Genetic analysis of *Staphylococcus aureus* from intravenous drug user lesions

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Multilocus sequence typing (MLST) of 48 methicillin-sensitive *Staphylococcus aureus* isolates from intravenous drug user abscesses/soft-tissue infections revealed 12 sequence types (STs) belonging to eight genetically distinct lineages. Only two novel STs were recovered (one isolate of each), indicating that isolates in this study were similar to those from previous studies of disease and carriage. However, ST59, the most common genotype recovered (from six individuals), may be adept at causing subcutaneous lesions in this patient population, as it is rare in carriage and disease. PCR detection of 22 toxin genes revealed a high prevalence of the gene for staphylococcal enterotoxin B compared with previous studies, indicating that this toxin may promote infections in this patient group.

Introduction

Abscesses and soft-tissue infections are very common among intravenous drug users (IVDUs), with the prevalence of abscesses as high as 32% in one San Francisco study (Binswanger *et al.*, 2000). *Staphylococcus aureus* is the most common cause of such infections (Lowy & Miller, 2002) and, although usually minor in nature, sequelae such as osteomyelitis and bacteraemia are not uncommon, and severe invasive diseases including infective endocarditis have been reported (Spikerman *et al.*, 1996). Little is known about the epidemiology of *S. aureus* amongst IVDUs, as modern molecular epidemiological methods have only been applied to the study of outbreaks among drug users (Fleisch *et al.*, 2001), rather than analysing endemic strains (Lowy & Miller, 2002).

Many toxins have been characterized in *S. aureus*, some of which appear to be associated with particular diseases. Panton–Valentine leukocidin (PVL) is common in *S. aureus* causing furunculosis and severe necrotizing pneumonia (Gillet *et al.*, 2002; Lina *et al.* 1999), isolates expressing toxic-shock syndrome toxin-1 (TSST-1) are predominant in patients with this syndrome and isolates causing food poisoning express superantigen enterotoxins such as enterotoxin B (Bohach *et al.*, 1997). Toxin production is regulated by the global gene regulator *agr* (accessory gene regulator) and alleles III and IV of the autoinducing peptide *agrD* have been associated with staphylococcal scalded skin syndrome and toxic-shock syndrome in previous studies (Jarraud *et al.*, 2000; Ji *et al.*, 1997).

The aims of this study were to examine associations between genetic background, toxin gene content and *agr* type in isolates from IVDU abscess and soft-tissue infections and to compare our results with those of studies of invasive disease and nasal carriage (Becker *et al.*, 2003; Peacock *et al.*, 2002) for evidence of associations between genotype and IVDU disease. Forty-eight isolates from 25 patients were analysed by the highly discriminatory, precise molecular typing method multilocus sequence typing (MLST), PCR analysis of 22 toxin gene loci, *agr* typing and antibiotic susceptibility testing.

Methods

**Bacterial strains.** Forty-eight *S. aureus* isolates were collected from abscesses/soft-tissue infections of 25 IVDUs attending Royal Sussex County Hospital Outpatients Department in a 4-month period in 1999–2000. Isolates were confirmed as *S. aureus* by positive tube coagulase and DNase tests. Antimicrobial susceptibility tests were performed by the agar dilution method of the National Committee for Clinical Laboratory Standards.

**MLST.** Chromosomal DNA was extracted from overnight growth on blood agar plates using DNeasy kits (Qiagen). MLST was performed as described previously (Enright *et al.*, 2000). Briefly, seven housekeeping gene fragments (~500 kb) were sequenced and compared with known alleles at each locus on the MLST web site (http://www.mlst.net). The resulting allelic profiles, each consisting of seven allele numbers, define sequence types (STs). ST5, for example, has the allelic profile 1-4-1-4-12-1-10. STs were compared with those of 1072 *S. aureus* isolates from disease and carriage held on the web-site database.

Abbreviations: CC, clonal complex; IVDU, intravenous drug user; MLST, multilocus sequence typing; PVL, Panton–Valentine leukocidin; ST, sequence type.
PCR-based methods. PCR was used to detect the genes for the following toxins: staphylococcal enterotoxins (SEs) A–E, G–J and M–O, TSST-1, exfoliative toxins A and B, α- and β-haemolysins, epidermal-cell differentiation inhibitor (EDIN) and PVL. Primer sequences and PCR conditions were those described by Jarraud et al. (2002). Alleles 1–IV of the autoinducing peptide agrD were detected by PCR using the method of Peacock et al. (2002).

