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1 **Multiple distinct outbreaks of Panton-Valentine leukocidin (PVL)-positive community-**

2 **associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Ireland**

3 **investigated by whole-genome sequencing**

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23 *Running Title:* PVL-+ve CA-MRSA outbreaks investigated by WGS

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33 Abbreviations; WGS, whole-genome sequencing; wgMLST, whole-genome multilocus

34 sequence typing

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35 **Summary**

36 **Background:** Panton-Valentine leukocidin (PVL)-positive community-associated

37 methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are increasingly associated with

38 infection outbreaks.

39

40 **Aim:** To investigate multiple suspected PVL-positive CA-MRSA outbreaks using whole-

41 genome sequencing (WGS).

42

43 **Methods:** Forty-six suspected outbreak-associated isolates from 36 individuals at three

44 separate Irish hospitals (H1-H3) and from separate incidents involving separate families

45 associated with H2 were investigated by whole-genome multilocus sequence typing

46 (wgMLST).

47

48 **Findings:** Two clusters (CH1 and CH2) consisting of 8/10- and 6/6- PVL-positive t008 ST8-

49 MRSA-IVa isolates from H1 and H2, respectively, were identified. Within each cluster,

50 neighbouring isolates were separated by ≤5 allelic differences; however ≥73 allelic

51 differences were identified between the clusters, indicative of two independent outbreaks.

52 Isolates from the H3 maternity unit formed two clusters (CH3-SCI and CH3-SCII) composed

53 of four PVL-negative t4667 ST5-MRSA-V and 14 PVL-positive t002 ST5-MRSA-IVc

54 isolates, respectively. Within clusters, neighbouring isolates were separated by ≤24 allelic

55 differences, whereas both clusters were separated by 1822 allelic differences, indicative of

56 two distinct H3 outbreaks. Eight PVL-positive t127 ST1-MRSA-V+*fus* and three PVL-

57 negative t267 ST97-MRSA-V+*fus* isolates from two distinct H2-associated families FC1

58 (*N*=4) and FC2 (*N*=7), formed three separate clusters (FC1 [t127], FC2 [t127] and FC2

59 [t267]). Neighbouring isolates within clusters were closely related and exhibited ≤7 allelic

60 differences. Intra-familial transmission was apparent, but the detection of ≥48 allelic

61 differences between clusters indicated no interfamilial transmission.

62

63 **Conclusion:** The frequent importation of PVL-positive CA-MRSA into healthcare settings,

64 transmission and association with outbreaks is a serious ongoing concern. WGS is a highly

65 discriminatory, informative method for deciphering such outbreaks conclusively.

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INTRODUCTION

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections were originally defined as those occurring in otherwise healthy populations without traditional healthcare-associated MRSA (HA-MRSA) risk factors[1]. CA-MRSA infections can range from superficial skin and soft tissue infections (SSTIs) to life-threatening illnesses[2].

Several genotypic characteristics of CA-MRSA strains previously indicated an evolutionary trajectory independent to that of HA-MRSA clones, which was subsequently confirmed by whole-genome sequence (WGS)-based phylogenetic studies [1,2]. CA-MRSA lineages typically carry smaller staphylococcal chromosomal cassette elements harbouring *mec* (SCC*mec*) such as SCC*mec* types IV and V, fewer antimicrobial resistance determinants and larger arsenals of virulence factor-encoding genes than HA-MRSA. These are considered contributory factors in the ability of CA-MRSA to infect otherwise healthy individuals. The boundaries between CA-MRSA and HA-MRSA have increasingly blurred as CA-MRSA lineages have diversified and become increasingly prevalent in hospitals and other healthcare settings[1].

The expression of the Panton-Valentine leukocidin (PVL) was originally considered one of the hallmark traits of CA-MRSA, however reports of PVL-negative CA-MRSA have been increasing[3]. PVL is encoded by the *lukF-PV* and *lukS-PV* genes located in the genomes of a range of lysogenic bacteriophages. SSTIs are considered the classic presentation of PVL-positive CA-MRSA strains, although more serious infections such as necrotizing pneumonia can ensue.

Methicillin-susceptible *S. aureus* (MSSA) isolates belonging to clonal complex (CC)8 and multilocus sequence typing (MLST) sequence type (ST) 8 emerged from Central Europe and spread to the United States of America approximately 160 years ago[4]. Here it developed into the well-documented CA-MRSA clone USA300, following the acquisition of SCC*mec* IVa, the *pvl* genes and the arginine catabolic mobile element (ACME)[4], the latter of which is thought to enhance its ability to persist on human skin[5]. The USA300 clone has since achieved global spread although it has yet to become endemic outside of North America, probably due to competition by native CA-MRSA clones in these regions[4,6]. Whilst the CA-MRSA population in Europe is diverse[7], the prevalence of USA300 is increasing[8].

