Distribution of meticillin-resistant *Staphylococcus aureus* spa types isolated from health-care workers and patients in a Scottish university teaching hospital

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Our study was aimed at comparing the meticillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from an anonymous group of health-care workers (HCWs) with those obtained from patient samples during a 3-month time interval. We employed spa typing and virulence gene profiling to characterize the MRSA strains. Our data revealed that a total of 14 discrete spa types were circulating in both patients and HCW groups. The t032 spa type, characteristic of EMRSA15 and the Barnim EMRSA ST 22 clones, accounted for over 70 % of isolates, and was equally distributed between patients and HCW groups. In addition, a number of epidemic and sporadic strains were identified, which highlighted the diversity of spa types that can be found within a health-care setting. Virulence profiling for the carriage of 7 genes by the 14 different spa types demonstrated that 10 types carried the *fnbA*, *cna*, *sdrE*, *hlg* and *ica* virulence factors. We concluded that there was no significant difference between the MRSA strains found circulating in the patients and the HCWs, and noted that the dominant spa types carried an identical set of virulence genes that included the key adhesins *fnbA*, *cna* and *sdrE*.

**INTRODUCTION**

*Staphylococcus aureus* is an important human pathogen and is one of the most significant causes of health-care-associated infections in the UK. In an attempt to control the high mortality and morbidity of systemic meticillin-resistant *S. aureus* (MRSA) infections, techniques have been developed to analyse the genetic relatedness of different strains and help inform infection control policies. The single most important identification method is PFGE; however, the complexity, cost and lack of inter-laboratory standardization have led to the appearance of alternative methods (Deurenberg et al., 2007). For long-term global studies on the evolution of strains, multilocus sequence typing is the most commonly used technique (Enright & Spratt, 1999), while for short-term studies on molecular evolution and nosocomial outbreaks, spa typing has proved an excellent tool (Koren et al., 2004; Malachowa et al., 2005; Shopsin et al., 1999).

Alongside the development of sequenced-based typing methods, attention has become focused on the comparison of different MRSA genomes. In particular, the number of studies on the distribution of genes that are directly involved in causing disease has increased (Kuhn et al., 2006; Nashev et al., 2004; Peacock et al., 2002). These genes encode a variety of products that are referred to as virulence factors. Examples include PVL, a toxin linked to the occurrence of necrotizing pneumonia (Brown et al., 2009; Labandeira-Rey et al., 2007); ETA, the toxin responsible for scalded skin syndrome (Amagai et al., 2000); and CNA, the collagen-binding protein that is associated with osteomyelitis (Elasri et al., 2002). In 2002, Peacock et al. (2002) compared the distribution of 33 putative virulence factors amongst *S. aureus* strains isolated from the nasal passages of blood donors and strains responsible for invasive disease. This work suggested that seven specific virulence factors were over-represented in invasive isolates.

Our study aimed at characterizing the MRSA strains carried transiently by health-care workers (HCWs), and comparing them with invasive and non-invasive strains isolated from patients. The 87 samples were collected over a 3-month period in the autumn of 2004 and were typed by spa sequencing. This analysis identified 14 different spa types, with one class, t032, accounting for over 70 % of all
our MRSA strains. We also screened 14 individual isolates representing each distinct spa type for the carriage of the 7 virulence factors identified by Peacock et al. (2002). This work showed that two of the most common virulence factor profiles found in the original study could be assigned to 12 of the 14 spa types identified in our samples.

METHODS

Screening and sample acquisition. The 87 MRSA strains analysed in this study were isolated from staff and patients associated with a Scottish teaching hospital. The largest single group of isolates (n=46) was obtained from an anonymous study analysing transient MRSA nasal colonization in 512 HCWs, which included staff who worked on wards where MRSA was considered to be either endemic or sporadic. The study was performed over 3 months, and to ensure anonymity, positive results were not followed up to investigate clearance. To compare the MRSA strains recovered from the HCWs with those found in the in-patients, we used two additional groups of samples obtained during the same period of time. The first group consisted of 20 MRSA strains that were isolated from the same wards during the routine analysis of nasal, perineal and axillary swabs obtained from in-patients sampled within 24 h of hospital admission. These patients had no previous history of deep-seated MRSA infection. The second group consisted of 21 MRSA isolates from blood culture. Each of these isolates was obtained from an individual subject, with no repeat isolates included. In addition, 19 of these blood cultures had previously been typed by PFGE at the Scottish Reference Laboratory, and consequently their identification by spa typing could be cross-referenced with the original results.

