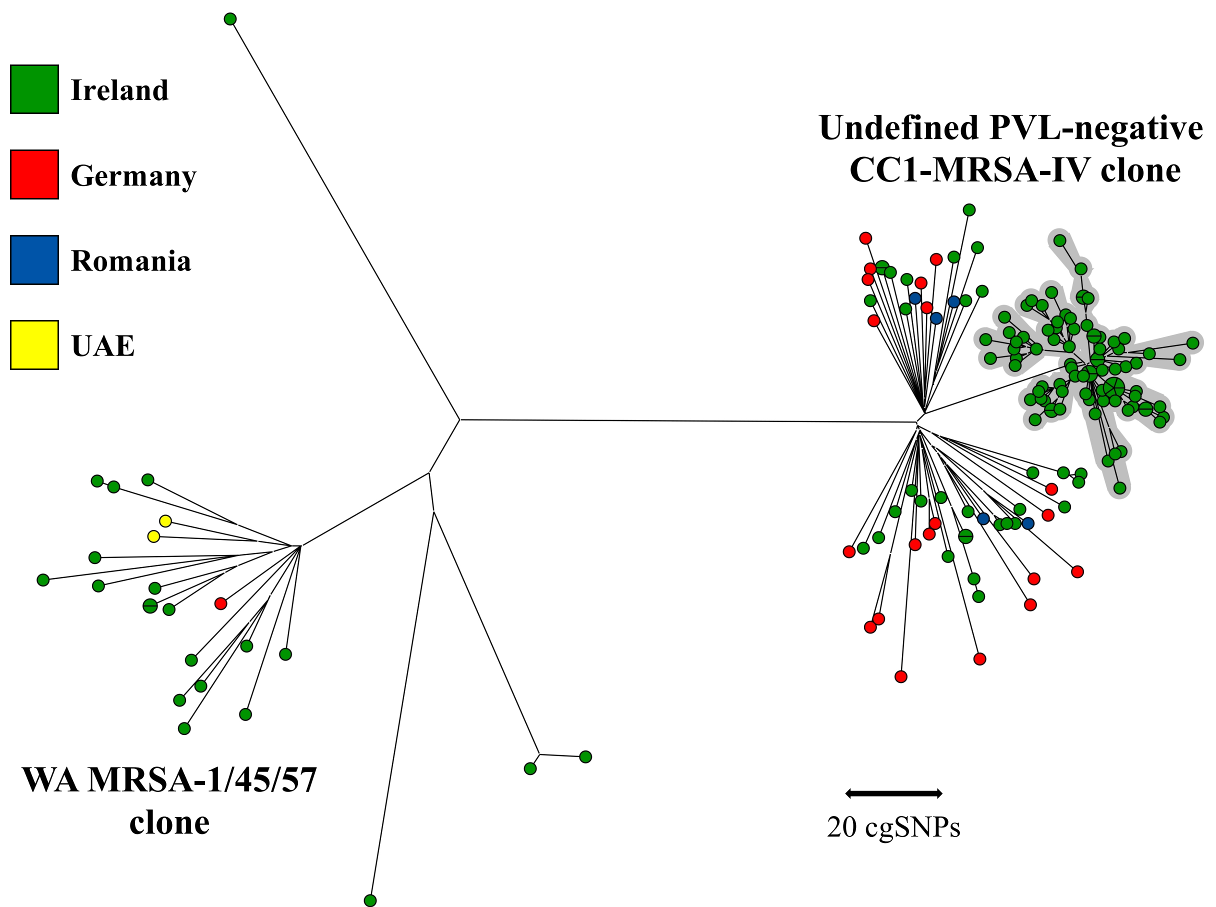


Figure S1. A neighbour-joining tree based on a cgSNP analysis of 167 CC1-MRSA-IV isolates. The countries in which the isolates were recovered are indicated in the colour legend. Outbreak isolates are shaded in grey. All branches yielded 100% permutation resampling support. Abbreviation: cgSNP, core-genome single nucleotide polymorphism.

Table S1. Epidemiological data associated with the 167 CC1-MRSA-IV isolates and 20 CC1-MSSA isolates investigated in the present study.

