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Panton-Valentine Leukocidin-Positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 Clones, Frequent Importation of Clones, Temporal Shifts of Predominant Methicillin-Resistant *S. aureus* Clones, and Increasing Multiresistance

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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-PV* and *lukS-PV*. This study investigated the clinical and molecular epidemiologies of *pvl*-positive MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates 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), 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% to 8.8%, and that of *pvl*-positive MSSA decreased from 20% to 2.5% during the study period. The *pvl*-positive MRSA and MSSA isolates belonged to 16 and 5 genotypes, respectively, with CC/ST8-MRSA-IV, CC/ST30-MRSA-IV, CC/ST80-MRSA-IV, CC1/ST772-MRSA-V, CC30-MSSA, CC22-MSSA, and CC121-MSSA predominating. Temporal shifts in the predominant *pvl*-positive MRSA genotypes and a 6-fold increase in multiresistant *pvl*-positive MRSA genotypes occurred during the study period. An analysis of patient data indicated that *pvl*-positive *S. aureus* strains, especially MRSA strains, had been imported into Ireland several times. Two hospital and six family clusters of *pvl*-positive MRSA were identified, and 70% of the 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* isolates into Ireland.

Usually, methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be a health care-associated (HCA) pathogen, and it is 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. Health care-associated MRSA isolates have been found to belong to five distinct clonal lineages, typically harbor the staphylococcal cassette chromosome *mec* (SCC*mec*) type I, II, or III, (or less frequently, SCC*mec* type 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, who exhibit none of the HCA risk factors (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 in many different populations, particularly those in 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*, which are carried on a variety of lysogenic bacteriophages (9). While outbreaks of PVL-producing methicillin-susceptible *S. aureus* (MSSA) isolates were reported in the 1950s and 1960s (10), PVL was first reported in newly emerging CA-MRSA strains in the 1990s (4, 11). While not all CA-MRSA isolates produce PVL, and there are conflicting data regarding the role of PVL in the pathogenesis of CA-MRSA infection, it is clear that the success of some CA-MRSA clones is associated with PVL, albeit not exclusively (12).

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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

yr	MRSA isolates			MSSA isolates		
	No. identified by NMRSARL	No. investigated for *pvl*[a]	No. (%) confirmed *pvl* positive[b]	No. identified by NMRSARL	No. investigated for *pvl*[a]	No. (%) confirmed *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	6,702	1,217	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 health care-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_{M1}.

[b] The values shown in parentheses indicate the percentages of *pvl*-positive MRSA or MSSA isolates identified among the total number of MRSA or MSSA isolates investigated by the NMRSARL each year during the study period.

[c] The MRSA isolates recovered between 2002 and 2005 were 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).

MRSA isolates carrying the PVL toxin genes (*pvl*) are predominantly genetically distinct from HCA-MRSA, as they belong to more diverse clonal lineages and harbor the smaller SCC*mec* elements type IV, V, or V_T, and they are frequently not multiresistant (1, 3, 13). Different *pvl*-positive MRSA clones predominate in different regions, e.g., sequence type 8 (ST8)-MRSA-IV (USA300) in the United States (14), ST59-MRSA-V_T in Asia (13, 15), ST30-MRSA-IV in New Zealand (16), ST93-MRSA-IV in Australia (17), ST80-MRSA-IV in Europe (18) and the Middle East (1), ST88-MRSA-IV in Africa (19), and ST22-MRSA-IV and ST772-MRSA-V in India (20). However, recent studies highlighted the complex and changing epidemiology of *pvl*-positive MRSA, including (i) considerable variation in the prevalence rates of *pvl*-positive MRSA in different regions of the world (2, 17), (ii) the increasing prevalence and polyclonal population structure of *pvl*-positive MRSA isolates in Europe (1, 21, 22), (iii) the increasing prevalence of ST8-MRSA-IV in Europe and the decreasing prevalence of ST80-MRSA-IV (21), (iv) the increasing prevalence of multiresistant *pvl*-positive MRSA (22), and (v) the spread of *pvl*-positive MRSA into hospitals (14, 23–25). Furthermore, there has been an increasing frequency of reports of infections associated with *pvl*-positive MSSA (26, 27) that produce similar clinical presentations as *pvl*-positive MRSA, and the former are a potential reservoir for the emergence of *pvl*-positive MRSA.