The PVL-positive ST5-MRSA-IV USA800 clone is well dispersed globally[9,10] and predominantly associated with CA- and HA-SSTIs. Recent WGS studies revealed a closely

104 related PVL-positive CC5-MRSA-IVc clade of the USA800 clone (known as the Sri Lankan
105 clade), comprising isolates from Sri Lanka, the United Kingdom and Australia[10], and the
106 association of the USA300 clone with infection outbreaks in neonatal intensive care
107 units[11].

108   In Ireland, PVL-negative ST22-MRSA-IV remains the predominant cause of MRSA
109 bloodstream infection (BSI), and the prevalence of PVL-positive MSSA is also low
110 (0.8%)[12]. The proportion of PVL-positive MRSA isolates submitted to the Irish National
111 MRSA Reference Laboratory (NMRSARL) has gradually increased since 2002 (Figure S1)
112 [13,14]. Between 2011 and 2017, the PVL genes were detected in an average of 9.5% of
113 MRSA isolates sent to the NMRSARL. These PVL-positive isolates harboured SCC*mec*
114 types IV or V and predominantly belonged to CC5 (14.7%), CC8 (31.4%) or CC30 (17.6%)
115 [13–15]. Outbreaks caused by PVL-positive ST772-MRSA-V (known as the pandemic
116 Bengal Bay clone)[16] and PVL-negative ST78-MRSA-IVa and ST1-MRSA-IV[17] CA-
117 MRSA lineages have been reported in Irish hospitals also. Earls *et al.* recently demonstrated
118 the transmission of multidrug-resistant PVL-negative ST1-MRSA-IV isolates, originally
119 considered a CA-MRSA lineage, within and between hospitals in Ireland and the
120 involvement of healthcare workers (HCWs) in transmission events[17].

121   The advent of WGS has revolutionised the epidemiological investigation of microbial
122 pathogens over the last decade. In laboratories where WGS is the most cost-effective method
123 for highly informative molecular typing and the required bioinformatic tools are available,
124 conventional molecular typing approaches have largely been replaced by WGS-based
125 techniques, although isolate lineages are still described according to conventional MLST.
126 WGS has facilitated the expansion of the traditional *S. aureus* MLST scheme based on seven
127 loci to a core genome (cg) MLST scheme based on 1,861 loci[18], providing a globally-
128 available, standardised and highly-discriminatory method for strain comparison and
129 population structure investigations. Strain discrimination resolution can be further enhanced
130 using whole-genome (wg) MLST, which analyses a total of 3,904 loci including the cgMLST
131 loci[19], or single nucleotide variation (SNV) analysis for the investigation of infection
132 outbreaks or transmission studies. Isolates exhibiting ≤24 wgMLST or cgMLST allelic
133 differences or ≤15 SNVs are deemed closely related and indicative of recent transmission
134 [20].

135   The purpose of the present study was to use WGS to investigate the genetic relatedness of
136 isolates recovered during distinct outbreaks caused by ST8, ST5 and ST1 PVL-positive
137 MRSA within healthcare and community settings in Ireland.

138 **METHODS**

139 **Bacterial isolates**. Forty-six MRSA isolates from 36 individuals suspected of being involved

140 in several distinct infection outbreaks between 2011 and 2020 were submitted to the

141 NMRSARL for routine analyses and investigated here (Table I). Thirty-five isolates were

142 from patients or HCWs who had previously attended or worked at one of three Irish hospitals

143 (H1-H3). Eleven isolates were recovered from members of two separate families, each of

144 which had at least one member that attended the emergency department or was hospitalised

145 in H2 during 2011, 2012 or 2018 due to an MRSA infection (Table SI).

146   Twenty MRSA isolates submitted to the NMRSARL were included as comparator

147 reference isolates (CRFs) including PVL-positive MRSA identified as *spa* types t002, t008

148 and t127 recovered between 2014 and 2019 from community general practice clinics,

149 regional Irish hospitals, or Dublin-based teaching hospitals other than those included in the

150 present study (Table SI).

151   Seven international CRFs were selected following comparison of the DNA

152 microarray profiling patterns (see below) of isolates recovered from each H2-associated

153 family cluster (FC) to a *S. aureus* DNA microarray profile database. This database comprises

154 approximately 25,000 microarray profiles of human and animal strains recovered

155 worldwide[21]. The seven CRFs selected exhibited highly similar array patterns to those of

156 the FC isolates and consisted of two PVL-positive ST5 MRSA from humans, two PVL-

157 positive ST1 MRSA from human (*N=1*) and bovine (*N=1*) hosts and three PVL-negative

158 ST97 MRSA from humans (*N=2*) and poultry meat (*N=1*) in three countries in the Middle

159 East (Table SI).

160

161 **Identification, molecular characterisation and antimicrobial susceptibility testing.**

162 Isolates were confirmed as *S. aureus* using the tube coagulase test and as MRSA using 30 μg

163 cefoxitin disks (Oxoid Ltd, Basingstoke, UK). Isolates underwent antimicrobial susceptibility

164 testing against 15 antimicrobial agents (Table S2) as described previously[22,23]. The PVL-

165 encoding *lukF-PV* and *lukS-PV* genes were detected by PCR [24], *spa* typing was performed

166 as previously described[25] and DNA microarray profiling was performed using the *S.*

167 *aureus* Genotyping Kit 2.0 [Abbott (Alere Technologies GmbH), Jena, Germany] according

168 to the manufacturer's instructions[26]. Isolate STs and SCC*mec* types were inferred based on

169 *spa* types and/or DNA microarray profiles prior to WGS-based confirmation.

