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Molecular diversity within clonal complex 22 methicillin-resistant *Staphylococcus aureus* encoding Panton–Valentine leukocidin in England and Wales

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Abstract

Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA) that are multi-locus sequence type clonal complex 22 (CC22) comprise a significant public health problem in the UK. In the present study we sought to determine the genetic diversity, and the respective patient demographics, among 47 PVL-MRSA with a CC22 pulsotype that occurred sporadically or in clusters in community and healthcare settings in eight of nine geographic regions in England and Wales between January 2005 and September 2007. Patient demographics and disease presentations were typical for PVL-*S. aureus* infections (mostly skin and soft tissue infections in individuals <40 years old); one patient with community-acquired pneumonia died. Although the isolates were closely genotypically related by *spa* typing and pulsed field gel electrophoresis, at least two variant groups were suggested. PCR detections demonstrated that the majority of the CC22 PVL-MRSA identified (n = 42; 89%) harboured SCC*mec*IVc, three had SCC*mec*IVd, one had SCC*mec*IV but was non-subtypeable, and one isolate harboured SCC*mec*V. At least three different PVL-encoding phages were detected: ΦPVL, Φ108PVL and an unidentified icosahedral phage. Agar dilution MIC determinations showed that the CC22 PVL-MRSA identified were typically resistant to gentamicin and trimethoprim (43 of 47 isolates) and ciprofloxacin resistance was also noted in six isolates. In conclusion, the CC22 PVL-MRSA tested were geographically disseminated but highly genetically related. The observed variances in acquired elements (most notably SCC*mec* and PVL-encoding phages) suggested that CC22 PVL-MRSA in England and Wales have evolved on multiple occasions.

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Introduction

Worldwide, community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have caused infections among previously healthy individuals, often with few or no traditional healthcare-associated risk factors for MRSA [1]; however, this situation is changing in some parts of the world at least [2]. Although the origins of many major MRSA lineages remain poorly understood, it is apparent that MRSA have emerged on numerous occasions [3]. CA-MRSA harbour *Staphylococcus* cassette chromosome *mec*, (SCC*mec*) type IV (24 kb), V (28 kb) or VII (41.3 kb) [4–8] and are typically susceptible to more antibiotics than healthcare-associated MRSA (HA-MRSA). Additionally, CA-MRSA can encode bi-component toxin Panton–Valentine Leukocidin (PVL) via lysogeny of one of several icosahedral or elongated head shape temperate PVL-encoding bacteriophages: ΦSa2958, ΦSa2mw, ΦPVL, Φ108PVL, ΦSLT and ΦSa2USA [10,11]. Although MRSA with PVL are typically associated with pyogenic skin and soft tissue infections (SSTI) [12] they can cause life threatening disease, most notably necrotizing pneumonia [13].

With the exception of the USA, the distribution of PVL-MRSA strains is markedly polyclonal within a particular region. In Europe, multilocus sequence type (MLST) ST80-MRSA-SCC*mec*IV (European clone) has been associated with severe disease and predominates among PVL-MRSA-related infections, but other strains, such as ST8-SCC*mec*IVa

(USA300) and ST30-SCC*mec*IVc (South West Pacific clone), also cause significant morbidity and mortality [9,14]. In some locales, other lineages of PVL-MRSA have been reported, including MLST CC22 in Germany and Australia [15,16]. Recent analysis of PVL-MRSA referred to the national *Staphylococcus* Reference Unit (SRU) for England showed that, among polyclonal PVL-MRSA found in England, isolates of MLST clonal complex (CC) 22 had caused significant public health problems [14]. In England and Wales, EMRSA-15 (ST22-IV) are dominant in the hospital setting, leading to significant public health implications [17]. This clone belongs to the same MLST CC as the PVL-positive strains reported in this study. Here we examined the molecular epidemiology of CC22 PVL-MRSA from across England to better understand the genetic variability within the lineage.