Isolate[a]	Year/date of recovery	Country of recovery	MRSA/ MSSA	Source[b]	Recovery site	Isolate[a]	Year/date of recovery	Country of recovery	MRSA/ MSSA	Source[b]	Recovery site
Subclade A1						Subclade A1 continued					
A1_01	05/11/2013	Ireland	MRSA	IRL_01	Colonisation	A1_74	15/02/2017	Ireland	MRSA	IE_01	Unknown
A1_02	31/03/2016	Ireland	MRSA	IRL_01	Colonisation	A1_75	16/02/2017	Ireland	MRSA	Community	Unknown
A1_03	12/01/2014	Ireland	MRSA	Community	Colonisation	A1_76	24/02/2017	Ireland	MRSA	IE_01	Colonisation
A1_04	16/02/2014	Ireland	MRSA	IRL_01	Osteomyelitis	A1_77	01/03/2017	Ireland	MRSA	IE_08	Unknown
A1_05	26/02/2014	Ireland	MRSA	IRL_01	Colonisation	A1_78	25/02/2017	Ireland	MRSA	IE_08	Unknown
A1_06	19/05/2014	Ireland	MRSA	IRL_01	SSTI	A1_79	02/03/2017	Ireland	MRSA	IE_02	BSI
A1_07	30/05/2014	Ireland	MRSA	IRL_01	SSTI	A1_80	06/03/2017	Ireland	MRSA	IE_01	Colonisation
A1_08	11/05/2014	Ireland	MRSA	IRL_01	Colonisation	A1_81	14/03/2017	Ireland	MRSA	IE_01	Wound
A1_09	24/06/2014	Ireland	MRSA	IRL_01	Colonisation	A1_82	16/03/2017	Ireland	MRSA	IE_01	Colonisation
A1_10	02/07/2014	Ireland	MRSA	IRL_01	Colonisation	A1_83	28/03/2017	Ireland	MRSA	IE_01	Unknown
A1_11	01/07/2014	Ireland	MRSA	IRL_01	Colonisation	A1_84	25/04/2017	Ireland	MRSA	IE_01	Unknown
A1_12	01/07/2014	Ireland	MRSA	IRL_01	Colonisation	A1_85	09/05/2017	Ireland	MRSA	Community	Unknown
A1_13	08/07/2014	Ireland	MRSA	IRL_01	Colonisation	A1_86	10/05/2017	Ireland	MRSA	IE_05	Unknown
A1_14	16/07/2014	Ireland	MRSA	IRL_01	Colonisation	Remaining clade A					
A1_15	16/07/2014	Ireland	MRSA	IRL_01	Colonisation	A_01	20/12/2007	Ireland	MRSA	Community	Unknown
A1_16	28/07/2014	Ireland	MRSA	IRL_01	Colonisation	A_02	13/01/2008	Ireland	MRSA	Community	Unknown
A1_17	28/07/2014	Ireland	MRSA	IRL_01	Colonisation	A_03	24/07/2012	Ireland	MRSA	IRL_08	Unknown
A1_18	05/08/2014	Ireland	MRSA	IRL_01	Colonisation	A_04	24/09/2012	Ireland	MRSA	IRL_03	Unknown
A1_19	14/08/2014	Ireland	MRSA	IRL_01	SSTI	A_05	12/10/2012	Ireland	MRSA	Community	Unknown
A1_20	25/11/2014	Ireland	MRSA	IRL_01	Colonisation	A_06	01/03/2013	Ireland	MRSA	IRL_01	Colonisation
A1_21	2014	Ireland	MRSA	IRL_01	Environmental	A_07	13/06/2013	Ireland	MRSA	Community	SSTI
A1_22	16/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_08	25/02/2014	Ireland	MRSA	IRL_01	Colonisation