Identification and purification of chromosomal DNA. All the MRSA isolates were recovered from −70 °C storage on Protech beads (Technical Services Consultants). The strains were streaked on blood agar and inoculated into 2 ml Luria broth for incubation overnight at 37 °C. Prior to DNA extraction, the live cultures were heat-inactivated by incubating at 70 °C for 30 min. The cells from each sample were then harvested by centrifugation and the pellet was resuspended in 180 μl enzyme lysis buffer supplemented with 10 μl achromopeptidase (10 U μl⁻¹). Chromosomal DNA was obtained using the DNeasy Tissue kit (Qiagen) protocol and the chromosomal DNA was eluted in a final volume of 400 μl H₂O.

PCR and DNA sequence analysis. To obtain the strain data for our study, we chose to employ spa typing, a DNA sequence-based method that has proved valuable in several epidemiological investigations (Frenay et al., 1996; Harmsen et al., 2005; Koreen et al., 2004). This technique has been developed as a universal approach for MRSA typing that allows inter-laboratory exchange of information via a standard software analysis package and central internet depository (www.ridom.de) (Frenay et al., 1996; Harmsen et al., 2003). For typing of the polymorphic region of protein A, the x region of the spa gene was amplified using primers 1113F and 1514R (CAGCA-GTAGTGCGGTGGTGGTT) as described previously (Harmsen et al., 2003). The PCR products were purified using the ChargeSwitch PCR Clean-Up kit (Invitrogen) and the resulting DNA fragments were quantified by gel electrophoresis. Bidirectional DNA sequences were obtained using an ABI 3730 Genetic Analyzer and data were analysed using the Ridom StaphType software (Harmsen et al., 2003). The Ridom software assigns a reliability score to the sequence data and places the data into one of 10 categories. For this study, we chose to use only data that the software considered to be either ‘very good’ or ‘excellent’, and therefore repeated the PCR amplification and sequence analysis of 7 spa products. To check the accuracy of our data, we compared our results with PFGE information generated by the Scottish MRSA Reference Laboratory as either part of their routine surveillance of blood cultures (n=19), or, in the case of 10 isolates, as a special request. In total, we compared PFGE and spa identification for 29 of our 87 isolates and the correlation with the Reference Laboratory results indicated that our spa typing was reliable. Data clustering of individual spa types into clonal complexes was achieved using the Based Upon Repeat Pattern (BURP) algorithm set at a cost setting of 4.

For the virulence gene profiling of individual spa types for the presence or absence of the cna, sdrE, sei, eta, hlg and ica genes, we used the PCR amplification procedure described by Peacock et al. (2002). To distinguish between the presence of the fnbA and fnbB genes, we performed in silico PCR analysis using the published S. aureus genomes and several primer sets described in the literature (Kuhn et al., 2006; Nashev et al., 2004; Peacock et al., 2002). Subsequently we chose to use the primers fnbAf and fnbAr (Kuhn et al., 2006). PCR detection of the lukF gene, which encodes the potent leukotoxin PVL, was performed as described previously (Lina et al., 1999). For each set of PCRs, we included one reaction using chromosomal DNA from a positive control strain. The presence or absence of a particular virulence factor was then confirmed by gel electrophoresis and the identification of the correct sized DNA product.

PFGE and antimicrobial susceptibility testing. The PFGE identification and antimicrobial susceptibility testing of the 14 distinct spa types was performed by the Scottish MRSA Reference Laboratory. PFGE typing of Smal (Gibco-BRL)-digested DNA was performed as described previously (Bannerman et al., 1995; MacKenzie et al., 2002), with MRSA identities assigned by comparison with in-house standards. The antimicrobial screening for penicillin, meticillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, tetracycline, gentamicin, fusidic acid, kanamycin, mupirocin, chloramphenicol, trimethoprim, rifampicin, tobramycin and linezolid was also performed at the Reference Laboratory.

RESULTS AND DISCUSSION

Molecular typing

Typing and analysis of MRSA strains responsible for serious infections is now routine in many parts of the world, including Scotland. Uniquely our study compared the MRSA strains transiently colonizing HCWs with MRSA strains circulating within a control group of patient samples. For the 87 isolates that we examined (n=46 HCWs, n=20 in-patient screening, n=21 blood cultures), we identified 14 different spa types that, according to BURP (Harmsen et al., 2003) analysis, clustered into five distinct groups (Table 1).