Materials and Methods

Bacterial isolates

The Health Protection Agency's SRU performs epidemiological typing and investigation of *Staphylococcus* isolates. Forty-seven sporadic and cluster-associated PVL-positive MRSA with a CC22-like pulsed-field gel electrophoresis (PFGE) pulsotype identified by the SRU between January 2005 and September 2007 were included. The isolates were referred from 21 centres across eight of nine regions of England and Wales designated by the Health Protection Agency (http://www.hpa.org.uk/HPA/ProductsServices/InfectiousDiseases/RegionalMicrobiologyNetwork/), and were selected to maximize phenotypic and genotypic diversity.

Antibiotic susceptibility testing

MICs were determined by E-test (AB Biodisk, Solna, Sweden) or agar dilution using Iso-Sensitest agar (Oxoid, Basingstoke, UK), according to the latest British Society for Antimicrobial Chemotherapy methods [18]. The antimicrobials tested were penicillin, cefoxitin, oxacillin, methicillin, chloramphenicol, linezolid, sulphamethoxazole, trimethoprim, amikacin, tobramycin, gentamicin, ciprofloxacin, erythromycin, clindamycin, rifampicin, tetracycline, minocycline, doxycycline, tigecycline, daptomycin, quinupristin-dalfopristin, vancomycin, teicoplanin, fusidic acid and mupirocin. Isolates resistant to erythromycin but sensitive to clindamycin were tested for inducible clindamycin resistance (D-zone test) [19].

Toxin gene profiling and *agr*, SCC*mec* and *ccr* typing

Multiplex PCRs were used to detect 14 *S. aureus* toxin genes: staphylococcal enterotoxin genes *sea*, *seb*, *sec*, *sed* and *see* (reaction 1) [20]; *seg*, *seh*, *sei* and *sej* (reaction 2) [21]; toxic shock syndrome toxin gene (*tst*) and exfoliatin toxin genes *eta* and *etb* (reaction 3) [20]; and *etd* and *lukS-PV lukF-PV* (reaction 4) [12,22] (enterotoxin L was not tested). The *agr* allotype [23], SCC*mec* cassette and *ccr* allotype of each CC22-like PVL-MRSA were determined by PCR [7,23–26].

DNA sequence-based typing and PFGE

Sequence typing of the *spa* gene repeat region (n = 47) and MLST (n = 7) was performed as described previously [27,28]. BioNumerics (Applied Maths, Ghent, Belgium) was used to analyse *spa* and MLST types by comparison with online databases available at http://www.ridom.de/ and http://www.mlst.net/ respectively. Banding patterns produced by *Sma*I PFGE of chromosomal DNA [29] were also analysed using BioNumerics to produce dendrograms via the unweighted-pair group method (1% optimization and 1.9% position tolerance).

Phage characterization

Eight PCRs were performed to detect five of the PVL-encoding phages (ΦSa2958, ΦSa2mw, ΦPVL, Φ108PVL and ΦSLT) [11]. Two PCRs were designed to identify the carriage of two morphologically distinct phages by targeting genes encoding icosahedral or elongated head shape. Two further PCRs linked these morphologies to the PVL genes and the remaining PCRs classified individual PVL phages [11]. Phages that were not identifiable by these extended characterization PCRs, but were positive for the icosahedral head type, were described as being unknown icosahedral phages. PCR products were visualized on 1.5% agarose gels including 0.4 mg/L ethidium bromide.

Results

The patient demographics and disease presentations for the 47 affected individuals with CC22 PVL-MRSA were typical for PVL-MRSA [1] (Table 1). Four patients presented with upper respiratory tract infection and one with bacteraemia (Table 1). In addition, one patient presented with community-onset pneumonia and subsequently died.