A1_23	22/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_09	01/05/2014	Ireland	MRSA	IRL_14	Colonisation
A1_24	27/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_10	10/05/2014	Ireland	MRSA	IRL_03	Unknown
A1_25	23/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_11	14/07/2014	Ireland	MRSA	IRL_08	Unknown
A1_26	22/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_12	23/09/2014	Ireland	MRSA	IRL_11	Colonisation
A1_27	25/11/2014	Ireland	MRSA	IRL_01	Graft infection	A_13	23/09/2014	Ireland	MRSA	IRL_11	Colonisation
A1_28	17/11/2014	Ireland	MRSA	IRL_01	Colonisation	A_14	13/01/2015	Ireland	MRSA	IRL_03	Unknown
A1_29	28/10/2014	Ireland	MRSA	IRL_01	Colonisation	A_15	25/01/2015	Ireland	MRSA	IRL_01	Colonisation
A1_30	25/11/2014	Ireland	MRSA	IRL_01	Colonisation	A_16	24/03/2015	Ireland	MRSA	Community	Unknown
A1_31	19/12/2014	Ireland	MRSA	IRL_01	Colonisation	A_17	01/04/2015	Ireland	MRSA	Community	SSTI
A1_32	02/12/2014	Ireland	MRSA	IRL_01	BSI	A_18	30/04/2015	Ireland	MRSA	IRL_05	Unknown
A1_33	20/01/2015	Ireland	MRSA	IRL_01	BSI	A_19	11/05/2015	Ireland	MRSA	IRL_02	Unknown
A1_34	18/08/2014	Ireland	MRSA	IRL_02	Unknown	A_20	14/05/2015	Ireland	MRSA	IRL_06	Unknown
A1_35	11/02/2015	Ireland	MRSA	IRL_01	Colonisation	A_21	17/06/2015	Ireland	MRSA	Community	SSTI
A1_36	04/01/2015	Ireland	MRSA	IRL_01	Colonisation	A_22	18/06/2015	Ireland	MRSA	IRL_07	Unknown
A1_37	16/02/2015	Ireland	MRSA	IRL_01	Colonisation	A_23	13/07/2015	Ireland	MRSA	IRL_12	Unknown
A1_38	22/08/2014	Ireland	MRSA	IRL_02	Unknown	A_24	24/12/2015	Ireland	MRSA	IRL_08	SSTI
A1_39	10/03/2015	Ireland	MRSA	IRL_01	Colonisation	A_25	05/02/2016	Ireland	MRSA	IRL_06	Colonisation
A1_40	16/03/2015	Ireland	MRSA	IRL_03	SSTI	A_26	31/03/2016	Ireland	MRSA	IRL_13	Cellulitis
A1_41	17/03/2015	Ireland	MRSA	IE_01	Unknown	A_27	16/07/2016	Ireland	MRSA	IRL_05	Unknown
A1_42	24/03/2015	Ireland	MRSA	IE_01	Colonisation	A_28	23/12/2016	Ireland	MRSA	IRL_05	Unknown
A1_43	24/03/2015	Ireland	MRSA	IE_01	Graft infection	A_29	02/03/2017	Ireland	MRSA	IRL_08	Unknown
A1_44	01/04/2015	Ireland	MRSA	IE_04	Unknown	A_30	06/03/2017	Ireland	MRSA	IRL_08	Colonisation
A1_45	27/05/2015	Ireland	MRSA	IE_01	SSTI	A_31	07/03/2017	Ireland	MRSA	IRL_08	Colonisation
A1_46	06/07/2015	Ireland	MRSA	IE_09	Unknown	A_32	16/05/2017	Ireland	MRSA	IRL_08	Unknown