170

171   **Passaged isolates.** To investigate the genomic stability of each lineage investigated in this

172   study, serial passaging of representative MRSA (CC1:M18/0051, CC5:M18/1033,

173   CC8:M18/0227 & CC97:M18/0578) was undertaken. Isolates were reactivated from storage

174   at -80°C on Microbank cryogenic bead vials (Pro-Lab Diagnostics, Cheshire, UK) and single

175   colony sub-cultured on fresh Colombia blood agar plates every 24 h for a total of 10 days. On

176   days, 2, 4, 8 and 10, a single colony was randomly selected and subjected to WGS.

177

178   **Whole-genome sequencing.** WGS was carried out on all isolates investigated using DNA

179   extracted as described previously[25]. Libraries were prepared using the Nextera DNA Flex

180   Library Preparation kit (Illumina, Eindhoven, The Netherlands) according to the

181   manufacturer's instructions. Prepared libraries were subjected to paired-end sequencing using

182   the MiSeq instrument (Illumina, The Netherlands) using the 500-cycle MiSeq Reagent kit v2

183   (Illumina). Libraries were scaled to yield a minimum of 70× coverage per isolate and the

184   quality of each sequencing run was determined according to cluster density and Q30 values

185   according to the manufacturer's instructions. All read datasets are available in the NCBI

186   Sequence Read Archive as BioProject PRJNA638834

187   (https://dataview.ncbi.nlm.nih.gov/object/PRJNA638834?reviewer=r1ti4589pbk67a8badrb1jf

188   tki).

189

190   **Bioinformatic analyses.**

191   The Bionumerics software package v7.7 and incorporated SPAdes assembly software v3.7.1

192   (Applied Maths, Belgium) was used to perform wgMLST, pairwise SNV analyses and

193   construct minimum spanning trees (MSTs) as previously described[25]. Both assembly-based

194   and assembly-free methods were used to detect and identify MLST alleles as described

195   previously[25]. Sequence reads were also assembled using the VELVET assembler

196   incorporated within SeqSphere+ software version 7 (Ridom, GmBH, Germany). Previously

197   identified s*pa* types and inferred STs were confirmed based on these assemblies using the

198   web-based SCC*mec*Finder tool (https://cge.cbs.dtu.dk/services/SCCmecFinder/)[27]. The

199   resistance and virulence task templates within SeqSphere+ were used to identify

200   antimicrobial resistance genes and virulence factor genes, respectively.

201 **RESULTS**

202 **Investigation of ST8-MRSA-IV outbreaks at hospitals H1 and H2**

203 Ten isolates recovered from eight patients at the outpatient (*N=3*), gynaecology (*N=1*),

204 emergency department (*N=1*) and neonatal intensive care unit (*N=5*) of a maternity hospital

205 in Dublin (H1) during a four-month period spanning 2017 and 2018 were investigated. All 10

206 isolates were identified as ACME- and PVL-positive ST8-MRSA-IVa, features characteristic

207 of the USA300 clone (Table SI). Six additional isolates recovered from two separate patients

208 at a separate Dublin hospital (H2) during one week within the same period of the H1

209 outbreak were also identified as ACME- and PVL-positive ST8-MRSA-IVa. One H1 isolate

210 was identified as *spa* type t723, whereas the remaining 15 H1 and H2 isolates were t008

211 (Table I). To investigate possible transmission events within and between hospitals H1 and

212 H2, all isolates underwent WGS.

213   A wgMLST-based MST tree was constructed for all 16 isolates, the USA300 type

214 strain FPR3757 (GenBank accession number CP000255.1) and 10 t008 PVL-positive MRSA-

215 IV CRFs. This MST revealed two discrete clusters (CH1 [8/10 H1 isolates] and CH2 [all 6

216 H2 isolates]) within which the majority of H1 and H2 isolates clustered, respectively (Figure

217 1a).

218   A separate SNV-based MST constructed for the same isolates but excluding the

219 FPR3757 type strain also revealed the same discrete clusters (Figure S2a). Isolates within

220 CH1 and CH2 were differentiated from the USA300 type strain FPR3757 by 73 and 80 allelic

221 differences, respectively (Figure 1a) and each cluster was separated by ≥127 SNVs (Figure

222 S2a). Isolates within CH1 were all closely related with a median of 0 (average 1.5, range 0-4)

223 allelic differences or 0 (average 1.1, range 0-6) SNVs. The two remaining H1 isolates

224 differed from CH1 isolates by ≥73 allelic differences (Figure 1a) and ≥120 SNVs (Figure

225 S2a). Isolates within CH2 were also closely related with a median of 0.5 (average 0.5, range

226 0-5) allelic differences (Figure 1a) or 0 (average 0.8, range 0-5) SNVs (Figure S2a).