In Ireland, MRSA is endemic in hospitals, and since 2002, the *pvl*-negative ST22-MRSA-IV clone has accounted for 70 to 80% of MRSA from bloodstream infections (BSIs) each year (28, 29). Between 1999 and 2005, a prevalence rate of 1.8% was reported for *pvl*-positive MRSA in Ireland, and six distinct *pvl*-positive MRSA clones (ST30, ST8, ST22, ST80, ST5, and ST154, all harboring SCC*mec* IV) were identified, some of which were probably imported (30). In 2011, 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 only a single report of a familial outbreak of *pvl*-positive MSSA in Ireland; no molecular epidemiological typing of the isolates was undertaken (32). The purpose of the present study was to investigate the clinical and molecular epidemiologies 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 7,103 *S. aureus* isolates (6,702 MRSA and 401 MSSA) between 2002 and 2011, of which 1,531 were examined for the presence of the *lukF-PV* and *lukS-PV* genes (*pvl*) (Table 1). An isolate was selected for *pvl* investigation if it was recovered from a suspected *pvl*-associated infection; for MRSA only, an isolate was selected if it exhibited an antibiogram-resistogram (AR) pattern and/or pulsed-field group (PFG) distinct from that of previously or currently predominant *pvl*-negative health care-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_{M1} (28, 33). Of the 1,532 isolates investigated for *pvl* (1,217 MRSA and 315 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. When possible, patient demographics and clinical data were collected from isolate submission forms, telephone follow-ups, and follow-up questionnaires. The isolates were defined as clusters if they were recovered from members of one family/household, within a hospital, or both. Within each cluster, the isolates were recovered between 3 months and 2 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-PV* and *lukS-PV* genes. On receipt by the NMRSARL, all *S. aureus* isolates were inoculated onto Protect beads (Technical Service Consultants Ltd., Heywood, United Kingdom) and stored at −70°C prior to subsequent investigation. The isolates were confirmed to be *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-PV* and *lukS-PV* genes was performed by PCR, as described previously (4); isolates recovered in the final quarter of 2011 were tested 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-PV* and *lukS-PV* genes were 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 multilocus sequence typing (MLST) (31). The 24 previously described *pvl*-positive MRSA isolates recovered between 2002 and 2005 included in the study underwent previous antimicrobial susceptibility, MLST, 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, rifampin, spectinomycin, streptomycin, sulfonamide, 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-PV*, *lukS-PV* and *mecA*), and SCC*mec*-associated genes and typing markers allowing isolates to be assigned to MLST sequence types (STs) and/or clonal complexes (CCs), and 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 for which 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), *aadD* (39), and *qacA* (40), and also novel primers to detect *qacC*, *msr*(A), *dfrS1*, *lnu*(A), *mph*(C), and *blaZ* (see Table S1 in the supplemental material).

Statistical analysis. A two-sample *z* test was used to assess the significance of the difference between the two population proportions. A *P* value of ≤0.05 was considered significant.

RESULTS

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

Genotyping. The *pvl*-positive MRSA ($n = 190$) and MSSA ($n = 39$) isolates were assigned to 11 and five MLST clonal complexes (CCs), respectively (Table 2). For MRSA, the isolates were assigned to either SCC*mec* type IV (79.5% [151/190]) or V (20.5% [39/190]), and to 16 genotypes (CC/ST-SCC*mec* types) (Table 2), with CC/ST8-MRSA-IV predominating (33.7% [64/190]), followed by CC/ST30-MRSA-IV (21.1% [40/190]), CC/ST80-MRSA-IV (14.2% [27/190]), CC1/ST772-MRSA-V (13.2% [25/190]), CC/ST22-MRSA-IV (6.3% [12/190]), ST59/952-MRSA-V (4.7% [9/190]), ST93-MRSA-IV (3/190 [1.6%]), and CC1-MRSA-IV (1.1% [2/190]) (Table 2). The remaining eight MRSA genotypes were each represented by one isolate only (Table 2).

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

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

The CC/ST30-MRSA-IV clone predominated and was at its most prevalent in 2004, when it accounted for 70% of *pvl*-positive MRSA isolates (7/10). Subsequently, the prevalence of CC/ST30-MRSA-IV varied significantly each year between 2005 and 2011, accounting for 33.3% (3/9) of the isolates in 2005 but just 5% (2/40) of the isolates in 2011 (Fig. 1). The CC/ST8-MRSA-IV clone predominated and was at its most prevalent in 2005, when it accounted for 66.7% of the isolates (6/9); afterwards, however, the prevalence of this clone varied dramatically each year between 2006 and 2011, e.g., despite a decrease to 33.3% (4/12) in 2006, a rise in the prevalence of this clone was noted between 2006 and 2009 to 46.9% (15/32), followed by an overall decrease to 27.5% (11/40) in 2011 (Fig. 1).

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

Prior to 2008, only one *pvl*-positive ST22-MRSA-IV isolate had been detected (in 2003). Despite the low numbers of the isolates, an increase in the prevalence of *pvl*-positive ST22-MRSA-IV was

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

Typing category results (n):									
	Genotype[a]						Antibiotic resistance profiles		
CC[a]	MRSA	MSSA	*spa*[b]	*agr*[a]	Capsule[a]	IEC[a,b,c]	Phenotype (% indicated when not 100%)[d]	Genotype (% indicated when not 100%)	Virulence genes (% indicated when not 100%)[e]
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-MSSA (10)	t005 (7), t891 (2), t1869 (1)	I	5	B	AMI (10), AMP, CAD (60), FUS (10), GEN (80), KAN, TOB (90), TMP (90)	*blaZ*, *aacA-aphD*, *dfrS1* (90)	*egc*
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*
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), *qacC* (33.3)	*CM14*
121		CC121-MSSA (7)	t159 (5), t435 (2)	IV	8	E	AMP, CAD (28.6) (1), 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*
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 the 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), 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, one ST154-MRSA-IV, and one ST5-MRSA-IV isolates (30, 31).

[b] The number of isolates (*n*) represented by each *spa* type or IEC type are indicated in parentheses 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), rifampin, spectinomycin (SPC), streptomycin (STR), sulfonamide, 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 previously (42).

