Panton-Valentine Leukocidin-Positive *Staphylococcus aureus* in Ireland 2002-2011: Twenty-One Clones, Frequent Importation of Clones, Temporal Shifts of Predominant Methicillin-Resistant *S. aureus* and Increasing Multiresistance

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ABSTRACT

There has been a worldwide increase in community-associated (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) infections. CA-MRSA isolates commonly produce the Panton-Valentine leukocidin toxin encoded by the *pvl* genes *lukF*/*S-PV*. This study investigated the clinical and molecular epidemiology of *pvl*-positive MRSA and methicillin-susceptible *S. aureus* (MSSA) identified by the Irish National MRSA Reference Laboratory (NMRSARL) between 2002 and 2011. All *pvl*-positive MRSA (*n* = 190) and MSSA (*n* = 39) isolates underwent antibiogram-resistogram typing, *spa* typing and DNA microarray profiling for multilocus sequence type, clonal complex (CC) and/or sequence type (ST) and staphylococcal cassette chromosome *mec* type assignment and virulence and resistance gene detection. Where available, patient demographics and clinical data were analyzed.

The prevalence of *pvl*-positive MRSA increased from 0.2%-8.8% and *pvl*-positive MSSA decreased from 20%-2.5% during the study period. The *pvl*-positive MRSA and MSSA belonged to 16 and five genotypes, respectively, with CC/ST8-MRSA-IV, CC/ST30-MRSA-IV, CC/ST80-MRSA-IV and CC1/ST772-MRSA-V and CC30-MSSA, CC22-MSSA and CC121-MSSA predominating. Temporal shifts in the predominant *pvl*-positive MRSA genotypes and a six-fold increase in multiresistant *pvl*-positive MRSA occurred during the study period. Analysis of patient data indicated that *pvl*-positive *S. aureus* strains, especially MRSA, were imported into Ireland several times. Two hospital and six family clusters of *pvl*-positive MRSA were identified and 70% of patient isolates for which information was available were from patients in the community.

This study highlights the increased burden and changing molecular epidemiology of *pvl*-positive *S. aureus* in Ireland over the last decade and the contribution of international travel to the influx of genetically diverse *pvl*-positive *S. aureus* into Ireland.

INTRODUCTION

Usually methicillin-resistant *Staphylococcus aureus* (MRSA) are considered to be a healthcare-associated (HCA) pathogen, frequently responsible for serious and often life-threatening infections in individuals with established risk factors such as prolonged hospital stay and antibiotic usage, older age, recent surgery or an immunocompromised state. Healthcare-associated MRSA have been found to belong to five distinct clonal lineages, typically harbor the staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II or III, or, less frequently, SCC*mec* types IV, VI or VIII and often exhibit resistance to multiple classes of antimicrobial agents (1).

However, during the last decade there has been a concurrent worldwide increase in the prevalence of community-associated (CA-) MRSA infections among otherwise healthy individuals, often children and young adults, with none of the HCA risk factors for (2, 3). These consist predominantly of skin and soft tissue infections (SSTIs), but also include necrotizing pneumonia, necrotizing fasciitis and sepsis (2, 4-6). The pathogenesis of CA-MRSA has in some studies been attributed to the ability of these organisms to express the Panton-Valentine leukocidin (PVL) toxin (3, 7). Panton-Valentine leukocidin-positive MRSA infections have been reported among many different groups particularly those with close contact or in poor socioeconomic situations (2).

Panton-Valentine leukocidin is a bicomponent beta-barrel toxin that causes leukocyte lysis or apoptosis via pore formation (8). PVL is encoded by two genes, *lukF-PV* and *lukS-PV*, that are encoded on a range of different lysogenic bacteriophages (9). While outbreaks of PVL-producing methicillin-susceptible *S. aureus* (MSSA) were reported in the 1950s and 1960s (10) it was in the 1990s when PVL was first reported in newly emerging CA-MRSA strains (4, 11). While not all CA-MRSA produce PVL and there is conflicting data regarding the role of PVL in

70 the pathogenesis of CA-MRSA infection, it is clear that the success of some CA-MRSA clones is
71 associated with PVL, albeit not exclusively (12).

72 MRSA carrying the PVL toxin genes (*pvl*) are predominantly genetically distinct from
73 HCA-MRSA, belong to more diverse clonal lineages and harbor the smaller SCC*mec* elements
74 type IV, V or V_T, as well as frequently being non-multiresistant (1, 3, 13). Different *pvl*-positive
75 MRSA clones predominate in different regions e.g. ST8-MRSA-IV (USA300) in the USA (14),
76 ST59-MRSA-VT in Asia (13, 15), ST30-MRSA-IV in New Zealand (16), ST93-MRSA-IV in
77 Australia (17), ST80-MRSA-IV in Europe (18) and the Middle East (1), ST88-MRSA-IV in
78 Africa (19) and ST22-MRSA-IV and ST772-MRSA-V in India (20). However, recent studies
79 have highlighted the complex and changing epidemiology of *pvl*-positive MRSA including (i)
80 considerable variation in the prevalence rates of *pvl*-positive MRSA in different regions of the
81 world (2, 17), (ii) the increasing prevalence and polyclonal population structure of *pvl*-positive
82 MRSA in Europe (1, 21, 22), (iii) the increasing prevalence of ST8-MRSA-IV in Europe and the
83 decreasing prevalence of ST80-MRSA-IV (21), (iv) the increasing prevalence of multiresistant
84 *pvl*-positive MRSA (22) and (v) the spread of *pvl*-positive MRSA into hospitals (14, 23-25).
85 Furthermore, there has been an increasing frequency of reports of infections associated with *pvl*-
86 positive MSSA (26, 27) in association with similar clinical presentations as *pvl*-positive MRSA
87 and the former are a potential reservoir for the emergence of *pvl*-positive MRSA.

88 In Ireland, MRSA is endemic in hospitals, and since 2002 *pvl*-negative ST22-MRSA-IV
89 has accounted for 70-80% of MRSA from bloodstream infections (BSIs) each year (28, 29).
90 Between 1999 and 2005, a prevalence rate of 1.8% was reported for *pvl*-positive MRSA in
91 Ireland and six distinct *pvl*-positive MRSA clones (ST30, ST8, ST22, ST80, ST5 and ST154, all
92 harboring SCC*mec* IV), were identified, some of which were probably imported (30). In 2011,
93 we reported multiple importations of the multiresistant *pvl*-positive ST772-MRSA-V clone into

Ireland and a cluster of this clone in a neonatal intensive care unit (NICU) in an Irish hospital (31). However, there have been no published data on the overall prevalence and molecular epidemiological characteristics of the *pvl*-positive MRSA population in Ireland since 2005 and just a single report of a familial outbreak of *pvl*-positive MSSA in Ireland with no molecular epidemiological typing of the isolates undertaken (32). The purpose of the present study was to investigate the clinical and molecular epidemiology of *pvl*-positive MRSA and MSSA identified by the Irish National MRSA Reference Laboratory (NMRSARL) between 2002 and 2011.

MATERIALS AND METHODS

Bacterial isolates. The NMRSARL investigated 7103 *S. aureus* isolates (6702 MRSA and 314 MSSA) between 2002 and 2011, of which 1531 were examined for the presence of the *lukF/S*-PV genes (*pvl*) (Table 1). An isolate was selected for *pvl* investigation if it was recovered from a suspected *pvl*-associated infection or, for MRSA only, if the isolate exhibited an antibiogram-resistogram (AR) pattern and/or pulsed-field group (PFG) distinct from that of previously or currently predominant *pvl*-negative healthcare-associated MRSA clones e.g. AR-PFG 06-01, indicative of ST22-MRSA-IV or AR-PFG 13/14-00, indicative of ST8-MRSA-IIA-E +/-SCC_{MI} (28, 33). Of the 1531 isolates investigated for *pvl* (1217 MRSA and 401 MSSA), 229 (190 MRSA and 39 MSSA) were *pvl*-positive and were investigated further (Table 1). This included 24/25 previously described *pvl*-positive MRSA isolates recovered between 2002 and 2005 (30) and 18 previously described *pvl*-positive ST772-MRSA-V isolates recovered between 2009 and 2011 (31). One MRSA isolate (E1760), previously reported as *pvl*-positive (30), was excluded from the present study because *pvl* was not detected despite several attempts using PCR and DNA microarray profiling. Only one isolate per patient was investigated unless AR and pulsed-field gel electrophoresis typing indicated the presence of a second strain from a particular patient.

Where possible, patient demographics and clinical data were collected from isolate submission forms, telephone follow-ups and follow-up questionnaires. Isolates were defined as clusters if they were recovered from members of the one family/household, or within a hospital or both. Within each cluster isolates were recovered between three months and two years apart. Each isolate within a cluster was recovered from a different person or environmental source. This paper does not include any identifying, or potentially identifying, patient information.