227   In contrast, the 10 ST8 CRFs and the FPR3757 reference exhibited an average of 74

228 allelic differences between neighbouring isolates and none clustered with CH1 or CH2

229 (Figure 1a). These findings indicated the occurrence of two separate outbreaks caused by

230 genetically distinct strains, one in H1 involving the 8/10 CH1 isolates and the other in H2

231 involving the six CH2 isolates.

232   Whilst all 16 isolates recovered from H1 and H2 exhibited ciprofloxacin and

233 ampicillin resistance, two distinct phenotypic antibiotic resistance patterns were identified. In

234 contrast, the 10 CRFs exhibited five distinct antibiotic susceptibility patterns (Table I).

Investigation of ST5-MRSA outbreak at hospital H3

235 Nineteen isolates from 17 separate patients and one HCW associated with the maternity unit
236
237 of a regional Irish hospital (H3) recovered between 2018 and 2020 were sent to the
238 NMRSARL for epidemiological analysis. Fifteen isolates identified as t002 ST5-MRSA-IVc
239 (commonly referred to as USA800) were recovered over a 15 month period during 2018-20
240 and four t4667 ST5-MRSA-V isolates were recovered during one month in 2019 (Table SI).
241 Eighteen were PVL-positive and one t002 isolate was PVL-negative. These isolates were
242 recovered from patients in the maternity (*N*=10), special care baby (*N*=5), outpatient (*N*=1),
243 emergency (*N*=1) and paediatric (*N*=1) units, and from a HCW (*N*=1) and subjected to WGS
244 to determine if the isolates were part of a protracted outbreak or two separate outbreaks.

245 Both wgMLST-based (Figure 1b) and SNV-based (Figure S2b) MSTs were
246 constructed based on all 19 isolates, five Irish t002 CRFs and two international PVL-positive
247 CC5-MRSA-IV CRFs (125-318618 and 37-158951; Table SI) which were selected on the
248 basis of having highly similar DNA array profiles to the t002 isolates investigated here.
249 These MSTs revealed two distinct sub-clusters consisting of either t4667 (sub-cluster CH3-
250 SCI, *N*=4 isolates) or t002 (sub-cluster CH3-SCII; *N*=14) isolates. The PVL-negative t002
251 ST5-MRSA-IVc isolate from H3 (M18/1063) did not cluster with any other isolates and
252 exhibited 324 allelic differences to the CH3-SCII sub-cluster (Figure 1b). All four t4667
253 ST5-MRSA-V isolates within CH3-SCI were closely related and exhibited a median of 1
254 (average 1, range 1-2) allelic difference (Figure 1b) and 0 (average 0.25, range 0-1) SNVs
255 (Figure S2b). These four isolates were recovered from three patients and one HCW in H3
256 during a one month period in 2019 (Table SI). All four yielded identical phenotypic antibiotic
257 susceptibility profiles, and exhibited resistance to ≥3 classes of antibiotics (Table I).
258 According to the wgMLST-based MST, the 14 t002 ST5-MRSA-IVc CH3-SCII isolates
259 exhibited a median of 1 (average 3.6, range 0-24) allelic difference (Figure 1b). Four isolates
260 within CH3-SCII were genetically indistinguishable (M18/033, M19/0889, M19/0930 and
261 M19/1191) despite being recovered from four separate patients at intervals of 11, one and
262 two months apart, respectively (Table SI). The seven CRFs (Table SI) exhibited ≥80 allelic
263 differences to the closest relative isolate within CH3-SCII (Figure 1b). The SNV-based MST
264 sub-clusters correlated with those of the wgMLST-based tree with the exception of isolate
265 M19/0811, which was separated by its nearest CH3-SCII neighbour by 24 allelic differences
266 (Figure 1b) or 28 SNVs (Figure S2b), respectively.

267 All 15 t002 isolates exhibited phenotypic ampicillin resistance and one also exhibited
268 ciprofloxacin resistance (Table I).

269   Collectively, these findings revealed the occurrence of two separate outbreaks in H3,
270   one involving ST5-MRSA-V (CH3-SCI) and the other involving ST5-MRSA-IVc (CH3-
271   SCII).

272

273   **Investigation of ST1-MRSA and ST97-MRSA isolates recovered from two separate**
274   **families associated with H4.**
275   These isolates were recovered from two separate, unrelated families; referred to as FC1 and
276   FC2. Four isolates were recovered from three FC1 members during a three month period
277   spanning 2011 and 2012. Of these four isolates, two were recovered from separate members
278   who presented at the H2 emergency department with SSTIs. Two were from screening
279   samples; one from an individual previously treated for a SSTI, and one from a third family
280   member (Table SI). All four isolates were identified as PVL-positive t127 ST1-MRSA and
281   exhibited phenotypic resistance to ≥3 classes of antibiotics (Table I).

