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 HCWs. 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*.

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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 (Koreen *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

Abbreviations: CA-MRSA, community-acquired MRSA; HCW, health-care worker; MRSA, meticillin-resistant *Staphylococcus aureus*; SMRSA, sporadic MRSA.

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 in 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 enzymic 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 dH_2O .

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-GTAGTGCCGTTTGCTT) 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*, *sej*, *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 *SmaI* (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

Table 1. Frequency of individual *spa* types

<i>spa</i> type	Repeat succession	HCWs	Patient screen	Blood cultures	Total
EMRSA15					
t022	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	3	1		4 (3.5 %)
t025	26-23-23-13-23-29-17-31-29-17-25-17-25-16-28	4	1		5 (4.5 %)
t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	31	14	16	61 (61.7 %)
t379	26-23-23-13-23-31-29-17-25-17-25-16-28	1			1 (0.9 %)
t531	26-23-23-13-23-31-29-17-31-29-17-25-17-16-28	2		1	3 (2.6 %)
t718	26-23-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	1			1 (0.9 %)
t756	26-23-13-23-29-17-31-29-17-25-17-25-16-28		1		1 (0.9 %)
t1302	26-23-23-29-17-31-29-17-25-17-25-16-28	1		1	2 (1.7 %)
txAA	26-23-29-17-25-16-28			1	1 (0.9 %)
EMRSA16					
t012	15-12-16-02-16-02-25-17-24-24	2	2		4 (3.5 %)
t018	15-12-16-02-16-02-25-17-24-24-24	1			1 (0.9 %)
EMRSA3					
t001	26-30-17-34-17-20-17-12-17-16		1		1 (0.9 %)
CA-MRSA					
t044	07-23-12-34-34-33-34			1	1 (0.9 %)
SMRSA					
t3209	04-34-24-34-22-25			1	1 (0.9 %)

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 txAA represented a new *spa* type. Although truncated, txAA 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 $n=2$, txAA $n=1$, 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

Table 2. Virulence profiling of individual *spa* type

<i>spa</i> type	PFGE	Antibiogram*	<i>fnbA</i>	<i>cna</i>	<i>sdrE</i>	<i>sej</i>	<i>eta</i>	<i>hlg</i>	<i>ica</i>	Peacock grouping†	<i>lukF</i>	Total no. of virulence factors
EMRSA15												
t022	15c	Pn, Mt, Cx, Cp	–	+	+	–	–	+	+	ND	–	4
t025	15a	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
t032	15(a,b,i,t)	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
t379	15-70	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
t531	15i	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
t718	15a	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
t756	15a	Pn, Mt, Cx, Cp	+	+	+	–	–	+	+	D	–	5
t1302	15-31	Pn, Mt, Cx, Er, Cp	+	+	+	–	–	+	+	D	–	5
txAA	15a	Pn, Mt, Cx, Cp	+	+	+	–	–	+	+	D	–	5
EMRSA16												
t012	16a	Pn, Mt, Cx, Er, Cl, Cp	+	+	+	–	–	+	+	D	–	5
t018	16n	Pn, Mt, Cx, Er, Cl, Cp	+	+	+	–	–	+	+	D	–	5
EMRSA-3												
t001	112-29	Pn, Mt, Cx, Er, Cl, Cp, Km	+	–	+	–	–	+	+	A	–	4
CA-MRSA												
t044	116a/c	Pn, Mt, Cx, Te, Fd, Km	–	–	+	–	–	–	+	ND	+	2‡
SMRSA												
t3209	118a/d	Pn, Mt, Cx, Er, Cl, Te, Fd, Km, Mp	+	–	+	–	–	+	+	A	–	4

*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). ND, Not described in the original paper.

‡In addition to encoding two of the seven virulence factors, the strain also encodes the gene *lukF*.

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 cytolyisin (*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

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

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 antibiogram 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 antibiograms 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 methicillin 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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REFERENCES

Amagai, M., Matsuyoshi, N., Wang, Z. H., Andl, C. & Stanley, J. R. (2000). Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. *Nat Med* **6**, 1275–1277.

Amorim, M. L., Faria, N. A., Oliveira, D. C., Vasconcelos, C., Cabeda, J. C., Mendes, A. C., Calado, E., Castro, A. P., Ramos, M. H. & other authors (2007). Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. *J Clin Microbiol* **45**, 2881–2888.

Bannerman, T. L., Hancock, G. A., Tenover, F. C. & Miller, J. M. (1995). Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* **33**, 551–555.

Brown, E. L., Dumitrescu, O., Thomas, D., Badiou, C., Koers, E. M., Choudhury, P., Vazquez, V., Etienne, J., Lina, G. & other authors (2009). The Pantone-Valentine leukocidin vaccine protects mice against lung and skin infections caused by *Staphylococcus aureus* USA300. *Clin Microbiol Infect* **15**, 156–164.

Dancer, S. J., Garratt, R., Saldanha, J., Jhoti, H. & Evans, R. (1990). The epidermolytic toxins are serine proteases. *FEBS Lett* **268**, 129–132.

Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A. & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* **13**, 222–235.

Elasri, M. O., Thomas, J. R., Skinner, R. A., Blevins, J. S., Beenken, K. E., Nelson, C. L. & Smeltzer, M. S. (2002). *Staphylococcus aureus* collagen adhesin contributes to the pathogenesis of osteomyelitis. *Bone* **30**, 275–280.