Results and Discussion

Twelve different allelic profiles defining STs were found (Table 1). Only two genotypes, ST104 and ST148, were absent from the MLST S. aureus databases. ST104 is closely related to ST59, sharing 100 % identity at 5/7 loci, and ST148 shares 6/7 loci with ST5. The MLST S. aureus databases currently contain sequence and demographic data on 1072 isolates from disease and nasal carriage from 25 different countries, with 459 isolates (43 %) from UK studies of carriage and invasive disease (Enright et al., 2000, 2002; Feil et al., 2003; Grundmann et al., 2002). With the exception of these two genotypes and ST5 and ST6, which share 6/7 MLST loci, genetic differences between isolates were high; the next most closely related genotypes, ST1 and ST5, differ at 4/7 loci (Table 1).

STs 104 and 148 apart, all MLST genotypes found in this study have previously been recovered from UK isolates from community carriage and/or invasive disease. The number of isolates in the MLST database corresponding to each ST recovered is shown in Table 1. ST59 was the most commonly recovered genotype, with nine isolates from six individuals. Four ST59 community isolates have previously been characterized, one of which was an isolate from impetigo and the remainder were from carriage. ST5 and ST12 isolates were each recovered from four IVDUs, ST30 and ST45 from three and ST15 from two. The remaining five STs were recovered from single individuals.

PCR detection of 22 toxin genes revealed marked heterogeneity between isolates of different MLST genotype (Table 2). The number of toxin genes present in each isolate varied from two in isolates of ST6 (seo and δ-haemolysin) to 12 in an ST5 isolate, with a median of nine toxins present per isolate. As isolates from the same patient were present in this study, we have disregarded later isolates from the same patient if they possessed the same genotype (ST). Data from 28 isolates were therefore used in calculating the relative frequency of each toxin (Table 2). Table 2 also shows isolates grouped by clonal complex (CC), defined as groups of STs sharing 100 % genetic identity at ≥5/7 MLST loci.

δ-Haemolysin was the only determinant present in every isolate, and other haemolysin genes were also present at high frequencies (64–100 % of isolates). The genes for enterotoxins B, O, G and I were present in 7/28 (25 %), 22/28 (78.6 %) and 20/28 (71.4 %) isolates (genes for enterotoxins G and I were always present together). Other enterotoxin genes were present in at least two isolates, with the exception of enterotoxin E, which was not present. No isolates had the exfoliative toxin B gene, tsst-1 or edin. lukS and lukF, the genes for PVL, were present in 6/28 isolates (21.4 %) of ST1, 5, 12 and 30. sed and sej were the only toxins that were limited to one clonal complex, CC5 (STs 5 and 148).

Detection of toxin genes in invasive disease and carriage isolates was performed in two recent large studies of disease and carriage. Peacock et al. (2002) examined 17 of the 22 toxin gene loci used in this study in 334 isolates in the Oxford region. Becker et al. (2003) studied the prevalence of pyrogenic toxin superantigen genes in 429 isolates from invasive disease and carriage, and 12 of the genes studied were common to this work, although the PCR primers used in this study were from an earlier work (Jarraud et al., 2002). The percentage prevalence of each toxin gene locus in this

<table>
<thead>
<tr>
<th>ST</th>
<th>MLST allele profile</th>
<th>Isolates (n)</th>
<th>Patients (n)</th>
<th>Isolates in database (n)*</th>
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<td>2</td>
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<td>2</td>
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<td>123</td>
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<td>148</td>
<td>1</td>
<td>4</td>
<td>1</td>
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</table>

*Community carriage or community-acquired disease.
Table 2. Toxin gene content and antibiotic susceptibility of 28 IVDU isolates

Isolates are PCR-negative or antibiotic-susceptible unless indicated. Totals are numbers of isolates testing PCR-positive. ND, Not done; Ery, erythromycin; Gen, gentamicin; Fus, fusidin.

| MLST ST | Isolate Patient | agr type | Sea | Seb | Sec | Sed | See | Seh | Sej | Sen | See | Sem | TSST | Eta | Etb | Hla | Hlb | Hld | Hlg | Hlg-2 | Edin | LukS/LukF | Ery | Gen | Fus |
|---------|----------------|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|-----|-----|-----|-----|-----|-----|--------|------|------|-----|
| CC1     | 1 44 23 III    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    | Ery | Gen | Fus |
| CC5     | 5 1 1 II       |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    | R   |     |     |
|         | 5 5 2 II       |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 5 11 6 II      |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 5 25 13 II     |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 6 3 1 I        |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 148 2 1 I      |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
| CC12    | 12 29 14 II    |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 12 41 21 II    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 12 42 22 II    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 12 47 24 II    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
| CC15    | 15 32 17 II    |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 15 35 20 II    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
| CC20    | 20 30 15 I     |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| CC30    | 30 10 5 III    |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 30 31 16 I     |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 30 48 25 II    |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
| CC45    | 45 6 3 I       |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 45 21 11 I     |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 45 24 12 I     |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
| CC51    | 104 18 10 I    |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| CC59    | 59 9 4 I       |          | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    | +    | +   | +   | +   | +   | +   | +    | +    |     |     |     |
|         | 59 13 7 I      |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 59 15 8 I      |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 59 17 9 I      |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|         | 59 33 18 I     |          | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