282   In an unrelated episode, seven MRSA isolates were recovered from five FC2
283   members during a seven month period spanning 2017 and 2018. Two isolates were recovered
284   from infections in two family members (one of whom was a H2 inpatient) and the remaining
285   five isolates were collected from screening samples of one of these patients and three other
286   family members (Table SI). All seven isolates were identified as PVL-positive t127 ST1-
287   MRSA (N=4) or PVL-negative t267 ST97-MRSA (N=3) and exhibited phenotypic resistance
288   to ≥3 classes of antibiotics (Table I).

289   To investigate the potential transmission of the isolates within FC2 and explore
290   potential relatedness of the t127 isolates to those from FC1, all 11 FC1 and FC2 isolates
291   underwent WGS. Three international PVL-negative ST97 CRFs, two international and five
292   Irish PVL-positive ST1 CRFs were also included. Separate wgMLST and SNV-based MSTs
293   revealed that within each distinct family cluster and *spa* type, isolates were separated from
294   their nearest neighbour by ≤7 allelic differences or ≤8 SNVs. Isolates belonging to FC1
295   (t127) were separated from their nearest neighbouring isolate by a median of 4 (average 4.6,
296   range 3-7) allelic differences (Figure 1c) or 1 (average 3, range 1-7) SNV (Figure S2c).
297   Isolates belonging to FC2 (t127) were separated from their nearest neighbour by a
298   median of 6 (average of 3.8, range 2-7) allelic differences (Figure 1b), 5 (average 4.7, range
299   1-8) SNVs (Figure S2c) and by 48 allelic differences or 52 SNVs to the FC1 (t127) isolates.
300   Each of the t127 and t267 CRFs investigated were separated from their neighbouring isolate
301   by ≥44 allelic differences (Fig. 1c). The three *spa* type t267 isolates from FC2 were separated
302   from each other by a median of 4 (average of 4, range 4-4) allelic differences (Figure 1c) and

303 4 (average 4, range 4-4) SNVs (Figure S2c). The three ST97 CRFs exhibited ≥72 allelic
304 differences or ≥76 SNVs to the FC2 isolates.

305 The FC1, FC2 and CRF t127 ST1 isolates harboured genes indicative of SCC*mec* IV,
306 V or the SCC*mec* V subtype (V+*fus*+*tirS*+*ccrA1*) (Table SI) previously identified in sporadic
307 PVL-positive CC1 isolates[28]. The FC2 and CRF t267 ST97 isolates harboured genes
308 indicative of SCC*mec* V+*fus* (Table SI) previously identified in sporadic CC97 isolates[28].

309 Collectively, these findings indicated the transmission of distinct MRSA strains
310 amongst two separate families. One t127 strain was transmitted amongst FC1 members,
311 whereas two distinct t127 and t267 strains were independently transmitted amongst several
312 FC2 members. Direct transmission of the t127 isolates between the two families investigated
313 was deemed unlikely due to the identification of 48 allelic differences between the two t127
314 clusters (Figure 1c).

315

316 **Serially Passaged Isolates**

317 The wgMLST analysis of colonies randomly selected from serial passage of the ST8-
318 MRSA-IVa isolate M18/0227, ST5-MRSA-IVc isolate M18/1033, ST1-MRSA-
319 V+*fus*+*tirS*+*ccrA1* isolate M18/0051 and ST97-MRSA- V+*fus* isolate M18/0578 revealed a
320 maximum of two, zero, three and three allelic differences between each passage, respectively.

321 **DISCUSSION**

322   Despite the decreasing reports of MRSA infections in Ireland in recent years, the
323 proportion of PVL-positive MRSA has continually increased since 2002 (Figure S1)[13,14].
324 The present investigation revealed the transmission of distinct PVL-positive CA-MRSA
325 lineages within separate Irish hospitals (H1-H3). Three of these lineages (ST8-MRSA-IVa,
326 ST5-MRSA-V and ST5-MRSA-IVc) were associated with outbreaks in neonatal care
327 facilities (Table SI). Outbreaks of ST5 and ST8 CA-MRSA infections have been reported in
328 neonatal and paediatric care units previously[11].

329   Multidrug resistance (resistance to ≥3 classes of clinically-relevant antibiotics) is not a
330 common characteristic of CA-MRSA, but is increasingly reported amongst PVL-positive
331 MRSA populations in Europe, North and South America, Asia and Australia[29]. In the
332 present study, multidrug resistance was detected in 22/53 (41.5%) isolates, including those
333 belonging to the ST1, ST5, ST8, and ST97 lineages (Table SI).