[e] Excluding *lukF-PV* and *lukS-PV*, which were detected in all isolates.

noted between 2009 (3.1% [1/32]) and 2011 (12.5% [5/40]) (Fig. 1). The first ST59/952-MRSA-V isolates were detected in 2006, and a small number of the isolates of this clone were subsequently detected each year apart from 2010 (Fig. 1). The highest prevalence of this clone occurred in 2009 (12.5% [4/32]). Only three ST93-MRSA-IV isolates were identified, one in 2009 and two in 2011. All other clones were represented by one or two isolates only (Fig. 1).

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

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

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

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

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

CC8. Although 12 *spa* types were identified among the CC/ST8-MRSA-IV isolates, t008 predominated (73.4% [47/64]). The majority of CC/ST8-MRSA-IV isolates exhibited resistances to kanamycin and neomycin encoded by *aphA3* and to erythromycin encoded by *msr*(A), and almost half of the isolates were resistant to ciprofloxacin. Slightly <10% of the CC/ST8-MRSA-IV isolates were tetracycline resistant and carried *tet*(K). One isolate carried *cfr* and *fexA* and exhibited chloramphenicol and linezolid resistances (42). The majority of the isolates carried the enterotoxin

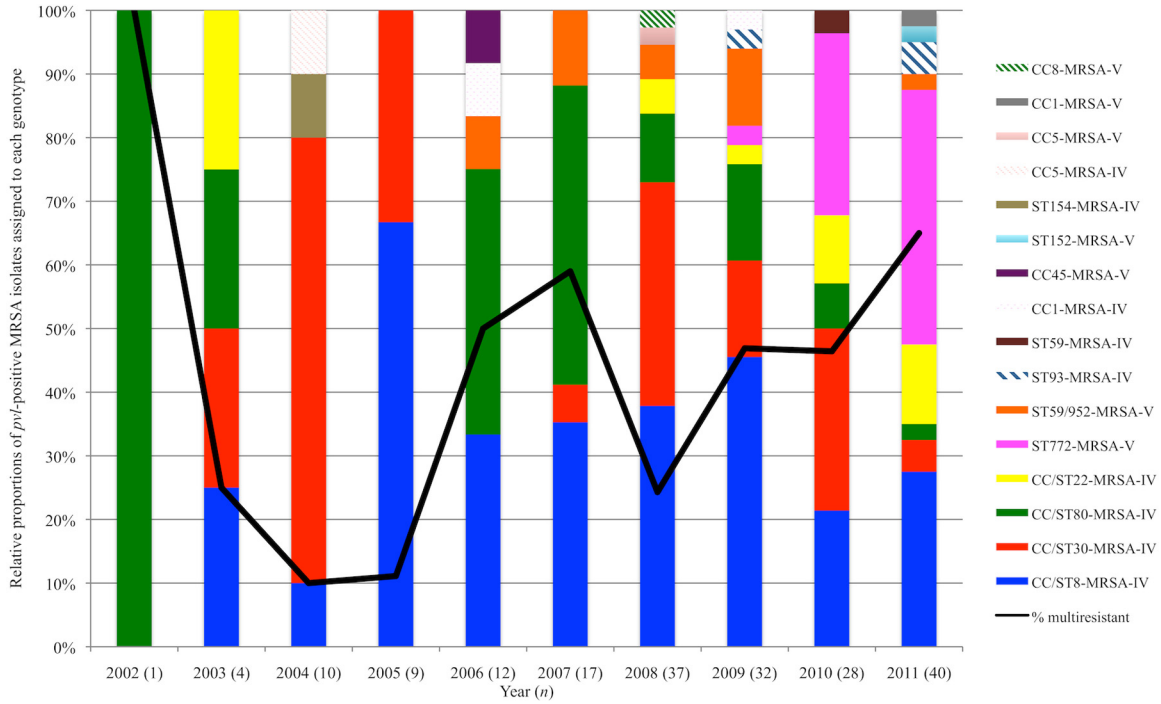

FIG 1 The relative proportions of the 190 *pvl*-positive MRSA isolates identified by the Irish National MRSA Reference Laboratory between 2002 and 2011 assigned to each genotype each year during the study period and the annual percentage of these MRSA isolates that exhibited multiresistance during this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

genes *sek* and *seq* and the arginine catabolic mobile element (ACME), and although they were less common, *sed*, *sej*, and *ser* were also identified.

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

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

CC30. The majority of CC/ST30-MRSA-IV isolates were assigned to *spa* type t019 (55% [22/40]) or t012 (30% [12/40]). Fewer than half of the isolates were resistant to fusidic acid encoded by *fusC*, and resistances to tetracycline and trimethoprim encoded by *tet*(K) and *dfrS1*, respectively, were detected in one isolate each. All CC/ST30-MRSA-IV isolates carried *egc*, and 35% (14/40) carried the toxic shock toxin gene *tst*, with only 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, resistances to tetracycline, trimethoprim, and erythromycin encoded by *tet*(K), *dfrS1*, and *mph*(C), respectively, were 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*, *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 the isolates exhibited resistances to multiple antimicrobial agents and carried multiple resistance genes, with erythromycin and lincomycin resistances encoded by *erm*(B), kanamycin and neomycin resistances 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 fewer resistance genes, but *aphA3* and *erm*(B) encoding resistances to aminoglycosides and erythromycin, respectively, were detected. Simi-