Confirmation of isolates as *S. aureus*, methicillin susceptibility testing and detection of the *lukF*/*S*-PV genes. On receipt by the NMRSARL all *S. aureus* isolates were inoculated onto Protect beads (Technical Service Consultants Limited, Heywood, United Kingdom) and stored at -70°C prior to subsequent investigation. Isolates were confirmed as *S. aureus* using the tube coagulase test and methicillin resistance was investigated with 10-µg and 30-µg cefoxitin discs (Oxoid Ltd, Basingstoke, United Kingdom), as described previously (30). The detection of the *lukF*/*S*-PV genes was performed by PCR as described previously (4) or, for isolates recovered in the final quarter of 2011, using an in-house real-time PCR assay designed to detect the *mecA*, *nuc* and *pvl* genes. The identification of isolates as *S. aureus*, the presence or absence of *mecA* and the presence of the *lukF*/*S*-PV genes was also confirmed in all isolates using DNA microarray profiling as described below.

Phenotypic and genotypic characterization of *pvl*-positive *S. aureus* isolates. All 229 *pvl*-positive *S. aureus* isolates underwent antimicrobial susceptibility testing, *spa* typing and DNA microarray profiling. For the 18 *pvl*-positive ST772-MRSA-V isolates included in the study this analysis was performed previously and three of these isolates also underwent MLST (31). The 24 previously described *pvl*-positive MRSA isolates recovered between 2002 and 2005 included in

the study previously underwent antimicrobial susceptibility, MLST and SCC*mec* typing and toxin gene profiling for a limited number of toxin genes (30).

Antimicrobial susceptibility testing. The susceptibility of each isolate to a panel of 23 antimicrobial agents was determined by disk diffusion as described previously (30). The antimicrobial agents tested were amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin, trimethoprim and vancomycin.

DNA microarray analysis. DNA microarray analysis was performed on all isolates using the StaphyType Kit (Alere Technologies GmbH, Jena, Germany) which simultaneously detects 333 *S. aureus* gene targets including species markers, antimicrobial resistance and virulence-associated genes (including *lukF/S*-PV and *mecA*) as well as SCC*mec*-associated genes and typing markers allowing isolates to be assigned to MLST sequence types (STs) and/or clonal complexes (CCs) as well as SCC*mec* types (34, 35). The DNA microarray procedure was performed according to the manufacturer's instructions.

PCR detection of antimicrobial resistance genes. Isolates that exhibited phenotypic resistance to particular antimicrobial agents for which associated resistance genes were not detected by the DNA microarray, or where resistance genes were detected but partial or none of the associated resistance phenotypes were detected were further investigated by PCR to confirm the presence or absence of these resistance genes. These investigations included PCRs using previously described primers to detect *mupA* (36), *aphA3* (37), *aacA-aphD* (37), *fusB* (38), *tet*(K) (36), *tet*(M) (36),

161 *aadD* (39) and *qacA* (40) and novel primers to detect *qacC*, *msr*(A), *dfrS1*, *lnu*(A), *mph*(C), and
162 *blaZ* (Supplemental Table S1).

163 **Statistical analysis.** A two-sample *z*-test was used to assess the significance of the difference
164 between two population proportions. A p-value ≤ 0.05 was considered significant at a
165 significance level of 0.05.

166 **RESULTS**

167 A total of 229 *pvl*-positive *S. aureus* isolates were identified by the NMRSARL between
168 2002 and 2011 including 190 MRSA and 39 MSSA representing 2.8% and 9.7% of all MRSA
169 and MSSA, respectively, submitted to the NMRSARL during this time (Table 1). Overall, the
170 prevalence of *pvl*-positive MRSA among all MRSA submitted to the NMRSARL increased
171 significantly during the study period (p-value < 0.0005) from 0.2% in 2002 (1/497) to 8.8%
172 (40/456) in 2011, with these two specific years recording the lowest and highest prevalence rates,
173 respectively (Table 1). In contrast, for *pvl*-positive MSSA, the prevalence rate among all MSSA
174 submitted to the NMRSARL decreased significantly (p-value < 0.0005) from 20% in 2004 (3/15)
175 to 2.5% (2/81) in 2011 (Table 1).

176 **Genotyping.** The *pvl*-positive MRSA (n =190) and MSSA (n = 39) isolates were assigned to 11
177 and five MLST clonal complexes (CCs), respectively (Table 2). Among the MRSA, the isolates
178 were assigned to either SCC*mec* types IV (79.5%, 151/190) or V (20.5%, 39/190), and 16
179 genotypes (CC/ST-SCC*mec* types) (Table 2) with CC/ST8-MRSA-IV predominating (33.7%,
180 64/190), followed by CC/ST30-MRSA-IV (21.1%, 40/190), CC/ST80-MRSA-IV (14.2%,
181 27/190), CC1/ST772-MRSA-V (13.2%, 25/190), CC/ST22-MRSA-IV (6.3%, 12/190),
182 ST59/952-MRSA-V (4.7%, 9/190), ST93-MRSA-IV (3/190, 1.6%) and CC1-MRSA-IV (1.1%,

183 2/190) (Table 2). The remaining eight MRSA genotypes were each represented by one isolate
184 only (Table 2).

185 Among the *pvl*-positive MSSA, CC30 was the dominant clone with 38.5% (15/39) of
186 isolates being assigned to this genotype (Table 2). CC22-MSSA accounted for 25.6% (10/39) of
187 MSSA isolates while CC121-MSSA, CC1-MSSA and CC88-MSSA accounted for 18% (7/39),
188 10.3% (4/39) and 7.7% (3/39) of isolates, respectively (Table 2)

189 **Temporal changes in the predominant clonal types of *pvl*-positive MRSA.** Figure 1
190 shows the percentage of *pvl*-positive MRSA isolates assigned to each genotype each year
191 between 2002 and 2011. Ten of the genotypes identified between 2006 and 2011 were not
192 identified between 2002 and 2005, including ST93-MRSA-IV, CC/ST59-MRSA-IV/V and
193 ST772-MRSA-V. The latter was identified for the first time in 2009 where it accounted for just
194 3.1% (1/32) of isolates but this increased to 28.6% (8/28) in 2010 and it was the predominant
195 genotype in 2011 accounting for 40% (16/40) of *pvl*-positive MRSA isolates (Fig. 1).

196 The CC/ST30-MRSA-IV clone predominated and was at its most prevalent in 2004 when
197 it accounted for 70% of *pvl*-positive MRSA isolates (7/10). Subsequently, the prevalence of
198 CC/ST30-MRSA-IV varied significantly each year between 2005 and 2011, accounting for
199 33.3% (3/9) of isolates in 2005 but just 5% (2/40) of isolates in 2011 (Fig. 1).

200 The CC/ST8-MRSA-IV clone predominated and was at its most prevalent in 2005 where
201 it accounted for 66.7% of isolates (6/9) but afterwards, the prevalence of this clone varied
202 dramatically each year between 2006 and 2011 e.g. despite a decrease to 33.3% (4/12) in 2006, a
203 rise in the prevalence of this clone was noted between this and 2009 to 46.9% (15/32) followed
204 by an overall decrease to 27.5% (11/40) in 2011 (Fig. 1).

205 Apart from 2002 when just one *pvl*-positive MRSA isolate was identified and was
206 assigned to CC80/ST80-MRSA-IV, the highest prevalence of this clone was in 2007 when it
207 accounted for 47.1% (8/17) of isolates. Subsequently the prevalence of this clone declined and by
208 2011 it accounted for just 2.5% of isolates (1/40) (Fig. 1).

209 Prior to 2008, just one *pvl*-positive ST22-MRSA-IV isolate was detected (2003). Despite
210 low numbers of isolates, an increase in the prevalence of *pvl*-positive ST22-MRSA-IV was noted
211 between 2009 (3.1%, 1/32) and 2011 (12.5%, 5/40) (Fig. 1). The first ST59/952-MRSA-V
212 isolates were detected in 2006 and a small number of isolates of this clone were subsequently
213 detected each year apart from 2010 (Fig. 1). The highest prevalence of this clone was in 2009
214 (12.5%, 4/32). Only three ST93-MRSA-IV isolates were identified, one in 2009 and two in 2011.
215 All other clones were represented by one or two isolates only (Fig. 1).

216 **Characteristics of *pvl*-positive *S. aureus* isolates.** The virulence and resistance gene profiles of
217 isolates identified within each genotype of *pvl*-positive MRSA and MSSA are shown in Table 2
218 and the main characteristics of isolates within lineages i.e. CCs, represented by more than one
219 isolate, are described below.

220 **CC1.** The majority of CC1/ST772-MRSA-V isolates exhibited *spa* type t657 (96%,
221 24/25) and all exhibited resistance to multiple antimicrobial agents including ciprofloxacin,
222 trimethoprim, erythromycin and aminoglycosides, the latter two encoded by *msr*(A)/*mph*(C) and
223 *aacA-aphD*/*aphA3*, respectively. The enterotoxins *sec* & *sel* and *egc* as well as the novel immune
224 evasion complex (IEC) type consisting of *scn* and *sea* were identified in all ST772-MRSA-V
225 isolates (41).