Enright, M. C. & Spratt, B. G. (1999). Multilocus sequence typing. *Trends Microbiol* **7**, 482–487.

Frenay, H. M., Bunschoten, A. E., Schouls, L. M., van Leeuwen, W. J., Vandenbroucke-Grauls, C. M., Verhoef, J. & Mooi, F. R. (1996). Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. *Eur J Clin Microbiol Infect Dis* **15**, 60–64.

Ghebremedhin, B., Konig, W., Witte, W., Hardy, K. J., Hawkey, P. M. & Konig, B. (2007). Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. *J Med Microbiol* **56**, 365–375.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D. & Vogel, U. (2003). 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* **41**, 5442–5448.

Harmsen, D., Claus, H. & Vogel, U. (2005). DNA sequence-based tandem repeat analysis of the *clfB* gene is less discriminatory than *spa* typing for methicillin-resistant *Staphylococcus aureus*. *Int J Med Microbiol* **294**, 525–528.

Johnson, A. P., Aucken, H. M., Cavendish, S., Ganner, M., Wale, M. C., Warner, M., Livermore, D. M. & Cookson, B. D. (2001). 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* **48**, 143–144.

Jonsson, K., Signas, C., Muller, H. P. & Lindberg, M. (1991). Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. *Eur J Biochem* **202**, 1041–1048.

Josefsson, E., McCrea, K. W., Ni Eidhin, D., O'Connell, D., Cox, J., Hook, M. & Foster, T. J. (1998). Three new members of the serine-aspartate repeat protein multigene family of *Staphylococcus aureus*. *Microbiology* **144**, 3387–3395.

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.

Koreen, L., Ramaswamy, S. V., Graviss, E. A., Naidich, S., Musser, J. M. & Kreiswirth, B. N. (2004). *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol* **42**, 792–799.

Kuhn, G., Francioli, P. & Blanc, D. S. (2006). Evidence for clonal evolution among highly polymorphic genes in methicillin-resistant *Staphylococcus aureus*. *J Bacteriol* **188**, 169–178.

Labandeira-Rey, M., Couzon, F., Boisset, S., Brown, E. L., Bes, M., Benito, Y., Barbu, E. M., Vazquez, V., Höök, M. & other authors (2007). *Staphylococcus aureus* Pantón-Valentine leukocidin causes necrotizing pneumonia. *Science* **315**, 1130–1133.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M. O., Gauduchon, V., Vandenesch, F. & Etienne, J. (1999). Involvement of Pantón-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* **29**, 1128–1132.

MacKenzie, F. M., Greig, P., Morrison, D., Edwards, G. & Gould, I. M. (2002). Identification and characterization of teicoplanin-intermediate *Staphylococcus aureus* blood culture isolates in NE Scotland. *J Antimicrob Chemother* **50**, 689–697.

Malachowa, N., Sabat, A., Gniadkowski, M., Krzyszton-Russjan, J., Empel, J., Miedzobrodzki, J., Kosowska-Shick, K., Appelbaum, P. C. & Hryniewicz, W. (2005). Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *Staphylococcus aureus* isolates. *J Clin Microbiol* **43**, 3095–3100.

Moore, P. C. & Lindsay, J. A. (2002). Molecular characterisation of the dominant UK methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16. *J Med Microbiol* **51**, 516–521.

Murchan, S., Aucken, H. M., O'Neill, G. L., Ganner, M. & Cookson, B. D. (2004). Emergence, spread, and characterization of phage variants of

epidemic methicillin-resistant *Staphylococcus aureus* 16 in England and Wales. *J Clin Microbiol* **42**, 5154–5160.

Nashev, D., Toshkova, K., Salasia, S. I., Hassan, A. A., Lammler, C. & Zschock, M. (2004). Distribution of virulence genes of *Staphylococcus aureus* isolated from stable nasal carriers. *FEMS Microbiol Lett* **233**, 45–52.

O'Gara, J. P. (2007). *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol Lett* **270**, 179–188.

Patti, J. M., Jonsson, H., Guss, B., Switalski, L. M., Wiberg, K., Lindberg, M. & Hook, M. (1992). Molecular characterization and expression of a gene encoding a *Staphylococcus aureus* collagen adhesin. *J Biol Chem* **267**, 4766–4772.

Peacock, S. J., Moore, C. E., Justice, A., Kantzanou, M., Story, L., Mackie, K., O'Neill, G. & Day, N. P. (2002). Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infect Immun* **70**, 4987–4996.

Shopsin, B., Gomez, M., Montgomery, S. O., Smith, D. H., Waddington, M., Dodge, D. E., Bost, D. A., Riehman, M., Naidich, S. & Kreiswirth, B. N. (1999). Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* **37**, 3556–3563.

Stranger-Jones, Y. K., Bae, T. & Schneewind, O. (2006). Vaccine assembly from surface proteins of *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* **103**, 16942–16947.

Tung, H., Guss, B., Hellman, U., Persson, L., Rubin, K. & Ryden, C. (2000). A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. *Biochem J* **345**, 611–619.

Zhang, S., Iandolo, J. J. & Stewart, G. C. (1998). The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). *FEMS Microbiol Lett* **168**, 227–233.