334   The USA300 isolates were recovered from neonatal, paediatric and adult patients in two
335 separate hospitals. All were identified as PVL-positive t008/t723, ST8-MRSA-IVa and
336 exhibited ampicillin and ciprofloxacin resistance (Table SI). Despite these similarities,
337 wgMLST revealed the presence of distinct clusters of isolates within each hospital and no
338 evidence of transmission between the two. HCW-associated CA-MRSA transmission
339 between hospitals in Ireland has previously been suggested, as transfer of staff between
340 distinct healthcare facilities is common[17]. Each cluster was separated from the USA300
341 type strain by a ≥73 allelic differences (Figure 1a) indicative of separate, unrelated outbreaks.
342 Due to the identification of t008 *spa* type and carriage of the SCC*mec* type IVa, these isolates
343 bear the closest similarity to the clone originating in North America[4], however no travel
344 histories were available for the patients investigated.

345   Isolates identified as PVL-positive ST5-MRSA-IVc were recovered from H3-associated
346 individuals over a 15 month period during 2018-20. These isolates were identified as *spa*
347 types t002 and t4667, which correlated with the two distinct sub-clusters identified by
348 wgMLST (Figure 1b). The t4667 isolates belonging to CH3-SCI were all recovered during
349 the same month, harboured SCC*mec* V, exhibited multidrug resistance and were recovered
350 from a HCW and patients. The PVL-positive t002 isolates were recovered between 2018 and
351 2020, harboured SCC*mec* IVc and exhibited ampicillin resistance (Table SI). The low
352 average number of allelic differences (3.4) detected amongst the H3 isolates compared to the
353 average 75.7 allelic differences among CRFs is indicative of a protracted ST5 MRSA
354 outbreak in H3.

355   The PVL-positive t127 ST1-MRSA isolates were from two separate families (FC1 and

356   FC2) with links to H2, however the FC1 t127 isolates were recovered during 2011 and 2012,

357   whereas the FC2 t127 isolates were recovered during 2018 (Table SI). Based on the detection

358   of 48 allelic differences between the FC1 and FC2 t127 isolates and the fact that these

359   isolates were recovered more than five years apart (Table SI), it is likely that these isolates

360   represent independent transmission networks. The ≤24 allelic differences or ≤15 SNVs

361   thresholds for inferring epidemiological relationships between *S. aureus* isolates[20] were

362   deemed appropriate for all lineages investigated and were supported by the available

363   epidemiological information.

364   Similar SCC*mec* type V+*fus*+*tirS*+*ccrA1* elements were detected in all FC1 and FC2

365   t127 isolates and SCC*mec* V+*fus* elements were detected in the PVL-negative FC2 t267 ST97

366   isolates. Genes indicative of similar elements[28] have also been identified in both PVL-

367   positive ST1 and PVL-negative ST97 isolates recovered in the Middle East[28,30], an area

368   where some members of FC2 had travelled, suggesting possible importation of these strains

369   from this geographical region and subsequent intra-familial transmission. Previous research

370   has shown that MRSA transmission is common in the household and can result in 67% of

371   household contacts of an index carrier becoming colonised with the same strain[31].

372   The present study demonstrated the high resolution offered by WGS for investigation of

373   outbreaks and transmission of MRSA strains both within and outside healthcare settings. For

374   each separate investigation, distinct wgMLST and SNV-based MSTs were congruent (Figure

375   1 and S2). The ST8 isolates recovered from H1 and H2 were deemed highly similar based on

376   phenotypic antibiotic susceptibility patterns and *spa* types, suggestive of possible

377   transmission between the two Dublin-based hospitals. However, these isolates were separated

378   into genotypically distinct clusters by wgMLST, indicating the independent transmission of

379   two distinct USA300 strains within each hospital and highlighting the advantage of WGS

380   over conventional molecular typing techniques. The unparalleled discriminatory power

381   offered by WGS also demonstrated the possibility of outbreak-associated isolates being

382   incorrectly excluded from outbreak investigations if based on *spa* types alone. In the present

383   investigation, isolate M18/0106 identified as t723 (repeat succession: 11-19-12-34-22-25)

384   exhibited between four-six allelic differences (Figure 1a), and between six-eight SNPs

385   (Figure S2a) to the seven t008 (repeat succession: 11-19-12-21-17-34-24-34-22-25) isolates

386   in CH1.

387   As WGS becomes more routinely available to more clinical microbiology laboratories,

388   the high resolution offered by the technology will help to inform and direct infection

389 prevention and control strategies for outbreak management in real-time, both within

390 healthcare facilities and/or private households. The high resolution offered by WGS can often

391 reveal transmission events directly. This information would be highly beneficial in the

392 implementation of routine strategies for (i) decontamination (e.g. improved cleaning and

393 monitoring of most frequently outbreak-associated fomites within healthcare facilities and

394 domestic settings), (ii) transmission risk minimisation (e.g. screening of HCWs, incoming

395 patients and the identification of long-term carriers) and (iii) decolonisation (e.g. use of

396 topical or systemic prophylactic therapies for management of endogenous infection risk prior

397 to admittance to healthcare facilities, which may also reduce the risk of outbreak-associated

398 infections in healthcare settings). In addition, the WGS data can be utilised to inform

399 approaches for antimicrobial treatments.