226 The other CC1 genotypes identified (CC1-MRSA-IV, CC1-MRSA-V and CC1-MSSA)
227 exhibited different *spa*, *agr*, capsule and IEC types to CC1/ST772-MRSA-V. The CC1-MRSA-V
228 isolate also exhibited resistance to multiple antimicrobial agents and carried multiple resistance
229 genes but apart from aminoglycoside resistance encoded by *aphA3* and *aacA-aphD*, these were
230 different to those detected in CC1/ST772-MRSA-V and included tetracycline resistance encoded
231 by *tet*(K) and *tet*(M) and fusidic acid resistance encoded by *fusC*. The two CC1-MRSA-IV
232 isolates carried less resistance genes with just one isolate carrying *tet*(K). The CC1-MRSA-IV/V
233 isolates lacked *egc* but various other enterotoxin genes were detected including *sea*, *sec* & *sel*, *sek*
234 & *seq* and *seh*.

235 Of the four CC1-MSSA isolates identified, two exhibited the same *spa* type, t127, as the
236 CC1-MRSA-V isolate. Multiple resistance genes were detected among these isolates including
237 *aphA3, fusC, ileS2* and *qacA* but for the latter two phenotypic resistance to mupirocin and
238 quaternary ammonium compounds was not detected. Similar toxin genes to those detected in
239 CC1-MRSA were detected among the CC1-MSSA isolates, namely *sea*, *sek* & *seq* and *seh*. In
240 fact, *seh* was unique to CC1 and was detected in all isolates except those belonging to ST772.

241 **CC5.** The two CC5 MRSA isolates identified, one with SCC*mec* IV and the other
242 SCC*mec* V, exhibited the same *spa*, *agr*, capsule and IEC type. Only the CC5-MRSA-V isolate
243 carried *dfrS1* and *tet*(K) and exhibited resistance to trimethoprim and tetracycline, respectively,
244 and both isolates carried *sea, egc* and the epidermolytic toxin gene *edinA*.

245 **CC8.** Although 12 *spa* types were identified among the CC/ST8-MRSA-IV isolates, t008
246 predominated (73.4%, 47/64). The majority of CC/ST8-MRSA-IV isolates exhibited resistance to
247 kanamycin and neomycin encoded by *aphA3* and erythromycin encoded by *msr*(A) and almost

248 half of the isolates were resistant to ciprofloxacin. Just under 10% of CC/ST8-MRSA-IV isolates
249 were tetracycline resistant and carried *tet*(K). One isolate carried *cfr* and *fexA* and exhibited
250 chloramphenicol and linezolid resistance (42). The majority of isolates carried the enterotoxin
251 genes *sek* and *seq* and the arginine catabolic mobile element (ACME) and, although they were
252 less common, *sed*, *sej* and *ser* were also identified.

253 The one remaining CC8-MRSA t008 isolate harbored SCC*mec* V and did not exhibit
254 resistance to multiple antimicrobial agents or harbor multiple resistance genes but *sek* & *seq* and
255 ACME were detected. ACME was only identified in one non CC/ST8-MRSA isolate (CC45-
256 MRSA-V).

257 **CC22.** The *spa* types t852 and t005 predominated among the CC22 MRSA (58.3%, 7/12)
258 and MSSA (70%, 7/10), respectively. Only one *spa* type, t005, was common to both CC22
259 MRSA and MSSA but only one t005 MRSA isolate was identified. Among the CC22-MRSA-IV
260 isolates resistance to aminoglycosides encoded by *aacA-aphD* and *aadD,* trimethoprim encoded
261 by *dfrS1*, erythromycin encoded by *erm*(C) and ciprofloxacin, were common. No ciprofloxacin
262 resistant CC22 MSSA were identified but they all exhibited aminoglycoside resistance encoded
263 by *aacA-aphD*, the majority were resistant to trimethoprim and carried *dfrS1* and one isolate
264 exhibited resistance to fusidic acid which was probably due to mutations in *fusA* as neither *fusB*
265 or *fusC* were detected. However, not all CC22 isolates harboring *aacA-aphD* and *aadD* exhibited
266 resistance to all of the appropriate aminoglycoside antimicrobial agents. All CC22 isolates carried
267 *egc* but no other toxin genes were detected.

268 **CC30.** The majority of CC/ST30-MRSA-IV isolates were assigned to *spa* type t019
269 (55%, 22/40) or t012 (30%, 12/40). Less than half of the isolates were resistant to fusidic acid

encoded by *fusC* and resistance to tetracycline and trimethoprim encoded by *tet*(K) and *dfrS1*, respectively, was detected in just one isolate each. All CC/ST30-MRSA-IV isolates carried *egc* and 35% (14/40) carried the toxic shock toxin gene *tst* with just two isolates harboring *sea*.

Among the CC30 MSSA *spa* types t021 (40%, 6/15) and t318 (26.7%, 4/15) predominated. The latter *spa* type (t318) was the only common *spa* type detected among CC30 MRSA and MSSA but was only detected in one CC30-MRSA isolate. While no fusidic acid resistance phenotype or genes were detected among the CC30-MSSA, resistance to tetracycline, trimethoprim and erythromycin encoded by *tet*(K), *dfrS1* and *mph*(C), respectively, was identified in one or two isolates each. CC30-MSSA isolates carried the greatest range of toxin genes i.e. the enterotoxin genes *sek* & *seq*, *egc*, *sea* and *sec* & *sel* and *tst*, but apart from *egc*, which was detected in the majority of CC30 MSSA, each of these were found in one or two CC30-MSSA isolates only. Overall, *tst* was unique to CC30 isolates.

CC59. All ST59/952-MRSA-V isolates exhibited a single *spa* type, t437, and the majority of isolates exhibited resistance to multiple antimicrobial agents and carried multiple resistance genes with erythromycin and lincomycin resistance encoded by *erm*(B), kanamycin and neomycin resistance encoded by *aphA3* and chloramphenicol resistance encoded by *cat*-pC223. Tetracycline resistance encoded by *tet*(K) was also common among these isolates. All ST59/952-MRSA-V isolates carried the enterotoxin genes *seb* and *sek* & *seq*.

The one ST59-MRSA-IV isolate identified carried less resistance genes but *aphA3* and *erm*(B) encoding resistance to aminoglycosides and erythromycin, respectively, were detected. Similar to the ST59/952-MRSA-V isolates, the ST59-MRSA-IV isolate carried *seb* and *sek* & *seq* but *sea* was also detected.

CC80. The majority of CC/ST80-MRSA-IV isolates exhibited *spa* type t044 (77.8%, 21/27). All isolates exhibited resistance to kanamycin and neomycin encoded by *aphA3*. Resistance to tetracycline, fusidic acid and erythromycin encoded by *tet*(K), *fusB* and *erm*(C), respectively, were also common. However, for a small number of isolates, *tet*(K) and *fusB* were identified but the appropriate resistance phenotype was not detected. Chloramphenicol and trimethoprim resistance encoded by *cat*-pC221 and *dfrS1*, respectively, as well as ciprofloxacin resistance were detected in just one isolate each. All CC/ST80-MRSA-IV isolates harbored the exfoliative toxin gene *etD* and the epidermolytic toxin *edinB*, which were identified in just one other isolate (ST152-MRSA-V).

CC88. Just three CC88 isolates, all MSSA, were identified and were assigned to two *spa* types. These isolates were the only isolates found to harbor IEC type F (*sep*, *sak*, *chp* and *scn*). Two isolates exhibited tetracycline resistance and carried *tet*(K) with just one isolate each exhibiting resistance to erythromycin and trimethoprim encoded by *erm*(C) and *dfrS1*, respectively. The enterotoxin genes *sek* & *seq* were detected in one CC88-MSSA isolate.

ST93. The three ST93-MRSA-IV isolates each exhibited a different *spa* type. Erythromycin resistance encoded by *msr*(A) and *mph*(C) was detected in just one isolate. The *qacC* gene was also detected in one isolate but the isolate did not exhibit resistance to ethidium bromide. The enterotoxin gene homolog *CM14* was the only toxin gene detected among ST93-MRSA-IV isolates.

CC121. All CC121 isolates identified were MSSA and the majority exhibited *spa* type t159 (71.4%, 5/7). Only CC121-MSSA isolates exhibited *agr* type IV. Just over half of the isolates exhibited erythromycin resistance encoded by *erm*(C) and tetracycline, trimethoprim and

chloramphenicol resistance encoded by *tet*(K), *dfrS1* and *cat*-pC221, respectively, were also detected among CC121 MSSA. All CC121-MSSA isolates harbored *egc* and *CM14* and just over half also carried *seb*.

Multiresistant *pvl*-positive MRSA. Multiresistance was identified among MRSA isolates only and was defined as phenotypic resistance to three or more classes of commonly used antimicrobial agents tested including fluoroquinolones (ciprofloaxacin), aminoglycosides (gentamicin/kanamycin/neomycin/tobramycin), macrolides/lincosamides (erythromycin-/lincomycin), tetracyclines, fusidic acid and mupirocin (22). Using this criterion, 43.7% (83/190) of *pvl*-positive MRSA isolates were multiresitant. These multiresistant *pvl*-positive MRSA isolates were assigned to six genotypes, with the majority belonging to CC/ST8-MRSA-IV (30.1%, 25/83), ST772-MRSA-V (30.1%, 25/83), and CC/ST80-MRSA-IV (25.3%, 21/83) with a small number of multiresistant isolates also belonging to CC/ST22-MRSA-IV (7.2%, 6/83), ST59/952-MRSA-V (6%, 5/83) and CC1-MRSA-V (1.2%, 1/83) (supplemental Fig. S1). An increase in the prevalence of multiresistant *pvl*-positive MRSA was observed between 2004 (10%, 1/10) and 2007 (59%, 10/17) ($p < 0.02$) and despite a decline in 2008 (24.3%, 9/37), this again increased between 2008 and 2011 to 65% (26/40) ($p < .001$) (Fig. 1). In fact, the highest prevalence of multiresistance among *pvl*-positive MRSA isolates was observed in 2011 and this was predominantly associated with isolates within ST772-MRSA-V (61.5%, 16/26) and to a lesser extent, CC/ST8-MRSA-IV (19.2%, 5/26) CC/ST22-MRSA-IV (11.5%, 3/26), ST59-MRSA-V (3.8%, 1/26) and CC1-MRSA-V (3.8%, 1/26).