A1_47	31/07/2015	Ireland	MRSA	IE_01	Colonisation	A_33	2009	Romania	MSSA	RM_1	BSI
A1_48	23/08/2015	Ireland	MRSA	IE_01	Colonisation	A_34	2010	Romania	MRSA	RM_1	BSI
A1_49	21/09/2015	Ireland	MRSA	IE_05	Unknown	A_35	2010	Romania	MRSA	RM_1	Wound
A1_50	25/09/2015	Ireland	MRSA	IE_10	Unknown	A_36	2010	Romania	MSSA	RM_1	Wound
A1_51	10/09/2015	Ireland	MRSA	IE_01	BSI	A_37	2010	Romania	MSSA	RM_1	Wound
A1_52	30/10/2015	Ireland	MRSA	IE_05	Unknown	A_38	2010	Romania	MRSA	RM_1	BSI
A1_53	10/09/2015	Ireland	MRSA	Community	Colonisation	A_39	2010	Romania	MSSA	RM_1	BSI
A1_54	04/12/2015	Ireland	MRSA	IE_01	Colonisation	A_40	2010	Romania	MSSA	RM_1	BSI
A1_55	09/02/2016	Ireland	MRSA	IE_02	Unknown	A_41	2011	Romania	MSSA	RM_1	Wound
A1_56	06/02/2016	Ireland	MRSA	IE_01	Colonisation	A_42	2011	Romania	MSSA	RM_1	Wound
A1_57	31/03/2016	Ireland	MRSA	Community	SSTI	A_43	2012	Romania	MSSA	RM_1	Wound
A1_58	11/05/2016	Ireland	MRSA	Community	Unknown	A_44	2012	Romania	MSSA	RM_1	Wound
A1_59	15/05/2016	Ireland	MRSA	Community	Unknown	A_45	2012	Romania	MSSA	RM_1	Colonisation
A1_60	03/06/2016	Ireland	MRSA	IE_05	Unknown	A_46	2012	Romania	MRSA	RM_1	Colonisation
A1_61	31/08/2016	Ireland	MRSA	IE_06	Unknown	A_47	Unknown	Romania	MRSA	RM_1	Unknown
A1_62	11/10/2016	Ireland	MRSA	IE_03	Unknown	A_48	2016	Germany	MRSA	GR_1	Unknown
A1_63	08/11/2016	Ireland	MRSA	IE_07	Unknown	A_49	2016	Germany	MRSA	GR_2	Colonisation
A1_64	02/01/2017	Ireland	MRSA	IE_01	Unknown	A_50	2016	Germany	MRSA	GR_2	Colonisation
A1_65	04/01/2017	Ireland	MRSA	IE_01	Unknown	A_51	2016	Germany	MRSA	Community	Colonisation
A1_66	01/02/2017	Ireland	MRSA	IE_01	Unknown	A_52	2016	Germany	MRSA	GR_3	Colonisation
A1_67	01/02/2017	Ireland	MRSA	IE_01	Unknown	A_53	2016	Germany	MRSA	GR_3	Colonisation
A1_68	07/02/2017	Ireland	MRSA	IE_07	Unknown	A_54	2016	Germany	MRSA	GR_3	Colonisation
A1_69	04/02/2017	Ireland	MRSA	IE_01	Unknown	A_55	2016	Germany	MRSA	GR_3	Wound
A1_70	04/02/2017	Ireland	MRSA	IE_01	Unknown	A_56	2017	Germany	MRSA	GR_2	Colonisation
A1_71	06/02/2017	Ireland	MRSA	IE_01	Unknown	A_57	2017	Germany	MRSA	GR_2	Infection
A1_72	07/02/2017	Ireland	MRSA	IE_01	Unknown	A_58	2017	Germany	MRSA	GR_2	Colonisation
A1_73	13/02/2017	Ireland	MRSA	IE_01	Unknown	A_59	2017	Germany	MRSA	GR_3	Colonisation