Overall, the largest individual group of spa types (79/87) belonged to the EMRSA15 complex, with one spa type, t032, predominating (61/87). This was consistent with the fact that EMRSA15 is the main cause of bacteraemias within the hospital, accounting for approximately 80% of the blood cultures typed at the Scottish MRSA Reference Laboratory, and is the dominant clone within the UK (Johnson et al., 2001; Murchan et al., 2004). Similarly, the prevalence of t032 was predictable as this is the second most common spa type deposited on the Spa Server, accounting for 9.83% of all the isolates, and is widely disseminated throughout Europe (Ghebremedhin et al., 2007; Moore & Lindsay, 2002; Murchan et al., 2004). The
PFGE analysis of 14 of our 61 t032 samples confirmed their identification (Table 2), and indicated the presence of four distinct PFGE band variants within one spa class (data not shown).

The other spa types that clustered with t032, with the exception of t022, have not been deposited on the spa server in large numbers (87). Indeed, three of the repeat patterns in the cluster had only been reported once or twice, and txAAs represented a new spa type. Although truncated, txAAs included the N-terminal 26-23 motif and C-terminal 17-25-16-28 motif that is common to all but one of the other EMRSA15 spa types identified in our study. The prediction that this totally novel spa type (txAA) was related to EMRSA15 was subsequently confirmed by a PFGE pattern (15a) that matched to our t025, t718, t756 isolates and several of our t032 strains.

A second cluster, containing five isolates, was identified by the BURP algorithm. This cluster consisted of the t012 and the t018 repeat patterns that are characteristic of EMRSA16. This identification was confirmed for all five samples by PFGE. The three remaining isolates belonged to individual spa types. The first of these, t001, is characteristic of EMRSA3. EMRSA3 is now considered to be a relatively infrequent cause of invasive disease in Scotland, and in our case, the single isolate was obtained during the routine screening of in-patients. The second individual isolate was identified as t044, a spa type that characterizes a widely disseminated PVL-positive community-acquired (CA-MRSA) MRSA found throughout Europe. Consistent with this, the corresponding PFGE identity was PF118a/d, a band pattern that is associated in Scotland with the ST80 European CA-MRSA. The third individual spa type encoded six repeats (04-34-24-34-22-25) and represented a rare spa type, t3209. PFGE of our isolate established it as having a band pattern associated with a particular MRSA strain recognized in Tayside, but rarely seen elsewhere in Scotland. The PFGE data also suggested that it may be closely related to the more common sporadic strain SMRSA108 (D. Morrison, pers. comm.).

Distribution of individual spa types

Our anonymous screen of HCWs revealed no clear difference in the carriage rate associated with staff attending wards where MRSA is considered to be either endemic or sporadic (data not shown). Crucially, a similar distribution of the dominant strain was found in all three sample groups, with spa type t032 accounting for 67% (31/46) of the HCWs isolates, 70% (14/20) of the strains obtained during in-patient screening, and 76% (16/21) of MRSA samples responsible for invasive disease. Statistical analysis confirmed this view (chi-square test; P-value=0.826). Besides the EMRSA15 cluster, we identified two spa types associated with EMRSA16 (n=5) and one associated with EMRSA3 (n=1). None of these samples were associated with invasive disease. Our results also revealed the presence of a number of rare spa types (t531 n=3, t756 n=1, t1302 n=2, tAA n=1, t3209 n=1) and therefore indicated that a variety of MRSA strains are circulating within a hospital environment sampled over a 3-month period.

Virulence profiling

In 2002, Peacock et al. (2002) investigated the distribution of virulence factors in MRSA samples isolated from blood
showed that 8 of the 9 EMRSA15 and both of the antibiogram data for all 14 strains (Table 2). The results showed that 8 of the 9 EMRSA15 and both of the EMRSA16 spa types encoded the ‘D combination’ of fnbA, cna, sdrE, hlg and ica. This combination of genes was the fourth most common in the original study, representing 10.7% of all strains. The one EMRSA15 spa type that did not match this pattern, t022, differed from the other isolates in the carriage of the fnbA gene. Although rare, isolates lacking fnbA and belonging to the CC22 complex, which includes EMRSA15 strains, were reported in the original study (Peacock et al., 2002). The EMRSA3 isolate, t001, lacked the cna gene but carried the other four virulence factors found in the EMRSA15 and EMRSA16 clusters. This ‘A combination’ of genes was the most common in the original study, representing 19.6% of all 334 samples, and the same profile could be assigned to the local sporadic MRSA (SMRSA) t3209. The remaining spa type, t044, lacked both fnbA and cna but did encode sdrE and ica, the only two virulence factors to be found in all 14 strains.

Overall, our results established that 10 of the 14 samples representing the distinct spa types identified in this study carried an identical pattern of virulence factors. Therefore, while the virulence factor profiling could not discriminate between 10 of our 14 individual spa types, it did confirm the presence of a number of key virulence factors that could prove to be ideal molecules for vaccine design and anti-infective drug development. Indeed, the significance of cultures associated with in-patients and nasal swabs obtained from blood donors. This work showed that a subset of seven specific virulence factors were over-represented in invasive MRSA strains.