400   In the present study, two PVL-positive ST8-MRSA-IVa MRSA infection outbreaks in

401 two Dublin-based hospitals were shown to be unrelated, a protracted PVL-positive ST5-

402 MRSA-IVc outbreak in a Dublin-based teaching hospital was revealed and the intra-familial

403 transmission of PVL-positive ST1-MRSA-V+*fus*+*tirS*+*ccrA1* and PVL-negative ST97-

404 MRSA-V+*fus* isolates in two separate families was described.

405

406 **CONCLUSIONS**

407 The prevalence of PVL-positive MRSA is continually increasing in Ireland. The frequent

408 importation of CA-MRSA strains into healthcare settings and increasing association with

409 HCWs and infection outbreaks is a serious concern. The application of WGS is highly

410 advantageous in the ongoing surveillance of MRSA strains and investigation of suspected

411 outbreaks.

412

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554

555 **Figure 1. Minimum spanning trees (MSTs) based on whole-genome multilocus**
556 **sequencing typing (wgMLST) analysis of the 42 Panton-Valentine leukocidin (PVL)**
557 **positive and four PVL-negative MRSA isolates investigated in addition to**
558 **epidemiologically unrelated but genotypically similar comparator reference isolates**
559 **(CRFs).**
560 In each MST, MRSA isolates recovered from separate hospitals or families and identified as
561 distinct *spa* types are indicated by separate colours. Genotypically similar but
562 epidemiologically unrelated CRFs included for comparative purposes are indicated in each
563 MST as red circles. Closely related clusters of isolates (≤24 wgMLST allelic differences
564 [20]) are outlined with grey shadowing. A black spot in the centre of each circle is used to
565 indicate PVL-negative isolates which were included in the study as they were identified as
566 the same *spa* types/STs as the outbreak-associated PVL-positive MRSA and were recovered
567 in the same hospital over the relevant time periods. The PVL-negative t267 isolates were
568 included as they were also recovered from one of the families affected by the PVL-positive
569 t127 MRSA lineage. The numbers on each branch indicate the numbers of wgMLST allelic
570 differences detected between neighbouring isolates. The epidemiological information for
571 each isolate and CRF investigated is provided in Table SI. (a) MST constructed from the
572 t008, ST8-IVa MRSA isolates associated with hospitals 1 and 2 (H1 [*N*=10] & H2 [*N*=6]),
573 CRFs (*N*=10) and the USA300 reference strain FPR3757. The two distinct clusters CH1 and
574 CH2, refer to isolates recovered from hospitals H1 and H2, respectively. (b) MST constructed
575 from the t002-ST5-IVc (*N*=15) and t4667-ST5-V (*N*=4) isolates associated with hospital 3
576 (H3) and CRFs (*N*=7). With the exception of the PVL-negative isolate M18/1063, all t002-
577 ST5-IVc isolates formed a distinct sub-cluster (CH3-SCI), and all four t4667-ST5-V isolates
578 formed a second sub-cluster (CH3-SCII). (c) MST constructed from the t127-ST1-
579 V+*fus*+*tirS*+*ccrA1* (*N*=8) and t267-ST97-V+*fus* (*N*=3) isolates recovered from multiple
580 members of two distinct families (FC1 [*N*=4] and FC2 [*N*=7]), of which each family had at
581 least one member that either attended the emergency department of, or was admitted to
582 hospital 2 (H2), and 10 epidemiologically unrelated CRFs. Three distinct sub-clusters were
583 apparent, FC1 (t127), FC2 (t127) and FC2 (t267) each of which consisted of all of the
584 isolates identified as each distinct *spa* type and from each separate family.
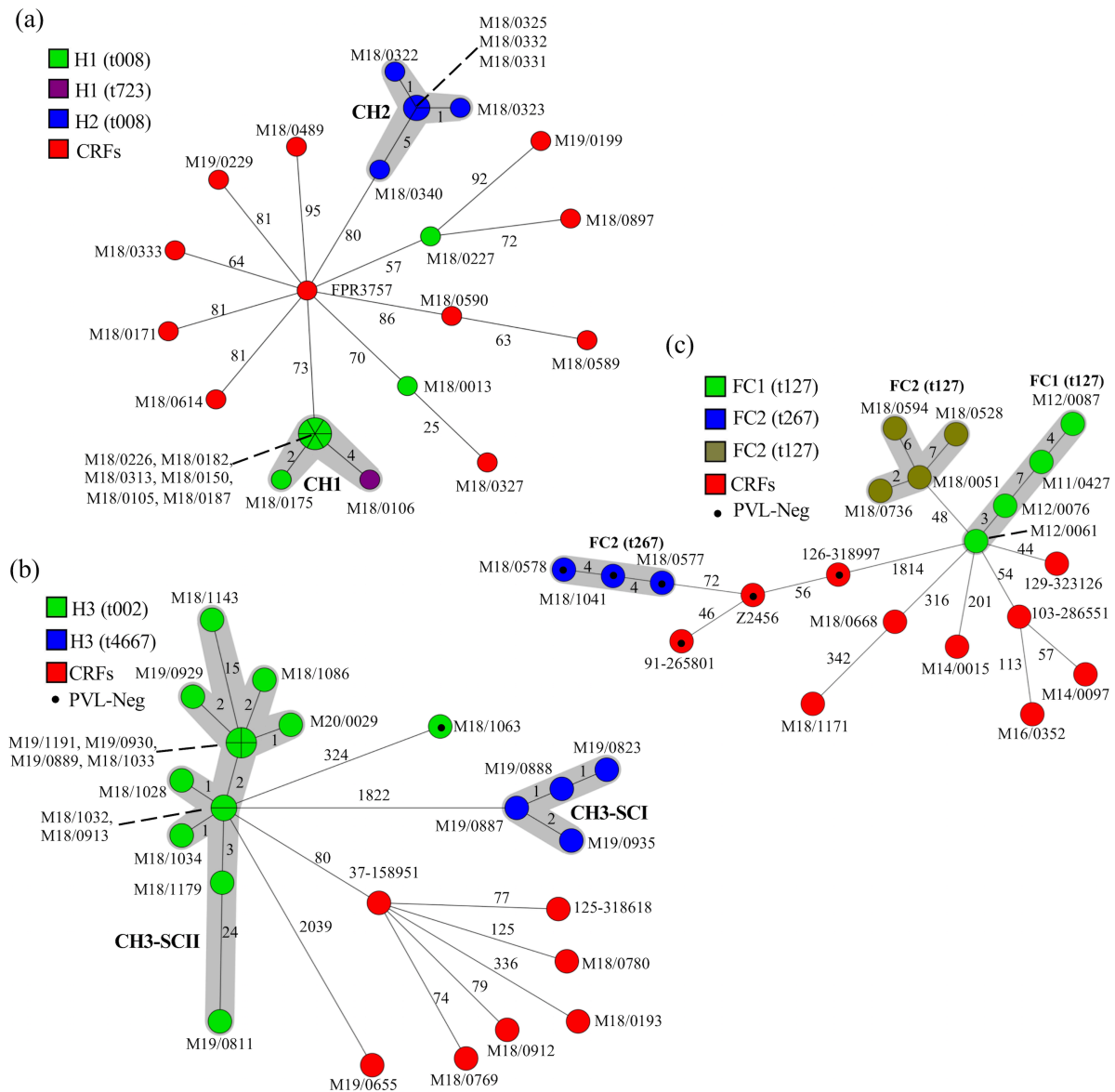
Figure 1