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

CC88. Only 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 only one isolate each exhibiting resistances 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 one isolate only. 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 resistances 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 (ciprofloxacin), 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 multiresistant. 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]) (see Fig. S1 in the supplemental material). 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 prevalence increased again between 2008 and 2011 to 65% (26/40) ($P < 0.001$) (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 (3.9%) from health care staff, 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 were 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 isolates being associated with females and males, respectively, and 53.3% (16/30) and 46.7% (14/30) of MSSA isolates being associated with females and males, respectively. The ages of patients from whom *pvl*-positive *S. aureus* isolates were recovered ranged from <1 month to 98 years, and the median age was 30 years (age data were available for 193 patients). Seventy percent (136/193) of the isolates were from patients who were <40 years of age (Table 3).

Isolate clusters. No clusters were identified among *pvl*-positive MSSA isolates, but seven isolate clusters were identified among *pvl*-positive MRSA isolates, either from two or more members of one family/household, within a hospital, or both (Table 3). Within each cluster, the isolates were recovered between 3 months and 2 years apart, and the 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 traveled internationally or had a country of origin other than Ireland (Table 3). Recent travel ranged from 2 weeks to 1 year prior to the recovery of the *pvl*-positive *S. aureus* isolates, 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 other than 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 associated exclusively 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 United States (4 isolates) (Table 3).

Clinical presentations. Clinical data were available for 159 isolates (135 MRSA and 24 MSSA) (see Fig. S2 in the supplemental material). 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] of the isolates including 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

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

Patient characteristics	No. of isolates	Genotype[a]
Gender		
Male	90	NA
Female	99	NA
Data not available	36	NA
Age group (yr)		
0–9	35	NA
10–19	19	NA
20–29	40	NA
30–39	42	NA
40–49	23	NA
50–59	11	NA
60–69	9	NA
70–79	7	NA
80–89	4	NA
90–99	3	NA
Data not available	32	NA
No. of clusters[b]		
1 (household)	4	CC/ST30-MRSA-IV
2 (household)	3	CC/ST30-MRSA-IV
3 (household)	4	CC/ST8-MRSA-IV
4 (household)	3	CC/ST8-MRSA-IV
5 (household)	4	CC80-MRSA-IV
6 (hospital)	6	ST772-MRSA-V
7 (hospital and household)[c]	11	ST772-MRSA-V
International travel to or country/region of origin		
United States	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
The Middle East	1	ST80-MRSA-IV
India	1	ST772-MRSA-V
	5	ST772-MRSA-V
Far East Asia	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
Brazil	1	ST8-MRSA-IV
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] NA, not applicable; CC, clonal complex; ST, sequence type.
[b] Isolates were defined as clusters if they were recovered from members of one family/household, within a hospital, or both. Within each cluster, isolates were recovered between 3 months and 2 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 were described previously (31).

(3.1% [5/159] of the isolates including CC30-MSSA, ST772-MRSA-V, CC/ST8-MRSA-IV, and CC/ST80-MRSA-IV), osteomyelitis (1.3% [2/159] of the isolates including CC121-MSSA and CC1-MSSA), necrotizing pneumonia (1.3% [2/159] of the isolates belonging to CC/ST8-MRSA-IV and CC1 MSSA), necrotizing fasciitis (0.6% [1/159] of the isolates belonging to CC30-MSSA), and endocarditis (0.6% [1/159] isolates belonging to CC22/ST22-MRSA-IV). Thirty-one isolates were recovered during patient screenings (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 isolates 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 to the higher rate. 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 that in the United States 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 United States 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 United States 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 to or ethnic origin from outside of Ireland were also identified, highlighting the continuing role of strain importation on the variety of *pvl*-positive MRSA strains found in Ireland.

While the prevalence of different *pvl*-positive MRSA clones identified in the present study, together with precise temporal shifts of predominant clones that 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, and 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 United Kingdom, 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 in the *pvl*-positive MRSA isolates in Ireland is of concern, as both clones appear to be highly transmissible, with a propensity to spread worldwide and 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 was found in association with three family clusters; 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), and also 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 isolates investigated in this study were multiresistant.

While the overall numbers of *pvl*-positive ST22-MRSA-IV isolates in this study were low, a 4-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), and it now predominates together with ST772-MRSA-V in hospitals in India (20). Although *pvl*-negative ST22-MRSA-IV is currently predominant in Irish hospitals (mainly *spa* type t032 [28]), *pvl*-positive ST22-MRSA-IV was genetically distinct (*spa* type t852) in the present study, indicating the independent evolution of these strains.