Patient demographics. Of the 229 isolates investigated, 216 (94.3%) were from patients, nine from healthcare staff (3.9%) and four (1.8%) from environmental sources. Information pertaining

to whether the *S. aureus* isolates were from patients based in the community or in hospitals were available for 175/216 isolates, 69.7% (122/175) of whom were based in the community.

Sex and age. Gender data was available for patients from whom 189 isolates were recovered, of which, 52.4% (99/189) were from females (Table 3). There was no significant difference between the genders of patients associated with *pvl*-positive MRSA and MSSA isolates with 52.2% (83/159) and 47.8% (76/159) of MRSA being associated with females and males, respectively, and 53.3% (16/30) and 46.7% (14/30) of MSSA being associated with females and males, respectively. The ages of patients from whom *pvl*-positive *S. aureus* was recovered ranged from <1 month to 98 years and the median age was 30 years (age data was available for 193 patients). Seventy-one percent (136/193) of isolates were from patients who were <40 years of age (Table 3).

Isolate clusters. No clusters were identified among *pvl*-positive MSSA but seven isolate clusters were identified among *pvl*-positive MRSA, either from two or more members of one family/household or within a hospital or both (Table 3). Within each cluster isolates were recovered between three months and two years apart and isolates were represented by a single genotype with indistinguishable *spa* and DNA microarray profiles in each case. ST772-MRSA-V accounted for almost half of all cluster-associated isolates identified (48.6%, 17/35) (Table 3).

International travel or country of origin outside of Ireland. Thirty-five individuals from whom *pvl*-positive isolates were recovered were known to have recently travelled internationally or had a country of origin outside of Ireland (Table 3). Recent travel ranged from two weeks to one year prior to the recovery of the *pvl*-positive *S. aureus* isolate, but for the majority of patients the time period since travel was not defined. The genotypes most commonly

associated with international travel or country of origin outside of Ireland were ST8-MRSA-IV (seven isolates), ST772-MRSA-V (six isolates) and ST30-MRSA-IV (five isolates) (Table 3). The ST8-MRSA-IV and ST30-MRSA-IV isolates were identified from patients with links to multiple regions worldwide, while the ST772-MRSA-V isolates were exclusively associated with India (Table 3).

Overall the most common travel destination or region of origin for patients with *pvl*-positive *S. aureus* was Asia **(**15 isolates**)** followed by Africa (six isolates) and the USA (4 isolates) (Table 3).

Clinical presentations. Clinical data was available for 159 isolates (135 MRSA and 24 MSSA) (supplemental Fig. S2). The most common infections were SSTIs (60.4%, 96/159) including unspecified SSTIs, abscesses, boils, furuncles, bursitis, folliculitis, sinusitis, eye and ear infections, inguinal lymphadenitis and wound infections. SSTIs were associated with isolates from all except three genotypes, CC1-MSSA (n = 4), ST152-MRSA-V (n =1) and ST59-MRSA-IV (n = 1). More serious manifestations were also identified including BSIs (10.7%, 17/159 isolates belonging to ST59-MRSA-IV, ST59/952-MRSA-V, ST30-MRSA-IV, ST22-MRSA-IV, ST8-MRSA-IV, CC1-MRSA-IV, ST772-MRSA-V and CC30-MSSA), pneumonia (3.1%, 5/159 isolates belonging to CC30-MSSA, ST772-MRSA-V, CC/ST8-MRSA-IV and CC/ST80-MRSA-IV), osteomyelitis (1.3%, 2/159 isolates; CC121-MSSA and CC1-MSSA), necrotizing pneumonia (1.3%, 2/159 isolates; CC/ST8-MRSA-IV and CC1 MSSA), necrotizing fasciitis (0.6%, 1/159, CC30-MSSA) and endocarditis (0.6%, 1/159 isolates, CC22/ST22-MRSA-IV). Thirty-one isolates were recovered during screening of patients (nose, throat and/or perineum sites) during hospital outbreaks or from persons with close contact with patients with *pvl*-positive *S. aureus*.

DISCUSSION

This study reports several novel findings in relation to *pvl*-positive MRSA including an increase in the prevalence and diversity of *pvl*-positive MRSA submitted to the NMRSARL between 2002 and 2011, and several temporal shifts in the predominant clonal types. A 44-fold increase in the prevalence of *pvl*-positive MRSA, from 0.2% to 8.8%, was observed between 2002 and 2011 (Fig. 1). While these findings may reflect a true increase in the prevalence of *pvl*-positive MRSA in Ireland over the last decade, enhanced clinical and laboratory awareness of *pvl* probably also contributed**.** A relatively low but increasing prevalence of *pvl*-positive MRSA has also been reported from Austria and Germany during the last decade (43, 44).

The polyclonal *pvl*-positive MRSA population structure identified in Ireland and in other European countries (21, 22, 43, 45) contrasts starkly with the USA and Australia where single epidemic *pvl*-positive clones predominate, specifically ST8-MRSA-IV/USA300 and ST93-MRSA-IV**,** respectively (17, 46). Many reasons have been proposed for this difference between the USA and Europe including environmental, host, social, economic and cultural factors (2, 21). However, direct evidence for these is somewhat lacking and many of the risk factors identified for *pvl*-positive MRSA/CA-MRSA in the USA may also apply to various communities in Europe (2). In the present study, while such specific parameters were not investigated, six familial/household outbreaks of *pvl*-positive MRSA were identified. Furthermore, links between several *pvl*-positive *S. aureus* isolates and patients with recent foreign travel or ethnic origin outside of Ireland were also identified highlighting the continuing role of strain importation on the variety of *pvl*-positive MRSA strains in Ireland.

While the prevalence of different *pvl*-positive MRSA clones identified in the present study together with precise temporal shifts of predominant clones are unique to Ireland,

similarities and differences were noted in comparison with polyclonal *pvl*-positive MRSA populations observed in other European countries. For example, a decline in the incidence of the *pvl*-positive European Clone ST80-MRSA-IV has been noted recently across Europe in association with an increase in ST8-MRSA-IV/USA300 (21). In the present study, an increase in the prevalence of ST8-MRSA-IV/USA300 was observed between 2006 and 2009, and it predominated in 2008 and 2009, decreased in 2010 but it was the second most common clone in 2011, surpassed only by ST772-MRSA-V. The emergence of ST772-MRSA-V as the dominant *pvl*-positive MRSA clone in 2011 in Ireland reflects a similar situation in the UK where ST772-MRSA-V was the predominant multiresistant *pvl*-positive clone between 2005 and 2008 (22). The predominance of ST772-MRSA-V and ST8-MRSA-IV/USA300 among *pvl*-positive MRSA in Ireland is of concern, as both clones appear to be highly transmissible with a propensity to spread worldwide and to displace hospital strains (14, 20). In the present study ST772-MRSA-V was found in association with two separate hospital clusters and one familial cluster and ST8-MRSA-IV/USA300 with three family clusters and both of these strains were found to have been imported frequently into Ireland. In addition, genetic characteristics that may enhance the virulence or ability of these clones to spread have been identified in this and other studies including ACME and the enterotoxin genes *sek* & *seq* in ST8-MRSA-IV/USA300 and an *sea*- and *pvl*-encoding bacteriophage (41) as well as multiple other enterotoxin genes in ST772-MRSA-V (Table 2). Lastly, isolates belonging to both clones can exhibit multiresistance (22) and all ST772-MRSA-V and 38.5% of ST8-MRSA-IV/USA300 investigated here were multiresistant.

While the overall numbers of *pvl*-positive ST22-MRSA-IV isolates in this study were low, a four-fold increase was noted between 2009 and 2011 (Fig. 1). Worryingly, *pvl*-positive ST22-MRSA-IV has been associated with hospital and community outbreaks elsewhere (47-49)

427 and it now predominates together with ST772-MRSA-V in hospitals in India (20). Although *pvl*-
428 negative ST22-MRSA-IV is currently predominant in Irish hospitals (mainly *spa* type t032 (28)),
429 in the present study *pvl*-positive ST22-MRSA-IV were genetically distinct (*spa* type t852)
430 indicating the independent evolution of these strains.