Overall, the isolates tested were all susceptible to linezolid, clindamycin, rifampicin, doxycycline, tigecycline, daptomycin, quinupristin-dalfopristin and vancomycin, and had variable levels of resistance to oxacillin (MIC: 16 to >128 mg/L). All isolates were accessory gene regulator type I and were indicated as being highly genetically related with a minimum of 84.5% similarity by PFGE (Fig. 1). One major PFGE group was identified showing limited banding changes resulting in sub-groups A and B (Fig. 1) and comprised 24 and 12 isolates, respectively (Fig. 1). Thirty-four (72%) of the 47 isolates were *spa* type

TABLE 1. Patient demographic data with disease pathology associated with 47 Panton–Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* presumptively identified as clonal complex 22 (identified from 2005 to 2007)

Patient characteristics		
Age (years)	**Sex**	**Individuals affected (*n* = 47)**
0–16	Male	15
	Female	10
17–40	Male	5
	Female	2
41–60	Male	3
	Female	7
>60	Male	1
	Female	1
Total		**44**
Disease pathology		
Skin and soft tissue infections		17
Community-acquired pneumonia		1
Upper respiratory tract infection		4
Bacteraemia		1
Colonization		20
Clinical data not available		4
Total		**47**

Age/gender data were not available for three patients.

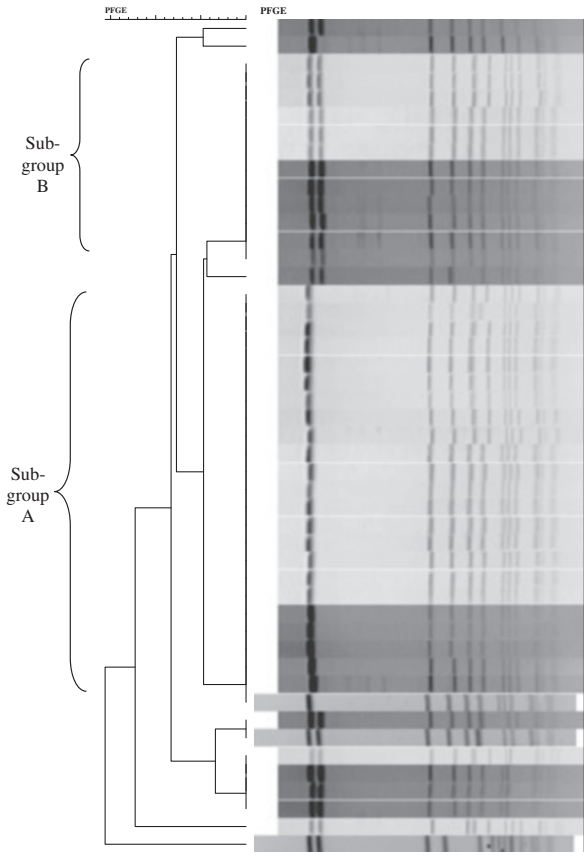
t005 or closely related and all of seven isolates selected to represent the maximal genetic diversity within the isolate set were confirmed as ST22. Forty-six isolates harboured SCC*mec*IV (*ccrα2*) (predominantly SCC*mec*IVc, *n* = 42) and all isolates harboured either ΦPVL, Φ108PVL or an unknown icosahedral phage (suggesting a PVL phage that is not detectable by the current PCRs) (Fig. 1).

All 24 PFGE sub-group A isolates harboured the same toxin genes and SCC*mec*IVc, and were highly related by *spa* type; the only detected difference was one single-nucleotide polymorphism (Fig. 1). At least three different PVL-encoding phages were identified in this group: ΦPVL phage (*n* = 16), Φ108PVL (*n* = 4) or unknown icosahedral phage (*n* = 5). All sub-group A isolates were resistant to oxacillin, trimethoprim, gentamicin and tobramycin (Fig. 1), in accordance with a previous study that identified *mecA*, *aacA-aphD*, *aadD* and *dfrA* in CC22 PVL-MRSA [30]; further variable resistance(s) were detected in some isolates (Fig. 1). All 12 PFGE sub-group B isolates harboured SCC*mec*IVc and the same toxin genes (Fig. 1); eleven had the same *spa* types (t005 and t852). One isolate (t1516) had six repeat unit differences compared to t005. The sub-group B isolates harboured either ΦPVL (*n* = 10) or Φ108PVL (*n* = 2). All isolates in sub-group B were resistant to oxacillin, gentamicin and tobramycin; resistance to fluoroquinolones macrolides and amikacin was variable (Fig. 1). By PFGE and other methods, the greatest genetic variability was apparent among the 11 remaining isolates; three different PVL-encoding phages were detected; five isolates had SCC*mec*IVc, four had SCC*mec*IVd, one had SCC*mec*IV that was not sub-typeable, and one had SCC*mec*V (Fig. 1). The four isolates with SCC*mec*IVd encoded enterotoxin C, in addition to the core pattern of enterotoxins G, I and PVL, were *spa* t005 (*n* = 1), t849 (*n* = 1) and t1790 (*n* = 2), and were resistant to oxacillin and fusidic acid; one isolate was also resistant to amikacin, gentamicin and tobramycin. As observed among other sub-groupings (above), the lysogenized PVL-encoding phages varied among the SCC*mec*IVd-positive isolates.