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

The diversity of *pvl*-positive MRSA clones increased in the second half of the study period, with 10/16 MRSA genotypes identified for the first time between 2006 and 2011, including CC/ST59-MRSA-V, ST93-MRSA-IV, and ST772-MRSA-V. Links between several isolates of these clones and the regions where they predominated were also noted. Although an increase in the Taiwanese clone (CC/ST59-MRSA-V), from 8.3% in 2006 to 12.5% in 2009, was observed, the number of isolates recovered each year remained low throughout (between one and four isolates each year). CC/ST59-MRSA-V is among the predominant CA-MRSA clones in some northern European countries (21). In contrast, similar to in the present study, ST93-MRSA-IV has only been reported sporadically in Europe (21, 53) but is the dominant *pvl*-positive MRSA strain in Australia, where it has spread into health care facilities (54). Increasing reports of outbreaks of *pvl*-positive MRSA, particularly in NICUs, highlights the ability of these strains to spread among vulnerable patient groups in hospitals (24, 25, 47, 48). In the present study, two NICU clusters in separate hospitals were due to the recently emerged *pvl*-positive multiresistant ST772-MRSA-V clone. In fact, 30% of *pvl*-positive isolates for which information was available were from patients in hospitals, a situation that requires close monitoring so that *pvl*-positive MRSA does not become widespread in Irish hospitals.

Despite a decrease in 2008, an overall 6-fold increase in the prevalence of multiresistant *pvl*-positive MRSA was identified between 2004 and 2011 (Fig. 1). Similarly, a 12.3-fold increase in the prevalence of multiresistant *pvl*-positive MRSA was noted in the United Kingdom between 2006 and 2008 (22). Both in Ireland and the United Kingdom, this was largely due to the emergence and predominance of ST772-MRSA-V, and in Ireland only, to the continued prevalence of ST8-MRSA-IV/USA300. Also of concern is the high prevalence of ciprofloxacin resistance identified among multiresistant *pvl*-positive MRSA isolates (67.1%). All of these findings highlight how non-multiantibiotic resistance and susceptibility to ciprofloxacin can no longer be considered to be reliable markers for *pvl*-positive MRSA.

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

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

Similar to a recent study in the United Kingdom, CC22 and CC30 were the most common *pvl*-positive MSSA clones identified in our study, accounting for 64.1% of the isolates (26). While not reported previously in the United Kingdom (26), the CC121-MSSA clone that accounted for 17.9% of *pvl*-positive MSSA isolates in the present study is a pandemic clone (55, 56). Interestingly, a link to Africa and the Far East was noted for 2/7 CC121 MSSA isolates, where that clone has been shown to dominate (56, 57). CC88-MSSA accounted for just 7.6% of the *pvl*-positive MSSA isolates and was reported previously in 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 highlights 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 obtaining detailed epidemiological information, in order to fully understand the burden of *S. aureus* infections that exists.