431 CC/ST30-MRSA-IV was the second most common *pvl*-positive MRSA clone identified
432 accounting for 21.1% of all isolates during the study period and predominating several times
433 (Fig. 1). Isolates of this pandemic clone are also common in the UK and have been associated
434 with a hospital outbreak where the probable index case was a staff member who had recently
435 travelled to the Philippines (24, 50, 51). In the present study a link between travel to, or ethnic
436 origin in Asia or Africa was identified for several CC/ST30-MRSA-IV isolates emphasizing the
437 role of travel in its spread. CC/ST30-MRSA-IV isolates were also associated with two familial
438 outbreaks, indicating its propensity to spread. In the present study the prevalence of CC/ST30-
439 MRSA-IV declined from 70% to 0% between 2004 and 2006 and from 28.6% to 5% between
440 2010 and 2011 (Fig. 1). A decline in the prevalence of this once predominant clone among CA-
441 MRSA was also recently reported in New Zealand where it was replaced by *pvl*-negative ST5-
442 MRSA-IV (52). It is now well established that not all CA-MRSA carry *pvl*. In the present study,
443 70% of *pvl*-positive *S. aureus* isolates for which information was available were from patients in
444 the community indicating that CA *S. aureus* have emerged as a significant problem in Ireland.
445 However, the true burden of CA *S. aureus* infections in Ireland will only be fully understood
446 when *pvl*-negative and *pvl*-positive CA *S. aureus* isolates are investigated systematically together
447 with detailed epidemiological information.

448 The diversity of *pvl*-positive MRSA clones increased in the second half of the study
449 period with 10/16 MRSA genotypes identified for the first time between 2006 and 2011 including

450 CC/ST59-MRSA-V, ST93-MRSA-IV and ST772-MRSA-V. Links between several isolates of
451 these clones and the regions where they predominate were also noted. Although an increase in the
452 Taiwan clone (CC/ST59-MRSA-V), from 8.3% in 2006 to 12.5% in 2009, was observed, the
453 number of isolates recovered each year remained low throughout (between one and four isolates
454 each year). CC/ST59-MRSA-V is among the predominant CA-MRSA clones in some Northern
455 European countries (21). In contrast, similar to the present study, ST93-MRSA-IV has only been
456 reported sporadically in Europe (21, 53) but it is the dominant *pvl*-positive MRSA strain in
457 Australia where it has spread into healthcare facilities (54).

458 Increasing reports of outbreaks of *pvl*-positive MRSA, particularly in NICUs highlights
459 the ability of these strains to spread among vulnerable patient groups in hospitals (24, 25, 47, 48).
460 In the present study two NICU clusters in separate hospitals were due to the recently emerged
461 *pvl*-positive multiresistant ST772-MRSA-V clone. In fact, 30% of *pvl*-positive isolates for which
462 information was available were from patients in hospitals, a situation that requires close
463 monitoring so that *pvl*-positive MRSA does not become widespread in Irish hospitals.

464 Despite a decrease in 2008, an overall six-fold increase in the prevalence of multiresistant
465 *pvl*-positive MRSA was identified between 2004 and 2011 (Fig. 1). Similarly, a 12.3-fold
466 increase in the prevalence of multiresistant *pvl*-positive MRSA was noted in the UK between
467 2006 and 2008 (22). Both in Ireland and the UK, this was largely due to the emergence and
468 predominance of ST772-MRSA-V and, in Ireland only, the continued prevalence of ST8-MRSA-
469 IV/USA300. Also, of concern is the high prevalence of ciprofloxacin resistance identified among
470 multiresitant *pvl*-positive MRSA isolates (67.1%). All of these findings highlight how non-
471 multiantibiotic resistance and susceptibility to ciprofloxacin can no longer be considered to be
472 reliable markers for *pvl*-positive MRSA.

473 This study has, for the first time, provided important insights into the molecular
474 epidemiology of *pvl*-positive MSSA in Ireland. The prevalence of *pvl*-positive MSSA decreased
475 8-fold from 20% in 2004 to 2.5% in 2011, and accounted for just 17% of all *pvl*-positive isolates
476 identified during the study period. In contrast, in the UK the prevalence of *pvl*-positive MSSA
477 increased 9-fold between 2005 and 2010 accounting for 61.5% of all *pvl*-positive *S. aureus* in
478 2009 (26) and in Africa *pvl*-positive MSSA are also common with 57% of MSSA isolates in one
479 study being identified as *pvl*-positive (27). However, MSSA isolates are not routinely referred to
480 the Irish NMRSARL and the number of MSSA isolates referred each year was low (Table 1).
481 Additional studies are required in order to determine the true burden of *pvl*-positive MSSA in
482 Ireland.

483 The results of this study also suggest that the importation of *pvl*-positive MRSA strains is
484 more significant than the local emergence of *pvl*-positive MRSA from *pvl*-positive MSSA with
485 just 1.6% (3/189) of MRSA isolates exhibiting the same *spa* type as MSSA isolates. Due to the
486 greater abundance of these *spa* types among *pvl*-positive MSSA it is reasonable to speculate that
487 this small number of *pvl*-positive MRSA may have evolved from the *pvl*-positive MSSA by the
488 acquisition of SCC*mec* rather than loss of SCC*mec* by MRSA, although both alternatives are
489 possible.

490 Similar to a recent UK study, CC22 and CC30 were the most common *pvl*-positive
491 MSSA clones identified accounting for 64.1% of isolates (26). While not reported previously
492 from the UK (26), the CC121-MSSA clone that accounted for 17.9% of *pvl*-positive MSSA
493 isolates in the present study is a pandemic clone (55, 56). Interestingly, a link to Africa and the
494 Far East was noted for 2/7 CC121 MSSA isolates where this clone has been shown to dominate
495 (56, 57). CC88-MSSA accounted for just 7.6% of *pvl*-positive MSSA isolates and have been

reported previously from India (58) but isolates of this lineage are more commonly reported as MRSA with SCC*mec* IV, particularly in Africa (19).

In conclusion, while this study has highlighted the changing molecular epidemiology of *pvl*-positive MRSA and MSSA in Ireland over the last decade it is clear that the actual burden of *pvl*-positive and CA *S. aureus* infections in Ireland may be even higher since this study investigated only *pvl*-positive isolates and only those submitted to the NMRSARL. There is a need for ongoing and systematic surveillance of *pvl*-positive and CA *S. aureus* infections in communities and hospitals in Ireland together with detailed epidemiological information in order to fully understand the burden of *S. aureus* infections that exists.

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TABLE 1 Numbers of *pvl*-positive MRSA and MSSA isolates identified each year between 2002 and 2011 by the Irish National MRSA Reference Laboratory

Year	No. of MRSA isolates			No. of MSSA isolates		
	Identified by NMRSARL	Investigated for *pvl*[a]	Confirmed as *pvl* positive (%)[b]	Identified by NMRSARL	Investigated for *pvl*[a]	Confirmed as *pvl* positive (%)[b]
2002	497	8	1 (0.2%)[c]	1	1	1 (100%)
2003	599	17	4 (0.7%)[c]	0	0	0 (0%)
2004	724	134	10 (1.4%)[c]	15	14	3 (20%)
2005	827	112	9 (1.1%)[c]	43	30	5 (11.6%)
2006	869	110	12 (1.4%)	41	31	5 (12.2%)
2007	782	120	17 (2.2%)	42	29	6 (14.3%)
2008	747	179	37 (5.0%)	58	53	7 (12.1%)
2009	605	187	32 (5.3%)[d]	44	35	5 (11.4%)
2010	596	160	28 (4.7%)[d]	77	61	5 (6.5%)
2011	456	190	40 (8.8%)[d]	81	61	2 (2.5%)
Total	6702	1217	190 (2.8%)	401	315	39 (9.7%)

[a]An isolate was selected for *pvl* investigation if it was from a suspected *pvl*-associated infection or, for MRSA only, if the isolate exhibited an antibiogram-resistogram (AR) and pulsed field group (PFG) pattern distinct from that of previously or currently predominant *pvl*-negative healthcare-associated MRSA clones e.g. AR-PFG 06-01, indicative of ST22-MRSA-IV or AR-PFG 13/14-00, indicative of ST8-MRSA-IIA-E +/-SCC_{MI}.

[b]The value shown in parenthesis indicates the percentage of *pvl*-positive MRSA or MSSA identified among the total number of MRSA or MSSA investigated by the National MRSA Reference Laboratory (NMRSARL) each year during the study period.

[c]The MRSA isolates recovered between 2002 and 2005 have been described previously (30). One MRSA isolate (E1760) from that study was excluded because *pvl* was not detected despite several attempts using PCR and DNA microarray profiling.

[d]One, eight, and nine *pvl*-positive isolates recovered in 2009, 2010 and 2011, respectively, were described previously (31).