Discussion

Subsequent to 2004, the SRU has observed the emergence of a PVL-positive MRSA clone belonging to CC22 [14]. Occasional reports of ST22 PVL-MRSA in Germany and, more recently, Australia [15,16] indicate their localized emergence elsewhere, which also correlates with the presence of EMRSA-15 in these areas. In the present study, our data show that the CC22 PVL-MRSA isolates studied comprised PFGE variant sub-groups (A and B) and 11 less closely-related isolates. The intra- and inter-group diversity in the combinations of PVL-encoding phage and the SCC*mec* type or subtype could suggest CC22 PVL-MRSA have emerged on multiple occasions.

Consideration of the structure of the SCC*mec*IVc and IVd cassettes found in 45 of the 47 isolates tested suggest that insertion/deletion events could cause inter-conversion between SCC*mec*IVc and IVd variants; however, the existence of multiple differences between them [25] favours the scenario that the different SCC*mec*IV subtypes represent independent SCC*mec* acquisition events and different instances of the evolution of ST22-PVL-MRSA (Fig. 2). The PVL status of MRSA belonging to CC22 has been documented, although the distribution of PVL-encoding phages within many populations of *S. aureus*, including CC22 MRSA, has not been mapped [31]. At least three variant PVL-encoding phages were detected among CC22-PVL-MRSA. The ΦPVL and Φ108PVL phage genomes are highly conserved and, as observed by Otter *et al.* [32], delineation between these variant phages may be hampered by polymorphisms in the primer binding sites for the detection of these two phages. Likewise, it cannot be ruled out that the exchange of (or recombination between) PVL phages in otherwise closely-related isolates of the same lineage may have occurred. Notwithstanding, we have mapped the PVL encoding phages within apparently closely-related isolates and have identified the same phages in CC22 MRSA and methicillin-sensitive *S. aureus* (MSSA) (data not shown), suggesting the PVL-encoding phages were acquired prior to SCC*mec* (Fig. 2). The presence of the same phages in isolates with



spa	PVL Phage	Antibiotic resistances	SCC*mec*	Toxin genes
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	CIP, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t852 [a]	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	MFX, ERY, TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	ERY, TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	ERY, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	ERY, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t1516 [a]	ΦPVL	CIP, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	MUP,ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Icosahedral	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005[a]	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	CIP, TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	CIP, MFX, ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	CIP, MFX, ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Icosahedral	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Icosahedral	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	TMP, AMI*,GEN, TOB, TET	IVc	*seg, sei , luk-PV*
t005	Φ108PVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Icosahedral	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	Icosahedral	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t852	ΦPVL	CIP, MFX, ERY, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t005	ΦPVL	CIP, MFX, TMP, GEN, TOB	IVc	*seg, sei , luk-PV*
t2816 [a]	ΦPVL	MFX, TMP, AMI*, GEN, TOB	IVc	*seg, sei , luk-PV*
t1790	ΦPVL	CIP, MFX, ERY, FUS	IVd	*seg, sei , luk-PV, sec*
t849 [a]	Φ108PVL	FUS	IVd	*seg, sei , luk-PV, sec*
t1790 [a]	ΦPVL	FUS	IVd	*seg, sei , luk-PV, sec*
t005	Φ108PVL	FUS	IVc	*seg, sei , luk-PV, sec*
t005	Icosahedral	TMP, GEN, TOB	IV-NT*	*seg, sei , luk-PV*
t005 [a]	ΦPVL	TMP, GEN, TOB	V	*seg, sei , luk-PV*