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REFERENCES

1. **Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S, Ehricht R.** 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One **6:**e17936. http://dx.doi.org/10.1371/journal.pone.0017936.
2. **Witte W.** 2009. Community-acquired methicillin-resistant *Staphylococcus aureus*: what do we need to know? Clin. Microbiol. Infect. **15:**17–25. http://dx.doi.org/10.1111/j.1469-0691.2009.03097.x.
3. **Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984. http://dx.doi.org/10.3201/eid0908.030089.
4. **Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132. http://dx.doi.org/10.1086/313461.
5. **Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piémont Y, Brousse N, Floret D, Etienne J.** 2002. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet **359:**753–759. http://dx.doi.org/10.1016/S0140-6736(02)07877-7.
6. **Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, Perl T, Ticehurst JR, Carroll K, Thomas DL, Nuermberger E, Bartlett JG.** 2005. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin. Infect. Dis. **40:**100–107. http://dx.doi.org/10.1086/427148.
7. **Boyle-Vavra S, Daum RS.** 2007. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Lab. Invest. **87:**3–9. http://dx.doi.org/10.1038/labinvest.3700501.
8. **Kaneko J, Kamio Y.** 2004. Bacterial two-component and heteroheptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Biosci. Biotechnol. Biochem. **68:**981–1003. http://dx.doi.org/10.1271/bbb.68.981.
9. **Boakes E, Kearns AM, Ganner M, Perry C, Hill RL, Ellington MJ.** 2011. Distinct bacteriophages encoding Panton-Valentine leukocidin (PVL) among international methicillin-resistant *Staphylococcus aureus* clones harboring PVL. J. Clin. Microbiol. **49:**684–692. http://dx.doi.org/10.1128/JCM.01917-10.
10. **Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC.** 2005. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired meticillin-resistant clone. Lancet **365:**1256–1258. http://dx.doi.org/10.1016/S0140-6736(05)74814-5.
11. **Groom AV, Wolsey DH, Naimi TS, Smith K, Johnson S, Boxrud D, Moore KA, Cheek JE.** 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. JAMA **286:**1201–1205. http://dx.doi.org/10.1001/jama.286.10.1201.
12. **Otto M.** 2013. Community-associated MRSA: what makes them special? Int. J. Med. Microbiol. **303:**324–330. http://dx.doi.org/10.1016/j.ijmm.2013.02.007.
13. **Boyle-Vavra S, Ereshefsky B, Wang CC, Daum RS.** 2005. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel staphylococcal chromosome cassette *mec* (SCC*mec*) type VT or SCC*mec* type IV. J. Clin. Microbiol. **43:**4719–4730. http://dx.doi.org/10.1128/JCM.43.9.4719-4730.2005.
14. **O'Hara FP, Amrine-Madsen H, Mera RM, Brown ML, Close NM, Suaya JA, Acosta CJ.** 2012. Molecular characterization of *Staphylococcus aureus* in the United States 2004–2008 reveals the rapid expansion of USA300 among inpatients and outpatients. Microb. Drug Resist. **18:**555–561. http://dx.doi.org/10.1089/mdr.2012.0056.
15. **Chen CJ, Unger C, Hoffmann W, Lindsay JA, Huang YC, Götz F.** 2013. Characterization and comparison of 2 distinct epidemic community-associated methicillin-resistant *Staphylococcus aureus* clones of ST59 lineage. PLoS One **8:**e63210. http://dx.doi.org/10.1371/journal.pone.0063210.
16. **Smith JM, Cook GM.** 2005. A decade of community MRSA in New Zealand. Epidemiol. Infect. **133:**899–904. http://dx.doi.org/10.1017/S0950268805004024.
17. **Coombs GW, Goering RV, Chua KY, Monecke S, Howden BP, Stinear TP, Ehricht R, O'Brien FG, Christiansen KJ.** 2012. The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* strain. PLoS One **7:**e43037. http://dx.doi.org/10.1371/journal.pone.0043037.
18. **Otter JA, French GL.** 2010. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. Lancet Infect. Dis. **10:**227–239. http://dx.doi.org/10.1016/S1473-3099(10)70053-0.
19. **Ghebremedhin B, Olugbosi MO, Raji AM, Layer F, Bakare RA, König B, König W.** 2009. Emergence of a community-associated methicillin-resistant *Staphylococcus aureus* strain with a unique resistance profile in southwest Nigeria. J. Clin. Microbiol. **47:**2975–2980. http://dx.doi.org/10.1128/JCM.00648-09.
20. **D'Souza N, Rodrigues C, Mehta A.** 2010. Molecular characterization of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. J. Clin. Microbiol. **48:**1806–1811. http://dx.doi.org/10.1128/JCM.01867-09.
21. **Rolo J, Miragaia M, Turlej-Rogacka A, Empel J, Bouchami O, Faria NA, Tavares A, Hryniewicz W, Fluit AC, de Lencastre H, CONCORD Working Group.** 2012. High genetic diversity among community-associated *Staphylococcus aureus* in Europe: results from a multicenter study. PLoS One **7:**e34768. http://dx.doi.org/10.1371/journal.pone.0034768.
22. **Ellington MJ, Ganner M, Warner M, Cookson BD, Kearns AM.** 2010. Polyclonal multiply antibiotic-resistant methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leucocidin in England. J. Antimicrob. Chemother. **65:**46–50. http://dx.doi.org/10.1093/jac/dkp386.
23. **Patel M, Thomas HC, Room J, Wilson Y, Kearns A, Gray J.** 2013. Successful control of nosocomial transmission of the USA300 clone of community-acquired meticillin-resistant *Staphylococcus aureus* in a UK paediatric burns centre. J. Hosp. Infect. **84:**319–322. http://dx.doi.org/10.1016/j.jhin.2013.04.013.
24. **Ali H, Nash JQ, Kearns AM, Pichon B, Vasu V, Nixon Z, Burgess A, Weston D, Sedgwick J, Ashford G, Mühlschlegel FA.** 2012. Outbreak of a South West Pacific clone Panton-Valentine leucocidin-positive meticillin-resistant *Staphylococcus aureus* infection in a UK neonatal intensive care unit. J. Hosp. Infect. **80:**293–298. http://dx.doi.org/10.1016/j.jhin.2011.12.019.
25. **Schlebusch S, Price GR, Hinds S, Nourse C, Schooneveldt JM, Tilse MH, Liley HG, Wallis T, Bowling F, Venter D, Nimmo GR.** 2010. First outbreak of PVL-positive nonmultiresistant MRSA in a neonatal ICU in Australia: comparison of MALDI-TOF and SNP-plus-binary gene typing. Eur. J. Clin. Microbiol. Infect. Dis. **29:**1311–1314. http://dx.doi.org/10.1007/s10096-010-0995-y.
26. **Otokunefor K, Sloan T, Kearns AM, James R.** 2012. Molecular characterization and Panton-Valentine leucocidin typing of community-acquired me-

thicillin-sensitive *Staphylococcus aureus* clinical isolates. J. Clin. Microbiol. **50:**3069–3072. http://dx.doi.org/10.1128/JCM.00602-12.