TABLE 2. Phenotypic and genotypic typing data for *pvl*-positive MRSA (*n* = 190) and MSSA (*n* = 39) isolates identified by the Irish National MRSA Reference Laboratory between 2002 and 2011

CC [a]	Genotype (*n*)[a]		*spa* types (*n*)[b]	*agr* type[a]	Capsule type[a]	IEC type (*n*)[a, b, c]	Antibiotic resistance		Virulence genes (excluding *lukF/S-PV* which was detected in all isoates)
	MRSA	MSSA					Resistance phenotype (% indicated when not 100%)[d]	Resistance genotype (% indicated when not 100%)	(% indicated when not 100%)
1	CC1-MRSA-IV (2)		t128 (1), t8968 (1)	III	8	D (1), B (1)	AMP, CAD , TET (50%)	*blaZ*, *sdrM*, *tet*(K) (50%)	*sea* (50%), *sec* & *sel* (50%), *sek* & *seq* (50%), *seh*
	ST772-MRSA-V (25)		t657 (24), t345 (1)	II	5	Novel: *scn* & *sea*	AMP, AMI, CAD (88%), CIP, ERY, GEN, KAN, NEO, TOB, TMP	*blaZ*, *sdrM*, *msr*(A), *mph*(C), *aacA-aphD*, *aphA3*&*sat*, *fosB*	*sea, sec* & *sel, egc*
	CC1-MRSA-V (1)		t127	III	8	D	AMP, FUS, GEN, KAN, NEO, TOB, TET	*blaZ*, *sdrM*, *aacA-aphD*, *aphA3* & *sat*, *tet*(K), *tet*(M), *fusC*	*sea, sek* & *seq*, *seh*
		CC1-MSSA (4)	t127 (2), t177 (1), t12303 (1)	III	8	D	AMP, CAD (75%), FUS, KAN (25%), NEO (25%)	*blaZ*, *sdrM*, *aphA3* & *sat* (25%), *ileS2* (50%), *fusC, qacA* (50%)	*sea, sek* & *seq, seh*
5	CC5-MRSA-V (1)		t311	II	5	A	AMP, CAD, TET, TMP	*blaZ*, *sdrM*, *tet*(K), *dfrS1*, *fosB*	*sea, edinA, egc*
	CC5-MRSA-IV (1)		t311	II	5	A	AMP, CAD	*blaZ*, *sdrM*, *fosB*	*sea, edinA, egc*
8	CC/ST8-MRSA-IV (64)		t008 (47), t051 (4), 121 (3), t068 (2), t4229 (1), t304 (1), t024 (1), t681 (1), t4306 (1), t11157 (1), t596 (1), t1635 (1)	I	5	B (63), neg (1)	AMI (21.8%), AMP, CAD (20.3%) (I), CHL (1.5%), CIP (46.9%), ERY (84.3%), GEN (1.5%), KAN (81.3%), LIN (3.1%), MC (6.3%), MUP (1.5%), NEO (81.3%), PMA (1.5%), TOB (1.5%), TET, (9.4%) TMP (3.1%), LNZ (1.6%)	*blaZ* (90.6%), *sdrM*, *tet*(K) (9.4%), *lnu*(A) (3.1%), *msr*(A) (84.3%), *mph*(C) (84.3%), *aacA-aphD* (1.5%), *aphA3* & *sat* (81.3%), *fosB* (100%), *merA* & *merB* (6.3%), *qacC* (4.7%), *ileS2* (1.5%), *cfr* & *fexA* (1.5%), *dfrS1* (3.1%)	*sek* & *seq* (96.9%), *sed, sej* & *ser* (4.7%), ACME (89.1%)
	CC8-MRSA-V (1)		t008	I	5	B	AMP	*sdrM, fosB*	*sek* & *seq,* ACME
22	CC/ST22-MRSA-IV (12)		t852 (7), t2480 (1), t3107 (1), t4463 (1), t5422 (1), t005 (1)	I	5	B (11), neg (1)	AMI (25%), AMP, CAD (50%), CIP (75%), ERY (66.7%), GEN (25%), KAN (91.7%), NEO (33.3%), TOB (91.7%), TMP (66.7%)	*blaZ*, *aacA-aphD* (91.7%), *dfrS1* (66.7%), *erm*(C) (66.7%), *aadD* (75%)	*egc*
		CC22-	t005 (7), t891 (2), t1869	I	5	B	AMI (10%), AMP, CAD (60%), FUS	*blaZ*, *aacA-aphD, dfrS1* (90%)	*egc*

CC [a]	Genotype (*n*)[a]		*spa* types (*n*)[b]	*agr* type[a]	Capsule type[a]	IEC type (*n*)[a, b, c]	Antibiotic resistance		Virulence genes (excluding *lukF/S-PV* which was detected in all isoates)
	MRSA	MSSA					Resistance phenotype (% indicated when not 100%)[d]	Resistance genotype (% indicated when not 100%)	(% indicated when not 100%)
		MSSA (10)	(1)				(10%), GEN (80%), KAN, TOB (90%), TMP (90%)		
30	CC/ST30-MRSA-IV (40)		t019 (22), t012 (12), 3800 (2), t122 (1), t275 (1), t318 (1), t9904 (1)	III	8	B (37), A (2), neg (1)	AMP, CAD (82.5%), CIP (5%), FUS (35%), TET (2.5%), TMP (2.5%)	*blaZ*, *sdrM*, *tet*(K) (2.5%), *fosB*, *dfrS1* (2.5%), *fusC* (35%)	*sea* (5%), *tst* (35%), *egc*
		CC30-MSSA (15)	t021 (6), t318 (4), t990 (1), t3502 (1), t1055 (1), t11156 (1), t433 (1)	III	8	A (2), B (11), neg (3)	AMP (73.3%), CAD, CIP, ERY (6.7%), TET (13.3%), TMP (13.3%)	*blaZ* (73.3%), *mph*(C) (6.7%), *sdrM*, *tet*(K) (13.3%), *fosB*, *dfrS1* (13.3%)	*sea* (13.3%), *sec* & *sel* (6.7%), *sek* & *seq* (6.7%), *tst* (13.3%), *egc* (86.7%)
45	CC45-MRSA-V (1)		t620	I	8	B	AMP, TET	*blaZ*, *sdrM*, *tet*(K)	*sec* & *sel,* ACME*, egc*
59	ST59/952-MRSA-V (9)		t437	I	8	C	AMP, CAD (20%), CHL (88.9%), ERY (88.9%), KAN (88.9%), LIN (88.9%), NEO (88.9%), STR (80%), TET (60%)	*blaZ* (11.1%), *sdrM*, *tet*(K) (66.7%), *aphA3* & *sat* (88.9%), *erm*(B) (88.9%), *cat*-pC223 (88.9%)	*seb, sek* & *seq*
	ST59-MRSA-IV (1)		t437	I	8	A	AMP, ERY, KAN, LIN, NEO, STR	*blaZ*, *sdrM*, *aphA3* & *sat*, *erm*(B)	*sea, seb, sek* & *seq*
78 80	CC80/ST80-MRSA-IV (27)		t044 (21) t376 (5) t131 (1)	III	8	E	AMI (3.7%), AMP, CAD (85.2%), CHL (3.7%), CIP (3.7%), ERY (40.7%), FUS (74.1%), KAN, NEO, TET (77.8%), TMP (3.7%)	*blaZ* (77.8%), *sdrM*, *dfrS1* (3.7%), *tet*(K) (70.4%), *aphA3* & *sat*, *fusB* (77.8%), *erm*(C) (40.7%), *tet*(K) (70.4%), *cat*-pC221 (3.7%), *dfrS1* (3.7%)	*etD, edinB*
88		CC88-MSSA (3)	t186 (2) t448 (1)	III	8	F	AMP, CAD (66.7%), ERY (33.3%), TET (66.7%), TMP (33.3%)	*blaZ*, *sdrM*, *tet*(K) (66.7%), *erm*(C) (33.3%), *dfrS1* (33.3%)	*sek* & *seq* (33.3%), *sep*
93	ST93-MRSA-IV (3)		t3949 (1) t1819 (1) t202 (1)	III	8	B	AMP, CAD (66.7%), ERY (33.3%)	*blaZ*, *sdrM*, *msr*(A) (33.3%)*, mph*(C) (33.3%)*, qac*C (33.3%)	*CM14*
121		CC121-MSSA (7)	t159 (5), t435 (2)	IV	8	E	AMP, CAD (28.6%) (I), CHL (14.3%), ERY (57.1%), LIN (14.3%), STR (14.3%), TET (28.6%), TMP (28.6%)	*blaZ*, *sdrM*, *tet*(K) (28.6%), *fosB*, *erm*(C) (57.1%), *cat*-pC221 (14.3%), *dfrS1* (28.6%)	*seb* (57.1%), *egc, CM14*

CC [a]	Genotype (*n*)[a]		*spa* types (*n*)[b]	*agr* type[a]	Capsule type[a]	IEC type (*n*)[a, b, c]	Antibiotic resistance		Virulence genes (excluding *lukF/S-PV* which was detected in all isoates)
	MRSA	MSSA					Resistance phenotype (% indicated when not 100%)[d]	Resistance genotype (% indicated when not 100%)	(% indicated when not 100%)
152	ST152-MRSA-V (1)		t355	I	5	E	AMP, CAD, GEN, KAN, TOB	*blaZ*, *sdrM, aacA-aphD*	*etD* & *edinB*
154	ST154-MRSA-IV (1)		t667	III	8	Neg	AMP, CAD, CIP, SPC, TET	*blaZ*, *sdrM, tet*(M), *cat*-pMC524	None detected

[a]The StaphyType DNA microarray Kit (Alere Technologies) was used for assigning isolates to a multilocus sequence type (MLST) sequence type (ST) and/or a clonal complex (CC), a staphylococcal cassette chromosome *mec* (SCC*mec*) type (for MRSA only) and accessory gene regulator (*agr*), capsule and immune evasion complex (IEC) types. Forty-three MRSA isolates previously underwent MLST and SCC*mec* typing, namely 18 ST772-MRSA-V, two ST22-MRSA-IV, 11 ST30-MRSA-IV, eight ST8-MRSA-IV, two ST80-MRSA-IV, the one ST154-MRSA-IV and the one ST5-MRSA-IV isolates (30, 31).