These included adhesins that bind either fibronectin (fnbA) (Jonsson et al., 1991), collagen (cna) (Patti et al., 1992) or a sialo-bone binding protein (sdrE) (Josefsson et al., 1998; Tung et al., 2000) and three toxins that act as either a serine protease (eta) (Dancer et al., 1990), a pyrogenic toxin superantigen (sej) (Zhang et al., 1998) or a cytolysin (hlg) (Kaneko & Kamio, 2004). The seventh virulence factor was the first gene in the ica operon that is required for efficient biofilm formation (O’Gara, 2007).

To investigate whether the carriage of these virulence factors varied between the different MRSA isolates identified during our study, we examined the carriage of the same 7 genes by the 14 individual spa types we had identified. For the 8 spa types that were found only once, we profiled the isolate available, while for the other 6 spa types, we chose one isolate as representative. In addition to the virulence factor profiling, we determined the carriage of the PVL genes, and obtained PFGE identification and antibiogram data for all 14 strains (Table 2). The results showed that 8 of the 9 EMRSA15 and both of the

<table>
<thead>
<tr>
<th>spa type</th>
<th>PFGE</th>
<th>Antibiogram*</th>
<th>fnbA</th>
<th>cna</th>
<th>sdrE</th>
<th>sej</th>
<th>eta</th>
<th>hlg</th>
<th>ica</th>
<th>Peacock grouping†</th>
<th>Total no. of virulence factors</th>
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<td>t022</td>
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<td>15i</td>
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<td>15a</td>
<td>Pn, Mt, Cx, Cp</td>
<td>+</td>
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<td>15-31</td>
<td>Pn, Mt, Cx, Er, Cp</td>
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<td>txAA</td>
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<tr>
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<td>16a</td>
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<td>D</td>
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<td>CA-MRSA</td>
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<td>t044</td>
<td>116a/c</td>
<td>Pn, Mt, Cx, Te, Fd, Km</td>
<td>–</td>
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<td>+</td>
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<td>–</td>
<td>+</td>
<td>ND</td>
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<td>SMRSA</td>
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<tr>
<td>t3209</td>
<td>118a/d</td>
<td>Pn, Mt, Cx, Er, Cl, Te, Fd, Km, Mp</td>
<td>+</td>
<td>–</td>
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<td>A</td>
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*Pn, Penicillin; Mt, meticillin; Cx, cefoxitin; Er, erythromycin; Cl, clindamycin; Cp, ciprofloxacin; Te, tetracycline; Gn, gentamicin; Fd, fusidic acid; Km, kanamycin; Mp, mupirocin.
†Peacock grouping refers to the combination of seven virulence genes found to be over-represented in clinical strains of MRSA (Peacock et al., 2002).
‡In addition to encoding two of the seven virulence factors, the strain also encodes the gene lukF.

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SdrE as a surface antigen has already been recognized in the development of a four-component subunit vaccine that is protective in mice (Stranger-Jones et al., 2006).

**PFGE and antiobiogram dataset**

The PFGE identification system used by the Scottish Reference Laboratory confirmed our BURP analysis, which had clustered 11 of our 14 distinct spa types into two classes. The PFGE pattern of two single spa types also corresponded to discrete strains associated with either SMRSA or CA-MRSA. The Scottish MRSA Reference Laboratory identified the remaining t001 isolate as a PFGE variant of EMRSA3 referred to as SMRSA112, a local subtype associated with Ninewells Hospital (G. Edwards, pers. comm.). We also noted that the PFGE identified four discrete band variants (a, b, i and t) within the 15 t032 isolates analysed (data not shown). One of these PFGE band patterns (15a) was also characteristic of the t025, t718 and t756 spa types. The antiobiograms of the 14 different spa types were typical of those seen by the Scottish MRSA Laboratory and broadly in line with the literature, which reports a narrowing resistance pattern in epidemic strains (Amorim et al., 2007). The SMRSA t3209 had an extended resistance pattern but borderline meticillin resistance. While this is typical of the strain, its importance as a reservoir of resistance genes appears to be limited by the fact that its occurrence in Scotland is rare and declining (G. Edwards, pers. comm.).

**Conclusion**

To the best of our knowledge, this work represents the first comparison of MRSA strains isolated from HCWs and patients associated with the same wards during the same period of time. Importantly, we established that there was no significant difference between the strains transiently colonizing the nasal tracts of both HCWs and patients, and those causing disease. We also report that four virulence factors are common to 10 of the 14 spa types identified, supporting the idea that these are key mediators of colonization and disease.

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