27. **Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene-Sarr F, Djibo S, Etienne J, Fonkoua MC, Perrier-Gros-Claude JD, Ramarokoto CE, Randrianirina F, Thiberge JM, Zriouil SB, Working Group on *Staphylococcus aureus* Infections, Garin B, Laurent F.** 2011. Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. Clin. Microbiol. Infect. **17:**633–639. http://dx.doi.org/10.1111/j.1469-0691.2010.03320.x.
28. **Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, Dolan A, Cunney R, Sullivan DJ, Goering RV, Humphreys H, Coleman DC.** 2010. Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining *spa*, *dru*, and pulsed-field gel electrophoresis typing data. J. Clin. Microbiol. **48:**1839–1852. http://dx.doi.org/10.1128/JCM.02155-09.
29. **Irish National MRSA Reference Laboratory.** 2011. National MRSA Reference Laboratory annual report. St. James's Hospital, Dublin, Ireland. http://www.stjames.ie/Departments/DepartmentsA-Z/N/NationalMRSAReferenceLaboratory/DepartmentinDepth/NMRSARL%20Annual%20Report%202011.pdf.
30. **Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC.** 2007. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. J. Clin. Microbiol. **45:**2554–2563. http://dx.doi.org/10.1128/JCM.00245-07.
31. **Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B.** 2012. Emergence of hospital- and community-associated Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. J. Clin. Microbiol. **50:**841–847. http://dx.doi.org/10.1128/JCM.06354-11.
32. **Heelan K, Murphy A, Murphy LA.** 2012. Panton-Valentine leukocidin-producing *Staphylococcal aureus*: report of four siblings. Pediatr. Dermatol. **29:**618–620. http://dx.doi.org/10.1111/j.1525-1470.2011.01522.x.
33. **Shore AC, Brennan OM, Deasy EC, Rossney AS, Kinnevey PM, Ehricht R, Monecke S, Coleman DC.** 2012. DNA microarray profiling of a diverse collection of nosocomial methicillin-resistant *Staphylococcus aureus* isolates assigns the majority to the correct sequence type and staphylococcal cassette chromosome *mec* (SCC*mec*) type and results in the subsequent identification and characterization of novel SCC*mec*-SCC*M1* composite islands. Antimicrob. Agents Chemother. **56:**5340–5355. http://dx.doi.org/10.1128/AAC.01247-12.
34. **Monecke S, Jatzwauk L, Weber S, Slickers P, Ehricht R.** 2008. DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Clin. Microbiol. Infect. Dis. **14:**534–545. http://dx.doi.org/10.1111/j.1469-0691.2008.01986.x.
35. **Monecke S, Slickers P, Ehricht R.** 2008. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol. Med. Microbiol. **53:**237–251. http://dx.doi.org/10.1111/j.1574-695X.2008.00426.x.
36. **McDougal LK, Fosheim GE, Nicholson A, Bulens SN, Limbago BM, Shearer JE, Summers AO, Patel JB.** 2010. Emergence of resistance among USA300 methicillin-resistant *Staphylococcus aureus* isolates causing invasive disease in the United States. Antimicrob. Agents Chemother. **54:**3804–3811. http://dx.doi.org/10.1128/AAC.00351-10.
37. **Vanhoof R, Godard C, Content J, Nyssen HJ, Hannecart-Pokorni E.** 1994. Detection by polymerase chain reaction of genes encoding aminoglycoside-modifying enzymes in methicillin-resistant *Staphylococcus aureus* isolates of epidemic phage types. Belgian Study Group of Hospital Infections (GDEPIH/GOSPIZ). J. Med. Microbiol. **41:**282–290.
38. **Chen CM, Huang M, Chen HF, Ke SC, Li CR, Wang JH, Wu LT.** 2011. Fusidic acid resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a Taiwanese hospital. BMC Microbiol. **11:**98. http://dx.doi.org/10.1186/1471-2180-11-98.
39. **Argudin MA, Mendoza MC, González-Hevia MA, Bances M, Guerra B, Rodicio MR.** 2012. Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. Appl. Environ. Microbiol. **78:**2930–2935. http://dx.doi.org/10.1128/AEM.07487-11.
40. **Smith K, Gemmell CG, Hunter IS.** 2008. The association between biocide tolerance and the presence or absence of *qac* genes among hospital-acquired and community-acquired MRSA isolates. J. Antimicrob. Chemother. **61:**78–84. http://dx.doi.org/10.1093/jac/dkm395.
41. **Prabhakara S, Khedkar S, Shambat SM, Srinivasan R, Basu A, Norrby-Teglund A, Seshasayee AS, Arakere G.** 2013. Genome sequencing unveils a novel *sea* enterotoxin-carrying PVL phage in *Staphylococcus aureus* ST772 from India. PLoS One **8:**e60013. http://dx.doi.org/10.1371/journal.pone.0060013.
42. **Shore AC, Brennan OM, Ehricht R, Monecke S, Schwarz S, Slickers P, Coleman DC.** 2010. Identification and characterization of the multidrug resistance gene *cfr* in a Panton-Valentine leukocidin-positive sequence type 8 methicillin-resistant *Staphylococcus aureus* IVa (USA300) isolate. Antimicrob. Agents Chemother. **54:**4978–4984. http://dx.doi.org/10.1128/AAC.01113-10.
43. **Berktold M, Grif K, Mäser M, Witte W, Würzner R, Orth-Höller D.** 2012. Genetic characterization of Panton-Valentine leukocidin-producing methicillin-resistant *Staphylococcus aureus* in Western Austria. Wien. Klin. Wochenschr. **124:**709–715. http://dx.doi.org/10.1007/s00508-012-0244-8.
44. **Witte W, Strommenger B, Cuny C, Heuck D, Nuebel U.** 2007. Methicillin-resistant *Staphylococcus aureus* containing the Panton-Valentine leucocidin gene in Germany in 2005 and 2006. J. Antimicrob. Chemother. **60:**1258–1263. http://dx.doi.org/10.1093/jac/dkm384.
45. **Brauner J, Hallin M, Deplano A, De Mendonça R, Nonhoff C, De Ryck R, Roisin S, Struelens MJ, Denis O.** 2013. Community-acquired methicillin-resistant *Staphylococcus aureus* clones circulating in Belgium from 2005 to 2009: changing epidemiology. Eur. J. Clin. Microbiol. Infect. Dis. **32:**613–620. http://dx.doi.org/10.1007/s10096-012-1784-6.
46. **Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, Dunman PM.** 2006. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J. Clin. Microbiol. **44:**108–118. http://dx.doi.org/10.1128/JCM.44.1.108-118.2006.
47. **Pinto AN, Seth R, Zhou F, Tallon J, Dempsey K, Tracy M, Gilbert GL, O'Sullivan MV.** 2012. Emergence and control of an outbreak of infections due to Panton-Valentine leukocidin positive, ST22 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. Clin. Microbiol. Infect. **19:**620–627. http://dx.doi.org/10.1111/j.1469-0691.2012.03987.x.
48. **Harris SR, Cartwright EJ, Török ME, Holden MTG, Brown NM, Ogilvy-Stuart AL, Ellington MJ, Quail MA, Bentley SD, Parkhill J, Peacock SJ.** 2012. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. Lancet Infect. Dis. **13:**130–136. http://dx.doi.org/10.1016/S1473-3099(12)70268-2.
49. **Yamamoto T, Takano T, Yabe S, Higuchi W, Iwao Y, Isobe H, Ozaki K, Takano M, Reva I, Nishiyama A.** 2012. Super-sticky familial infections caused by Panton-Valentine leukocidin-positive ST22 community-acquired methicillin-resistant *Staphylococcus aureus* in Japan. J. Infect. Chemother. **18:**187–198. http://dx.doi.org/10.1007/s10156-011-0316-0.
50. **Ellington MJ, Perry C, Ganner M, Warner M, McCormick Smith I, Hill RL, Shallcross L, Sabersheikh S, Holmes A, Cookson BD, Kearns AM.** 2009. Clinical and molecular epidemiology of ciprofloxacin-susceptible MRSA encoding PVL in England and Wales. Eur. J. Clin. Microbiol. Infect. Dis. **28:**1113–1121. http://dx.doi.org/10.1007/s10096-009-0757-x.
51. **Pantelides NM, Gopal Rao G, Charlett A, Kearns AM.** 2012. Preadmission screening of adults highlights previously unrecognized carriage of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in London: a cause for concern? J. Clin. Microbiol. **50:**3168–3171. http://dx.doi.org/10.1128/JCM.01066-12.
52. **Williamson DA, Roberts SA, Ritchie SR, Coombs GW, Fraser JD, Heffernan H.** 2013. Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in New Zealand: rapid emergence of sequence type 5 (ST5)-SCC*mec*-IV as the dominant community-associated MRSA clone. PLoS One **8:**e62020. http://dx.doi.org/10.1371/journal.pone.0062020.
53. **Ellington MJ, Ganner M, Warner M, Boakes E, Cookson BD, Hill RL, Kearns AM.** 2010. First international spread and dissemination of the virulent Queensland community-associated methicillin-resistant *Staphylococcus aureus* strain. Clin. Microbiol. Infect. **16:**1009–1012. http://dx.doi.org/10.1111/j.1469-0691-2009.02994.x.
54. **Munckhof WJ, Nimmo GR, Carney J, Schooneveldt JM, Huygens F, Inman-Bamber J, Tong E, Morton A, Giffard P.** 2008. Methicillin-susceptible, non-multiresistant methicillin-resistant and multiresistant methicillin-resistant *Staphylococcus aureus* infections: a clinical, epidemi-