[b]The number of isolates (*n*) represented by each *spa* type or IEC type are indicated in parenthesis only when more than one *spa* or IEC type was identified within a genotype.

[c]Immune evasion complex (IEC) types were defined as described previously (59): A= *sea*, *sak*, *chp* and *scn*; B = *sak, chp* and *scn*; C = *chp* and *scn*; D = *sea, sak* and *scn;* E = *sak* and *scn*; F = *sep, sak, chp* and *scn*; novel = novel IEC type consisting of *sak* and *sea* (41); neg (negative) = no IEC genes detected.

[d]The susceptibility of each isolate to a panel of 23 antimicrobial agents was determined by disk diffusion as described previously (30). The antimicrobial agents tested were amikacin (AMI), ampicillin (AMP), cadmium acetate (CAD), chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), ethidium bromide (ETBR), fusidic acid (FUS), gentamicin (GEN), kanamycin (KAN), lincomycin (LIN), mercuric chloride (MC), mupirocin (MUP), neomycin (NEO), phenyl mercuric acetate (PMA), rifampicin, spectinomycin (SPC), streptomycin (STR), sulphonamide, tetracycline (TET), tobramycin (TOB), trimethoprim (TMP) and vancomycin. The ST8-MRSA-IV *cfr*-positive isolate M05/0060 was tested for linezolid resistance (LNZ) as described (42).

791 TABLE 3. Patient demographics associated with *pvl*-positive MRSA and MSSA identified by
792 the Irish National MRSA Reference Laboratory between 2002 and 2011

Category	No. of isolates	Genotype[a]
Gender		
Male	90	n/a
Female	99	n/a
Data not available	36	n/a
Age		
Age range (yrs)		
0-9	35	n/a
10-19	19	n/a
20-29	40	n/a
30-39	42	n/a
40-49	23	n/a
50-59	11	n/a
60-69	9	n/a
70-79	7	n/a
80-89	4	n/a
90-99	3	n/a
Data not available	32	n/a
Clusters[b]		
Cluster 1 (Household)	4	CC/ST30-MRSA-IV
Cluster 2 (Household)	3	CC/ST30-MRSA-IV
Cluster 3 (Household)	4	CC/ST8-MRSA-IV
Cluster 4 (Household)	3	CC/ST8-MRSA-IV
Cluster 5 (Household)	4	CC80-MRSA-IV
Cluster 6 (Hospital)	6	ST772-MRSA-V
Cluster 7 (Hospital & household)[c]	11	ST772-MRSA-V
International travel to or country/region of origin		
USA	1	CC1-MRSA-IV
	3	ST8-MRSA-IV
Africa	1	ST8-MRSA-IV
	1	CC5-MRSA-IV
	2	ST30-MRSA-IV
	1	CC121-MSSA
	1	CC5-MRSA-V
Asia-The Middle East	1	ST80-MRSA-IV
Asia-India	1	ST772-MRSA-V
	5	ST772-MRSA-V
Asia-The Far East	1	ST80-MRSA-IV
	1	ST154-MRSA-IV
	3	ST30-MRSA-IV
	1	CC121-MSSA
	1	ST59-MRSA-V
	1	ST8-MRSA-IV
Australia	1	CC22-MRSA-IV
	1	ST93-MRSA-IV
New Zealand	1	ST8-MRSA-IV
South America-Brazil	1	ST8-MRSA-IV
Europe-the Czech Republic	1	CC1-MRSA-IV
Undefined (outside of Ireland)	2	ST59/952-MRSA-V
	1	ST22-MRSA-IV
	1	CC22-MSSA
	1	CC30-MSSA

[a]CC, MLST clonal complex; ST, sequence type.

[b]Isolates were defined as clusters if they were recovered from members of the one family/household, or within a hospital or both. Within each cluster isolates were recovered between three months and two years apart. Each isolate within a cluster was recovered from a different person or environmental source.

[c]The 11 *pvl*-positive ST772-MRSA-V isolates in cluster 7 have been described previously (31).

821 **Figure legend**

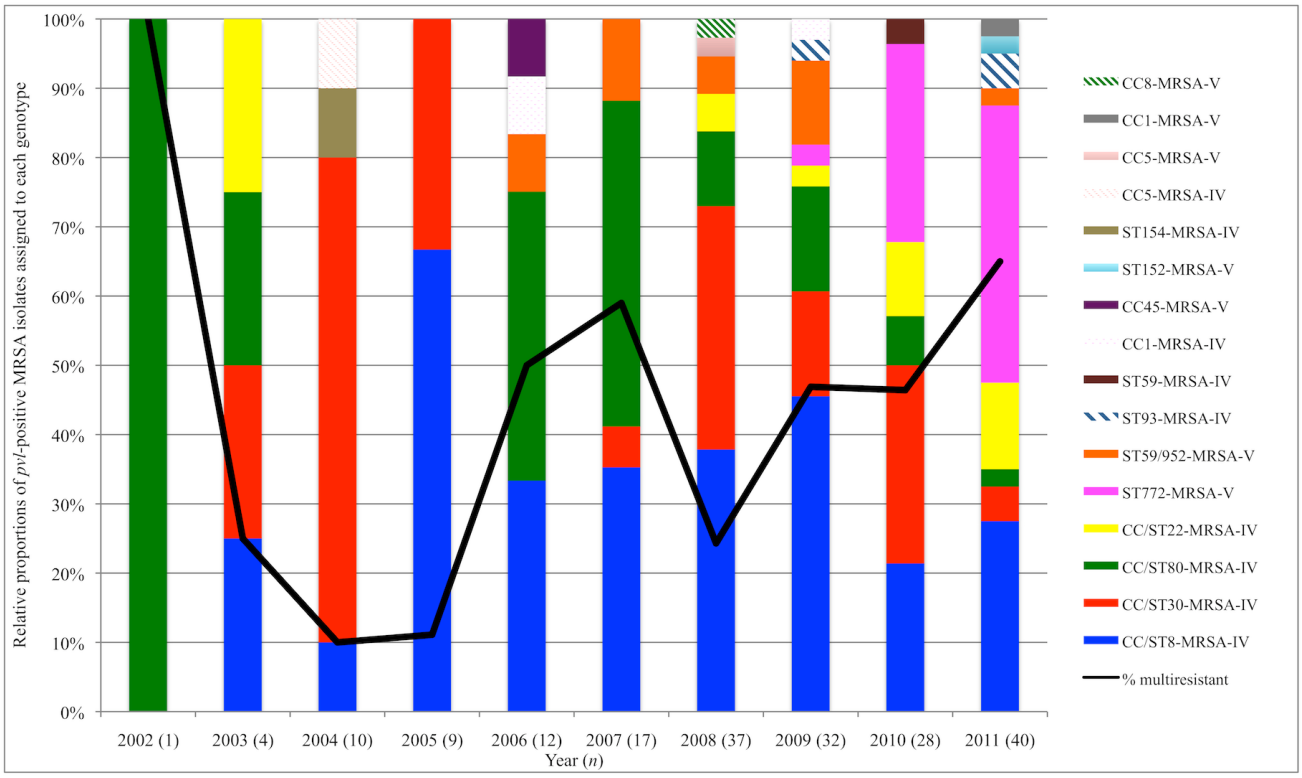
822

823 FIG 1. The relative proportions of the 190 *pvl*-positive MRSA isolates identified by the Irish National
824 MRSA Reference Laboratory between 2002 and 2011 assigned to each genotype each year between
825 during the study period and the annual percentage of these MRSA isolates that exhibited
826 multiresistance during this time period. Multiresitant MRSA were defined as those exhibiting
827 resistance to three of more classes of commonly used antimicrobial agents including fluorquinolones,
828 aminoglycosides, macrolides/lincosamides, tetracyclines, fusidic acid and mupirocin (22). Numbers in
829 parenthesis (*n*) indicate the number of *pvl*-positive MRSA isolates identified each year.