Figure 1. Minimum spanning trees (MSTs) based on whole-genome multilocus sequencing typing (wgMLST) analysis of the 42 Panton-Valentine leukocidin (PVL) positive and four PVL-negative MRSA isolates investigated in addition to epidemiologically unrelated but genotypically similar comparator reference isolates (CRFs). In each MST, MRSA isolates recovered from separate hospitals or families and identified as distinct *spa* types are indicated by separate colours. Genotypically similar but epidemiologically unrelated CRFs included for comparative purposes are indicated in each MST as red circles. Closely related clusters of isolates (≤24 wgMLST allelic differences [20]) are outlined with grey shadowing. A black spot in the centre of each circle is used to indicate PVL-negative isolates which were included in the study as they were identified as the same *spa* types/STs as the outbreak-associated PVL-positive MRSA and were recovered in the same hospital over the relevant time periods. The PVL-negative t267 isolates were included as they were also recovered from one of the families affected by the PVL-positive t127 MRSA lineage. The numbers on each branch indicate the numbers of wgMLST allelic differences detected between neighbouring isolates. The epidemiological information for each isolate and CRF investigated is provided in Table SI. (a) MST constructed from the t008, ST8-IVa MRSA isolates associated with hospitals 1 and 2 (H1 [N=10] & H2 [N=6]), CRFs (N=10) and the USA300 reference strain FPR3757. The two distinct clusters CH1 and CH2, refer to isolates recovered from hospitals H1 and H2, respectively. (b) MST constructed from the t002-ST5-IVc (N=15) and t4667-ST5-V (N=4) isolates associated with hospital 3 (H3) and CRFs (N=7). With the exception of the PVL-negative isolate M18/1063, all t002-ST5-IVc isolates formed a distinct sub-cluster (CH3-SCI), and all four t4667-ST5-V isolates formed a second sub-cluster (CH3-SCII). (c) MST constructed from the t127-ST1-V+*fus*+*tirS*+*ccrA1* (N=8) and t267-ST97-V+*fus* (N=3) isolates recovered from multiple members of two distinct families (FC1 [N=4] and FC2 [N=7]), of which each family had at least one member that either attended the emergency department of, or was admitted to hospital 2 (H2), and 10 epidemiologically unrelated CRFs. Three distinct sub-clusters were apparent, FC1 (t127), FC2 (t127) and FC2 (t267) each of which consisted of all of the isolates identified as each distinct spa type and from each separate family.