FIG. 1. Molecular characterization of clonal complex 22 (CC22)-like Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA). All isolates were tested for staphylococcal enterotoxins A–E (*sea*-e), and G–J (*seg-j*), toxic shock syndrome toxin-1 (*tst*), exfoliative toxins A, B and D (*eta, etb* and *etd*), and PVL toxin (*lukS-PV lukF-PV*); all were positive for *seg, sei* and *luk-PV*. Antibiotic susceptibilities were determined for penicillin, cefoxitin, oxacillin, methicillin, chloramphenicol, linezolid, sulphamethoxazole, trimethoprim, amikacin, tobramycin, gentamicin, ciprofloxacin, erythromycin, fusidic acid, clindamycin, rifampicin, teicoplanin, doxycycline, tetracycline, tigecycline, minocycline daptomycin, quinupristin-dalfopristin, vancomycin and mupirocin. SCC*mec* cassette type was determined by PCR. [a]Isolates selected for multilocus sequence typing (n = 7). AMI*, Not susceptible to amikacin; IVd*, typed by Okuma *et. al.* [24], IV-NT*, not subtypeable by the PCR scheme described by Milheirico *et. al.* [25] or Okuma *et. al.* [24]. Dendrogram generated using BioNumerics software showing cluster analysis of 47 CC22 PVL-MRSA based on pulsed field gel electrophoresis, with 1% optimization and 1.9% position tolerance.

different SCC*mec* types also supports this hypothesis, further upholding the notion that the CC22 PVL-MRSA circulating in the UK [14] have evolved on multiple occasions (Fig. 2).

Although EMRSA-15 (the predominant HA-MRSA in the UK) also belongs to CC22, it has a different epidemiological profile; it is commonly associated with wound infections and bacteraemia in elderly or severely ill hospitalized patients. Although the acquisition of PVL by EMRSA-15 would increase the virulence of a clone that is already highly transmissible and poses a significant public health concern, there are clear genetic differences between these two lineages. Ciprofloxacin and erythromycin resistance, t032 (or related) *spa* type and carriage of SCC*mec*IVh are common traits among EMRSA-15 but differ from the CC22-PVL-MRSA investigated in the present study (Fig. 2). A detailed study of the population structure of EMRSA-15 would provide further evidence to the evolutionary relationship between these two distinct groups of isolates. Collectively, the data obtained suggest that PVL positive-MRSA belonging to CC22 that have been investigated here have emerged from PVL-positive MSSA on multiple occasions, rather than from EMRSA-15 acquiring PVL [17,25,33].