Table S1 continued

Isolate[a]	Year/date of recovery	Country of recovery	MRSA/ MSSA	Source[b]	Recovery site
Remaining clade A continued					
A_60	2017	Germany	MRSA	GR_3	Colonisation
A_61	2017	Germany	MRSA	GR_3	Wound
A_62	2017	Germany	MRSA	GR_3	Colonisation
A_63	2017	Germany	MRSA	GR_3	Wound
A_64	2017	Germany	MRSA	GR_2	Colonisation
A_65	2017	Germany	MRSA	GR_2	Colonisation
A_66	2018	Germany	MRSA	GR_3	Colonisation
A_67	2018	Germany	MRSA	GR_2	Colonisation
Clade B1					
B1_01	09/08/2012	Ireland	MRSA	IRL_15	Unknown
B1_02	18/01/2013	Ireland	MRSA	IRL_16	Unknown
B1_03	25/03/2013	Ireland	MRSA	IRL_03	Unknown
B1_04	09/08/2013	Ireland	MRSA	IRL_03	Unknown
B1_05	25/07/2014	Ireland	MRSA	Community	SSTI
B1_06	30/10/2014	Ireland	MRSA	IRL_17	Unknown
B1_07	27/11/2014	Ireland	MRSA	IRL_15	BSI
B1_08	17/12/2014	Ireland	MRSA	IRL_17	Unknown
B1_09	28/01/2015	Ireland	MRSA	IRL_18	Unknown
B1_10	28/01/2015	Ireland	MRSA	IRL_18	Colonisation
B1_11	06/02/2015	Ireland	MRSA	IRL_19	Colonisation
B1_12	19/06/2015	Ireland	MRSA	IRL_13	Unknown
B1_13	14/10/2015	Ireland	MRSA	IRL_20	Unknown
B1_14	02/03/2016	Ireland	MRSA	Community	BSI
Clade B1 continued					
B1_15	08/03/2016	Ireland	MRSA	IRL_03	Unknown
B1_16	15/12/2016	Ireland	MRSA	IRL_01	Unknown
B1_17	29/05/2017	Ireland	MRSA	IRL_21	Unknown
B1_18	2007	Germany	MRSA	GR_1	SSTI
B1_19	2009	UAE	MRSA	UE_1	Infection
B1_20	2009	UAE	MRSA	UE_1	Infection
Remaining clade B					
B_01	20/10/2004	Ireland	MSSA	IRL_08	Unknown
B_02	18/01/2010	Ireland	MSSA	IRL_17	Unknown
B_03	24/10/2013	Ireland	MRSA	IRL_23	BSI
B_04	20/08/2014	Ireland	MSSA	IRL_03	Unknown
B_05	03/11/2015	Ireland	MSSA	IRL_01	Unknown
B_06	08/01/2016	Ireland	MRSA	IRL_13	Unknown
B_07	18/10/2016	Ireland	MRSA	IRL_03	Unknown
B_08	26/02/2017	Ireland	MSSA	IRL_03	Unknown
B_09	21/05/2017	Ireland	MSSA	IRL_01	Wound
Clade C					
C_01	31/07/2013	Ireland	MSSA	IRL_19	Unknown
C_02	28/10/2013	Ireland	MSSA	IRL_22	Unknown
C_03	18/04/2016	Ireland	MRSA	IRL_21	Unknown
Clade D					
D_01	15/03/2017	Ireland	MSSA	IRL_08	Colonisation
D_02	15/03/2017	Ireland	MSSA	IRL_08	Colonisation

[a]Isolates are grouped according to their position in the core-genome single nucleotide polymorphism-based neighbour-joining tree generated in the present study (Fig. 1).
[b]Isolates sourced from the community were defined as those recovered in a general medical practitioner's surgery, an emergency department or an outpatient's department. All other isolates were associated with specific hospitals which are indicated using a two-letter country code and a number.
Abbreviations: BSI, bloodstream infection; GR, Germany; IRL, Ireland; RM, Romania; SSTI, skin and soft tissue infection; UE, United Arab Emirates

Table S2. Oligonucleotide primers used to confirm the genetic organisation of the p140355 plasmid

Primer name	Sequence (5'-3')	Thermal cycle[a]	Region amplified (nt)[b]	Expected amplimer size (bp)
0F	GTTGAGGTGGTTGAATGGATTG	1	41986-42499	513
0R	CTGCCATAGTCCCCATAGAACC			
1F	GGGGACTATGGCAGGTAG	2	42485-2549	5988
1R	CATTATCAGTTTGACGTGTTATTAG			
2F	GATAATGAAAATAGAAGAACTGTGC	2	2543-8559	6016
2R	AACCTCTAATTCAACTGGTAAGC			
3F	TGGCTTACCAGTTGAATTAGAG	2	8535-14556	6021
3R	AGCTCATATTTGTGTTCTCAGATC			
4F	AGGATCTGAGAACACAAATATGAG	2	14351-20546	6015
4R	CATCTTGTTGTGTAAAATCGTC			
5F	ACAGTGATATTAATGAAGTTGACG	2	20505-26505	6000
5R	AATAGTAACAACAATACCTAAAGCG			
6F	TCGCTTTAGGTATTGTTGTTAC	2	26480-32378	5898
6R	TGTCTTAATCATTGGCTCATC			
7F	TGGAGGTTTAAATATTACTGGTG	2	32255-38249	5994
7R	AATGACGTGATAAGTTAATTCCTAC			
8F	AGGGCTAGAGAATTAAGTGATTG	2	38198-42001	3803
8R	ATTCAACCACCTCAACTTTC			

[a]Thermal cycling conditions: **1.** 94°C for 2 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s. Final elongation of 72°C for 5 min. **2.** 92°C for 2 min; 10 cycles of 92°C for 10 s, 59°C for 30 s, 68°C for 4 min, 20 cycles of 92°C for 15 s, 59°C for 30 s, 68°C for 5.5 min. Final elongation of 68°C for 7 min.
[b]Based on GenBank entry KY465818.1.