ological and microbiological comparative study. Eur. J. Clin. Microbiol. Infect. Dis. **27:**355–364. http://dx.doi.org/10.1007/s10096-007-0449-3.

55. **Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehricht R.** 2007. High diversity of Panton-Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S. aureus*. Clin. Microbiol. Infect. **13:**1157–1164. http://dx.doi.org/10.1111/j.1469-0691.2007.01833.x.
56. **Kurt K, Rasigade JP, Laurent F, Goering RV, Žemličková H, Machova I, Struelens MJ, Zautner AE, Holtfreter S, Bröker B, Ritchie S, Reaksmey S, Limmathurotsakul D, Peacock SJ, Cuny C, Layer F, Witte W, Nübel U.** 2013. Subpopulations of *Staphylococcus aureus* clonal complex 121 are associated with distinct clinical entities. PLoS One **8:**e58155. http://dx.doi.org/10.1371/journal.pone.0058155.
57. **Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z, Yun-Khoon L, Aziz MN, Hamat RA, Melles DC, van Belkum A, Shamsudin MN, Neela V.** 2011. Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. Int. J. Med. Microbiol. **301:**347–353. http://dx.doi.org/10.1016/j.ijmm.2010.10.004.
58. **Afroz S, Kobayashi N, Nagashima S, Alam MM, Hossain AB, Rahman MA, Islam MR, Lutfor AB, Muazzam N, Khan MA, Paul SK, Shamsuzzaman AK, Mahmud MC, Musa AK, Hossain MA.** 2008. Genetic characterization of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in Bangladesh. Jpn. J. Infect. Dis. **61:**393–396.
59. **van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA.** 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J. Bacteriol. **188:** 1310–1315. http://dx.doi.org/10.1128/JB.188.4.1310-1315.2006.