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

E. Boakes1, A. M. Kearns1, M. Ganner1, C. Perry1, M. Warner2, R. L. Hill2 and M. J. Ellington1,3

1) Staphylococcus Reference Unit, 2) Antibiotic Resistance Evaluation Unit, Centre for Infections, Health Protection Agency, London, NW, UK and 3) Health Protection Agency, Cambridge Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge, UK

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 SCCmecIVc, three had SCCmecIVd, one had SCCmecIV but was non-subtypeable, and one isolate harboured SCCmecV. At least three different PVL-encoding phages were detected: UPVL, U108PVL 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 SCCmec and PVL-encoding phages) suggested that CC22 PVL-MRSA in England and Wales have evolved on multiple occasions.

Keywords: Clonal complex 22, community, MRSA, PVL, Staphylococcus aureus

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Corresponding author: E. Boakes, Staphylococcus Reference Unit, Laboratory of Healthcare Associated Infections, Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK
E-mail: eve.boakes@hpa.org.uk

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, (SCCmec) 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: FmPVL, F108PVL and F108Sa2USA [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-SCCmecIV (European clone) has been associated with severe disease and predominates among PVL-MRSA-related infections, but other strains, such as ST8-SCCmecIV
(USA300) and ST30-SCCmecVc (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) pulsedotype 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, lin-ezolid, 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, SCCmec 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 (tsst) 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], SCCmec 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 Math, 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 Smol 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 1 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)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Individuals affected (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–16</td>
<td>Male</td>
<td>15</td>
</tr>
<tr>
<td>17–40</td>
<td>Male</td>
<td>5</td>
</tr>
<tr>
<td>41–60</td>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>&gt;60</td>
<td>Male</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

Disease pathology
- Skin and soft tissue infections: 17
- Community-acquired pneumonia: 1
- Upper respiratory tract infection: 4
- Bacteremia: 1
- Colonization: 20
- Clinical data not available: 4
- Total: 47

Age/sex 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 SCCmecIV (ccr=2) (predominantly SCCmecIVc, n = 42) and all isolates harboured either dfrA, 0108PVL 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 SCCmecIVc, 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: 0PVL phage (n = 16), 0108PVL (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 dfa in CC22 PVL-MRSA [30]; further variable resistance(s) were detected in some isolates (Fig. 1). All 12 PFGE sub-group B isolates harboured SCCmecIVc 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 0PVL (n = 10) or 0108PVL (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 SCCmecIVc, four had SCCmecIVd, one had SCCmecV that was not sub-typeable, and one had SCCmecV (Fig. 1). The four isolates with SCCmecIVd 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 SCCmecIVd-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 SCCmec type or subtype could suggest CC22 PVL-MRSA have emerged on multiple occasions.

Consideration of the structure of the SCCmecIVc and IVd cassettes found in 45 of the 47 isolates tested suggest that insertion/deletion events could cause inter-conversion between SCCmecIVc and IVd variants; however, the existence of multiple differences between them [25] favours the scenario that the different SCCmecIV subtypes represent independent SCCmec 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 0PVL and 0108PVL 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 SCCmec (Fig. 2). The presence of the same phages in isolates with...
different SCCmec 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 SCCmecVh 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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References


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 SCCmecIV non-typeable (IV-NT) is unclear because the origins of this SCCmec type are unknown. *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).