(continued overleaf)
study and in those of Peacock et al. (2002) and Becker et al. (2003) is shown in Table 2. Statistical comparisons between the three studies would be confounded by differences in the primer sequences used and the comparatively small size of this study, but some large differences in prevalence are of note. \textit{tssr-1} was not found in this study, although present in 27 and 20.3\% of isolates in the studies of Peacock et al. (2002) and Becker et al. (2003), respectively. \textit{seb} was found in 25\% of IVDU genotypes, but was found in only 8.1\% (Peacock et al., 2002) and 6.8\% (Becker et al., 2003) of isolates from the other studies. Similarly, \textit{sed} was present in 17.9\% of IVDU isolates compared with 5.1\% (Peacock et al., 2002) and 7\% (Becker et al., 2003). Four of seven isolates carrying \textit{seb} belonged to ST59, and the remainder were single isolates of three unrelated STs.

Three of the four recognized \textit{agr}D alleles were present in isolates of this study: 15/28 (53.6\%) isolates (from different individuals) possessed \textit{agr}I, 11/28 (39.3\%) had \textit{agr}II and 2/28 (7.1\%) had \textit{agr}III. \textit{agr}III was found only in ST1 and ST3C, whereas \textit{agr}I was found in seven STs and \textit{agr}II in four STs (Table 2). All isolates within STs have the same \textit{agr} subtype with the exception of ST30 (\textit{agr}I, II and III). All isolates were resistant to penicillin and sensitive to flucloxacillin and vancomycin. One gentamicin-resistant isolate (ST5), one fusidin-resistant isolate (ST123) and three erythromycin-resistant isolates (STs 12, 45 and 123) were present, although the majority of isolates of each genotype were sensitive.

\textit{S. aureus} isolates from abscesses and soft-tissue infection in IVDUs in this study were polyclonal, with most genotypes corresponding to those found in disease and carriage in the UK. The relatively high frequency of ST59 in the study population is interesting, as this genotype has been recovered before only from carriage (three cases) and one case of impetigo. However, ST59 could be a very common clone carried by IVDUs and even the general population in the area surveyed, but little is currently known of geographical variation in \textit{S. aureus} population structure outside hospitals. ST59 isolates varied markedly in their toxin gene content, with all isolates included in Table 2 having different toxin gene profiles. This ST is also notable in that most isolates had both \textit{sea} and \textit{seb}. This heterogeneity and the importance of ST59 as a cause of IVDU disease could be addressed by larger studies using prospective sampling of the IVDU population.

The increased presence of \textit{seb} and, to a lesser extent, \textit{sed}, compared with isolates from invasive disease and carriage indicates a possible role for these superantigen toxins in abscess/soft-tissue infection formation, possibly involving T-cell activation promoting inflammation. The presence of \textit{sed} in study isolates is, however, wholly due to the ubiquity of this toxin in CC5 isolates, as it was exclusive to this lineage. \textit{seb}, found in a quarter of isolates, was most common in ST59, and the high prevalence of this genotype in the study may account for the presence of this gene, although it is possible that \textit{seb} may be associated with abscess/soft-tissue infections, as this toxin is much rarer in studies of invasive disease and carriage [9 and 7\%, respectively, reported by Peacock et al. (2002) and Becker et al. (2003)].

<table>
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<th>MLST Isolate</th>
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<th>Toxin gene PCR resistance</th>
<th>Resistance</th>
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<tr>
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<td>123</td>
<td>22 22 22 22 22</td>
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<tr>
<td>Totals ((n))</td>
<td>77 25 19 14 14 14 14 14 14 14</td>
<td>18 18 18 18 18 18 18 18 18 18</td>
<td>18 18 18 18 18 18 18 18 18 18</td>
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<tr>
<td>Prevalence (%)</td>
<td>25.0 59.0 21.0 49.0 21.0 21.0 21.0 21.0 21.0 21.0</td>
<td>59.0 64.0 31.0 45.0 45.0 45.0 45.0 45.0 45.0 45.0</td>
<td>59.0 59.0 59.0 59.0 59.0 59.0 59.0 59.0 59.0 59.0</td>
</tr>
</tbody>
</table>
This study demonstrates the polyclonal nature of S. aureus causing lesions in IVDU patients and the often marked variability in toxin gene content between closely related isolates, especially those of ST59. Our results suggest that ST59 and isolates expressing enterotoxin B may be predisposed to cause such lesions, although larger case-control studies are required in order to demonstrate this conclusively.

Acknowledgements

We would like to thank Dr D. Ashley Robinson for critically reviewing the manuscript and Paul Wilkinson for technical help. This research was funded by the Wellcome Trust. M. C. E is a Royal Society University Research Fellow.

References