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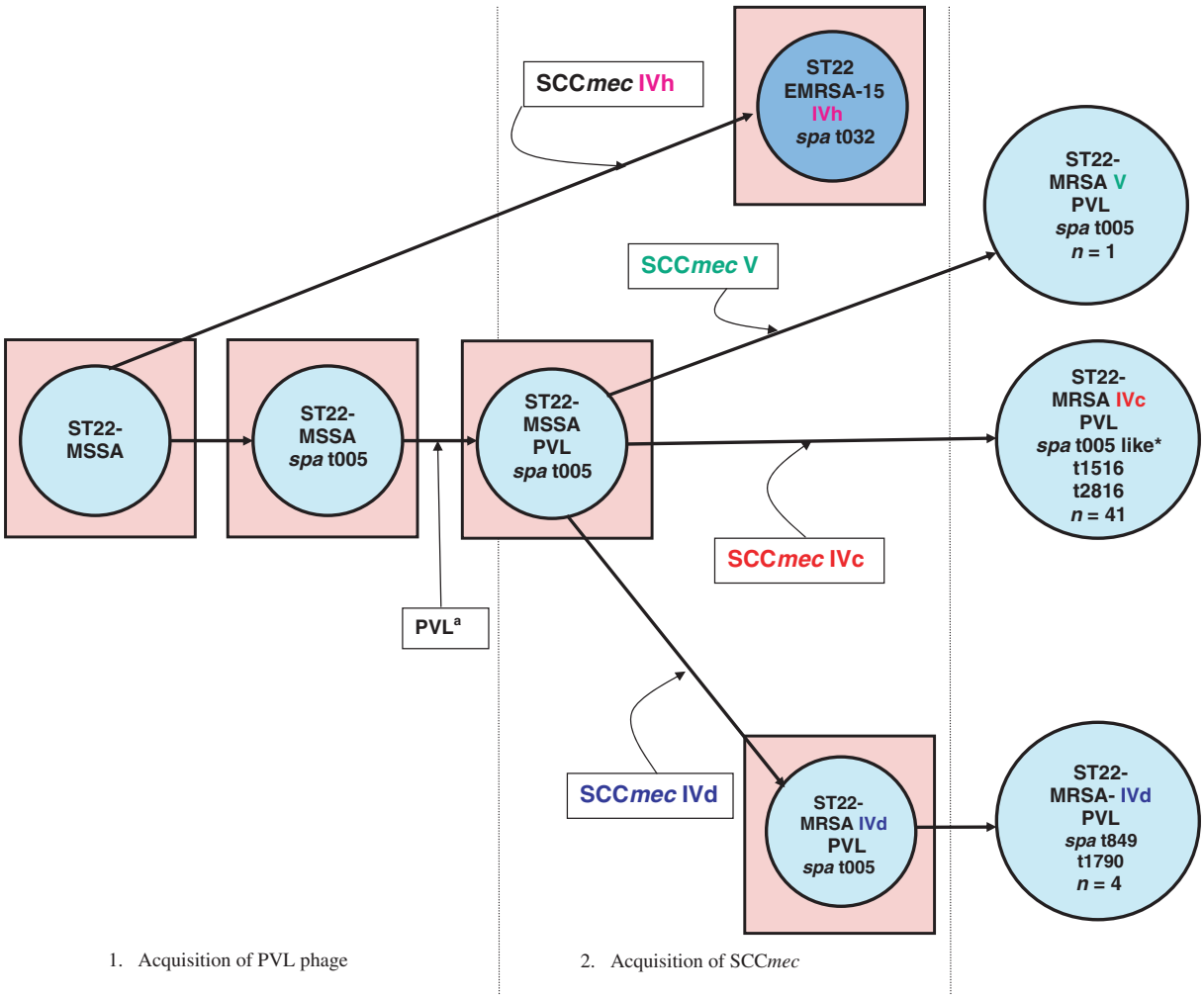

FIG. 2. Hypothetical evolutionary pathway for clonal complex 22 (CC22) Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus*. The red shaded area shows the progenitor clones that are not part of the CC22 isolate set described here but have been previously characterized in the *Staphylococcus* Reference Unit or elsewhere; the remaining groups are part of this data set. The evolutionary pathway of the SCC*mec*IV non-typeable (IV-NT) is unclear because the origins of this SCC*mec* type are unknown. [a]One of three possible PVL phages: ΦPVL, Φ108PVL or unknown icosahedral phage. At present, the relationship between these phages is unknown and classification is subject to limitations of the Ma *et al.* PCR scheme [11,32]. *t005-like, Less than five repeat differences between t005 and other spa types (evolutionary significance of repeat differences in *spa* is currently unknown).

Transparency Declaration

This work was funded by the Health Protection Agency, UK. All authors declare that they have no conflicts of interest.

References

1. Herold BC, Immergluck LC, Maranan MC *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998; 279: 593–598.
2. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis* 2008; 46: 787–794.
3. Musser JM, Kapur V. Clonal analysis of methicillin-resistant *Staphylococcus aureus* strains from intercontinental sources: association of the *mec* gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *J Clin Microbiol* 1992; 30: 2058–2063.
4. Ma XX, Ito T, Tiensasitorn C *et al.* Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002; 46: 1147–1152.
5. Daum RS, Ito T, Hiramatsu K *et al.* A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus*