830

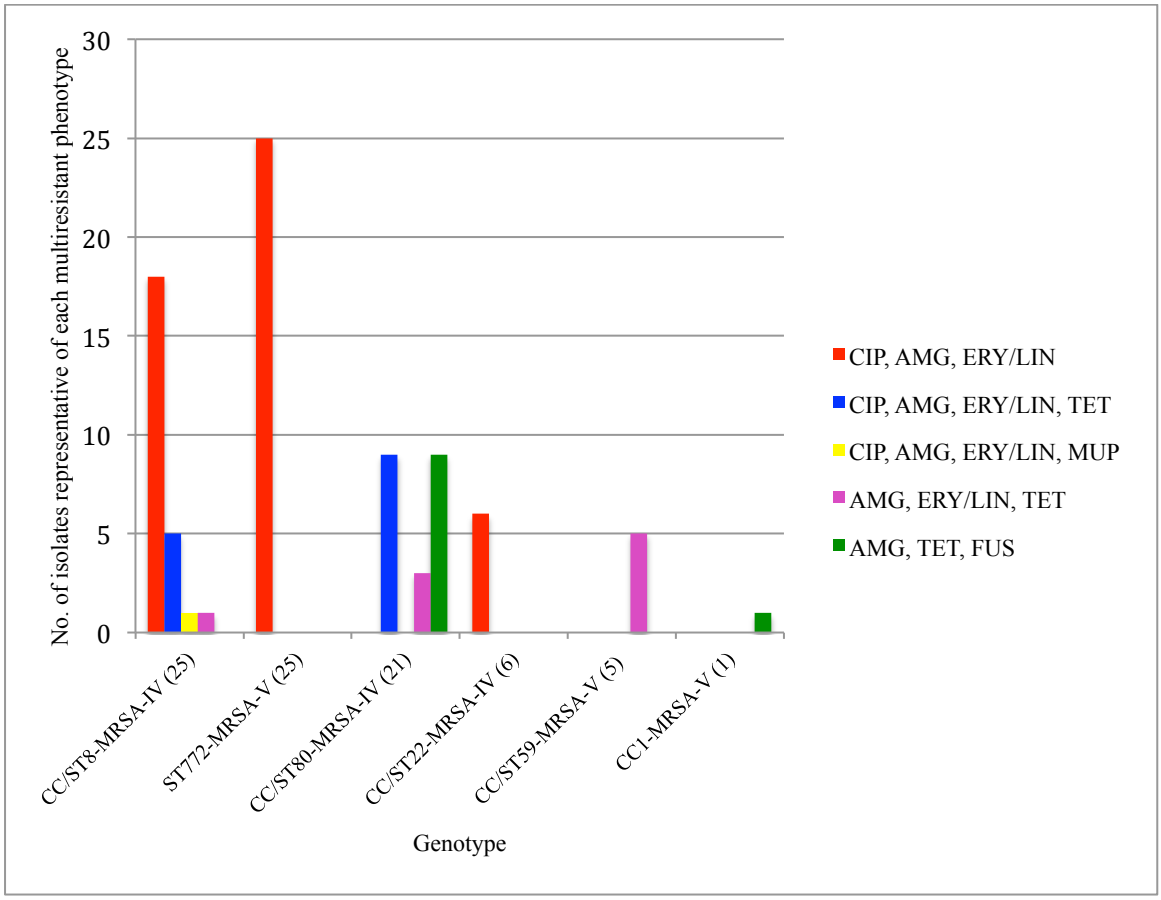
831

832

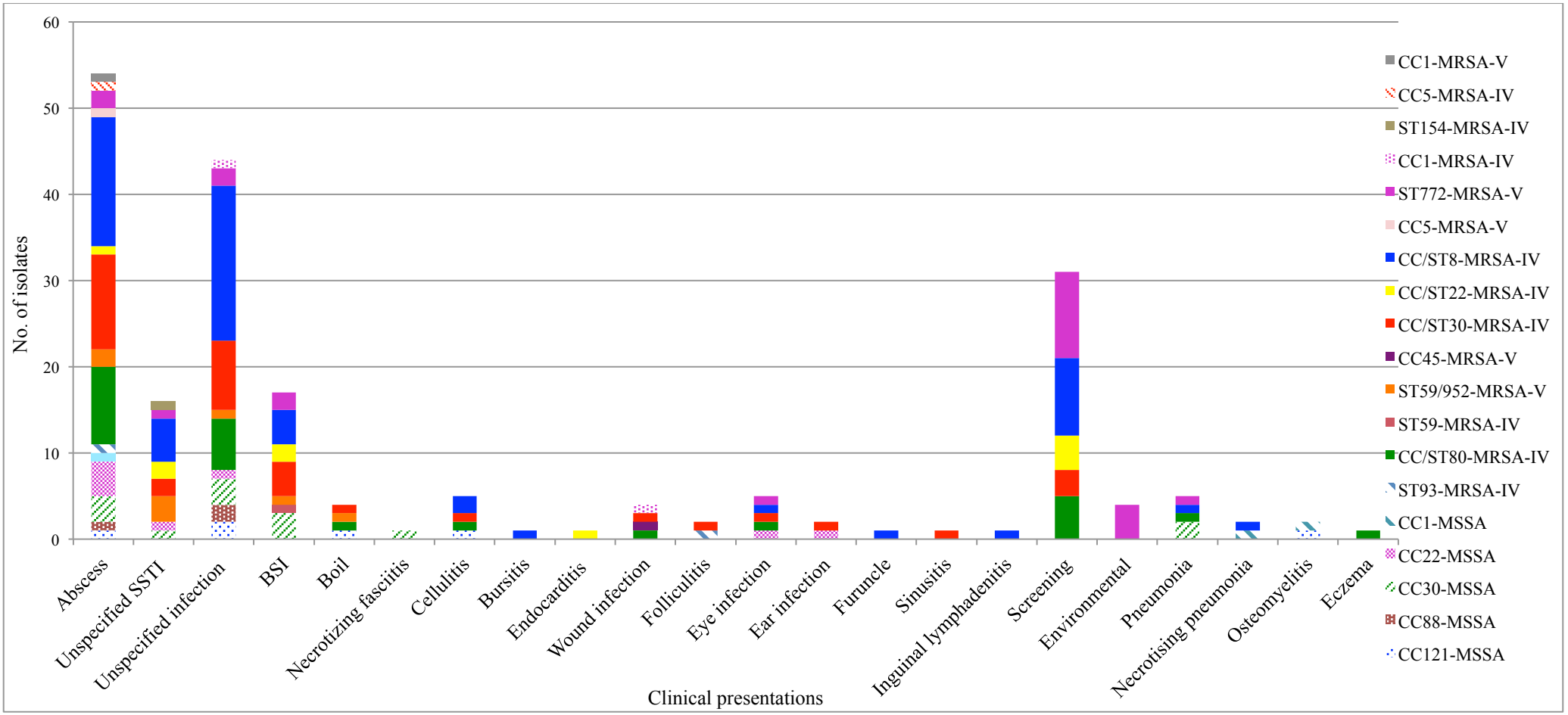

Relative proportions of pvl-positive MRSA isolates assigned to each genotype
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2002 (1)
2003 (4)
2004 (10)
2005 (9)
2006 (12)
2007 (17)
2008 (37)
2009 (32)
2010 (28)
2011 (40)
Year (n)
CC8-MRSA-V
CC1-MRSA-V
CC5-MRSA-V
CC5-MRSA-IV
ST154-MRSA-IV
ST152-MRSA-V
CC45-MRSA-V
CC1-MRSA-IV
ST59-MRSA-IV
ST93-MRSA-IV
ST59/952-MRSA-V
ST772-MRSA-V
CC/ST22-MRSA-IV
CC/ST80-MRSA-IV
CC/ST30-MRSA-IV
CC/ST8-MRSA-IV
% multiresistant

Supplemental Table S1. Novel PCR primers used in the present study

Antimicrobial resistance gene target	Primer name	Nucleotide sequence (5'-3')	Product size (bp)	Genbank accession no.
qacC	qacCF	CCATACCGATTTCAATGATTCCTT	525	Y16944.1
	qacCR	GCATGATGAAGCTGTAAGGC		
msr(A)	msrAF	GCACTTATTGGAGGTAATGGTACTGGC	503	AB013298.1
	msrAR	TGACGTTGTTGTTCTAACTGTTCTT		
dfrS1	dfrS1F	GTCGCTCACGATAAACAAAGAGT	160	AB049452.1
	dfrS1R	TACGTCTATTTGGCAATGGCTTCCC		
lnu(A)	lnuAF	AAGTTGAGCTTCTTTGGAAATGC	345	AM184101.1
	lnuAR	ACTCATTGGTTAGATGGAGGC		
mph(C)	mphCF	ATCAATTACACATCCAACCTCAAAC	348	AB013298.1
	mphCR	CGAGTGTTTCAGCTAATGTGTTAAT		
blaZ	blaZF	TTCAAACAGTTCACATGCCAAAGAG	384	AB074882.1
	blaZR	AGAACCGTTTGCTGTATTATCAC		



Supplemental Fig. S1. Multiresistant phenotypes detected among *pvl*-positive MRSA. Eighty-three of the 190 *pvl*-positive MRSA isolates exhibited multiresistance (43.7%) i.e. exhibited resistance to three or more classes of clinically used antimicrobial agents tested including fluoroquinolones (ciproxfloxacin, CIP), aminoglycosides (AMG), erythromycin/lincomycin (ERY/LIN), tetracycline (TET), mupirocin (MUP) and fusidic acid (FUS). Numbers (*n*) in parenthesis indicate the number of multiresistant isolates within each genotype.



Supplemental Fig. S2. Clinical details for each genotype identified among the *pvl*-positive MRSA and MSSA isolates investigated. Clinical details were available for 170 MRSA and 32 MSSA isolates. Abbreviations: SSTI, skin and soft tissue infection; BSI, bloodstream infection.