isolates of diverse genetic backgrounds. *J Infect Dis* 2002; 186: 1344–1347.
6. Boyle-Vavra S, Ereshefsky B, Wang CC, Daum RS. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel staphylococcal chromosome cassette mec (SCCmec) type VT or SCCmec type IV. *J Clin Microbiol* 2005; 43: 4719–4730.
7. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob Agents Chemother* 2004; 48: 2637–2651.
8. Higuchi W, Takano T, Teng LJ, Yamamoto T. Structure and specific detection of staphylococcal cassette chromosome *mec* type VII. *Biochem Biophys Res Commun* 2008; 377: 752–756.
9. Witte W, Braulke C, Cuny C *et al.* Emergence of methicillin-resistant *Staphylococcus aureus* with Panton–Valentine leukocidin genes in central Europe. *Eur J Clin Microbiol Infect Dis* 2005; 24: 1–5.
10. Diep BA, Gill SR, Chang RF *et al.* Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. *Lancet* 2006; 367: 731–739.
11. Ma XX, Ito T, Kondo Y *et al.* Two different Panton–Valentine leukocidin phage lineages predominate in Japan. *J Clin Microbiol* 2008; 46: 3246–3258.
12. Lina G, Piemont Y, Godail-Gamot F *et al.* Involvement of Panton–Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999; 29: 1128–1132.
13. Gillet Y, Issartel B, Vanhems P *et al.* Association between *Staphylococcus aureus* strains carrying gene for Panton–Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002; 359: 753–759.
14. Ellington MJ, Perry C, Ganner M *et al.* Clinical and molecular epidemiology of ciprofloxacin-susceptible MRSA encoding PVL in England and Wales. *Eur J Clin Microbiol Infect Dis* 2009; 28: 1113–1121.
15. Ghebremedhin B, Konig W, Witte W, Hardy KJ, Hawkey PM, Konig B. Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. *J Med Microbiol* 2007; 56: 365–375.
16. Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehricht R. 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* 2007; 13: 1157–1164.
17. Johnson AP, Aucken HM, Cavendish S *et al.* Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chemother* 2001; 48: 143–144.
18. Andrews JM. BSAC standardized disc susceptibility testing method (version 6). *J Antimicrob Chemother* 2007; 60: 20–41.
19. Nathwani D, Morgan M, Masterton RG *et al.* Diagnosis and Principles of management of MRSA Infections Presenting in the Comunity: Guidelines for UK Practice, Working Party* on Behalf of the British Society for Antimicrobial Chemotherpy (BSAC). 11-10-2007.
20. Becker K, Roth R, Peters G. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol* 1998; 36: 2548–2553.
21. Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J Clin Microbiol* 1999; 37: 3411–3414.
22. Yamaguchi T, Nishifuji K, Sasaki M *et al.* Identification of the *Staphylococcus aureus etd* pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. *Infect Immun* 2002; 70: 5835–5845.
23. Peacock SJ, Moore CE, Justice A *et al.* Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infect Immun* 2002; 70: 4987–4996.
24. Okuma K, Iwakawa K, Turnidge JD *et al.* Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002; 40: 4289–4294.
25. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCC*mec* IV multiplex'. *J Antimicrob Chemother* 2007; 60: 42–48.
26. Milheirico C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007; 51: 3374–3377.
27. Harmsen D, Claus H, Witte W *et al.* Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 2003; 41: 5442–5448.
28. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38: 1008–1015.
29. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton–Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J Clin Microbiol* 2005; 43: 2384–2390.
30. Monecke S, Berger-Bachi B, Coombs G *et al.* Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton–Valentine leukocidin. *Clin Microbiol Infect* 2007; 13: 236–249.
31. Witte W, Enright M, Schmitz FJ, Cuny C, Braulke C, Heuck D. Characteristics of a new epidemic MRSA in Germany ancestral to United Kingdom EMRSA 15. *Int J Med Microbiol* 2001; 290: 677–682.
32. Otter JA, Kearns AM, French GL, Ellington MJ. Panton–Valentine leukocidin-encoding bacteriophage and gene sequence variation in community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2009; 1: 68–73.
33. Khandavilli S, Wilson P, Cookson B, Cepeda J, Bellingan G, Brown J. Utility of spa typing for investigating the local epidemiology of MRSA on a UK intensive care ward. *J Hosp Infect* 2009; 71: 29–35.