EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE

Molecular characterisation of the dominant UK methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16

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Epidemic methicillin-resistant *Staphylococcus aureus* types 15 and 16 (EMRSA-15 and EMRSA-16) are the dominant types of MRSA found in UK hospitals, but accurate designation of strains has been difficult. Restriction fragment length polymorphism (RFLP) profiles of seven core virulence genes were used to classify unambiguously isolates of MRSA from St George's Hospital into two groups corresponding to EMRSA-15 and EMRSA-16. Variants of both EMRSA-15 and EMRSA-16 isolates occurred that had lost virulence genes encoded on mobile genetic elements. EMRSA-16 isolates had core gene profiles identical to a cluster of previously characterised MSSA (methicillin-sensitive *S. aureus*) isolates from St George's Hospital, suggesting that they have arisen from this source, or that loss of the accessory genetic element encoding methicillin resistance is frequent. EMRSA-15 and EMRSA-16 strains were distinct from other MRSA strains previously identified in UK hospitals, and always carried a mobile genetic element encoding multiple superantigens. These results contribute to the understanding of the types of MRSA found in UK hospitals, how they vary and how they arose.

Introduction

Now that the sequencing of six *Staphylococcus aureus* genomes is nearly complete [1] (www.sanger.ac.uk, www.tigr.org, www.genome.ou.edu), it is possible to compare strains to see how they differ genetically. About 78% of genes are conserved between strains with some allelic variation, whereas 22% of genes are accessory, i.e., entirely absent in other strains [2]. The allelic variation in core backgrounds is relatively stable and can be used as an indicator of genetic relatedness between strains. In contrast, the accessory regions are often large stretches of DNA carrying virulence genes, known as genetic elements. Many are mobile and can be identified as bacteriophages, pathogenicity islands, integrated plasmids or transposons [3, 4]. Some are known to transfer at high frequency and might do so in the hospital environment [3, 5]. Therefore, *S. aureus* genomes can be regarded as having relatively stable core backgrounds, but vary significantly by the

incorporation of a selection of accessory regions. This raises several interesting questions about how a strain or clone of *S. aureus* should be defined, particularly for epidemiological purposes.

Allelic variation in the relatively stable core backgrounds has been used to group isolates recently evolved from a common ancestor (clones). Enright *et al.* [6] and Day *et al.* [7] used multi-locus sequencing typing (MLST), the sequencing and comparison of seven housekeeping genes, for this purpose. They grouped isolates into related clusters and explored relationships between clusters. A previous report used a variation on this approach, comparing the sizes of *Cla*I fragments carrying seven core virulence genes among MSSA isolates from hospitals [5]. Instead of detecting every sequence change in each gene, this method identifies sequence polymorphisms at restriction endonuclease sites, re-arrangements, deletions, insertions or duplications in each gene and the surrounding region. The method allowed isolates to be grouped into clusters and relationships between clusters could be established.

Mobile accessory genetic elements can move frequently between isolates and, possibly, even within patients

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during infection [5, 8]. This frequent movement explains some of the difficulties associated with the current epidemiological typing methods, pulsed-field gel electrophoresis (PFGE) and phage typing, in determining the relatedness of *S. aureus* strains. For example, a bacteriophage integrating into the chromosome will cause a PFGE band to 'disappear' and a new band to appear that is 45 kb larger [9]. This is defined as a two-band change. Alternatively, if the phage contains a single *SmaI* site, one PFGE band will be converted to two new bands with a combined increase in size (three-band change), and so on. These patterns can rapidly become difficult to interpret. Similarly, an integrated phage or pathogenicity island will carry 'phage immunity', preventing related phages from lysing the host bacterium and altering the phage typing pattern [3, 4].

Whereas PFGE and phage typing have value in showing whether isolates from an outbreak are related, they are not useful for classifying epidemiologically unrelated isolates. For example, EMRSA-15 is a major clonal group of *S. aureus* in UK hospitals, but 8% of isolates identified by phage typing as EMRSA-15 were probably unrelated to typical EMRSA-15 strains identified by PFGE profile [10]. Of 145 isolates assigned as EMRSA-15 by PFGE typing, 22 different PFGE patterns were identified, each with up to five band differences from the nearest profile. These isolates also showed variation in phage typing pattern, or the loss of a 15-kb pathogenicity island carrying the gene for enterotoxin C (*sec*), or both.

The characterisation of EMRSA-15 and EMRSA-16 strains is important because their emergence in UK hospitals has coincided with a substantial increase in the incidence of MRSA infections over the last 5 years, from 1–2% to 40% of all *S. aureus* infections in UK hospitals [11]. It is estimated that EMRSA-15 and EMRSA-16 now account for >95% of MRSA bacteraemias in the UK [11]. These two types of MRSA are particularly adapted to cause problems in hospitals compared with other MRSA, but the basis for this is unknown. To address these issues accurate classification is necessary together with an estimate of the amount of variation within each clonal type.

In this study the RFLP method (Southern blotting) was used to investigate MRSA isolates at St George's Hospital, a 1200-bed teaching hospital in south London. The seven core gene profiles were used to classify the isolates unambiguously as either EMRSA-15 or EMRSA-16. Variation in the accessory virulence gene carriage was detected within each of these clonal groups. Finally, by comparing these strains with less dominant epidemic UK MRSA strains, accessory genes associated with EMRSA-15 and EMRSA-16 were identified.

Materials and methods

Isolates

Forty-three MRSA isolates from different patients were obtained from the Department of Medical Microbiology at St George's Hospital, London, chosen to reflect the spectrum of hospital MRSA isolates. The isolates were from patients on a range of wards and comprised 26 isolates from blood cultures, 7 from sputum samples, 4 nasal isolates, 6 wound swab isolates, 1 MRSA groin screening swab isolate, 1 drain fluid isolate and 1 catheter urine isolate. Isolates were recovered from nutrient agar slopes at room temperature and transferred to storage at -70°C on MicrobankTM beads (Pro-Lab diagnostics). Representative strains of EMRSA-1 to EMRSA-14 and EMRSA-17 [12] were kindly provided by the Staphylococcal Reference Laboratory, Laboratory for Hospital Infection, Central Public Health Laboratory, Colindale, London.

Methicillin resistance testing

Methicillin resistance determination was based on the modified Stokes method [13]. Briefly, test and control strains were grown on nutrient agar with salt 5% with a 10- μg methicillin disk at 37°C for 18 h, and a resistant strain had a zone of growth inhibition at least 3 mm smaller than the sensitive control. The presence of the *mecA* gene was confirmed by Southern blotting [5].

Southern blotting

Seven core genes were investigated: (i) the accessory gene regulator (*agr*) effector molecule, *rnaIII*; (ii) the regulator *sarA*; (iii) V8 serine protease, *ssp*; (iv) surface protein A, *spa*; (v) intercellular adhesin component, *icaA*; (vi) fibrinogen binding protein A, *fmbA*; and (vii) γ -haemolysin, *hlg*. A range of accessory virulence genes was also tested, including α - and β -haemolysins (*hla*, *hly*), surface-binding proteins (*cna*, *sbi*, *efb*), capsule types 5 and 8 (*cap5*, *cap8*), heat-shock protein 70 (*hsp70*), enterotoxins A–I (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*), toxic shock syndrome toxin-1 (*tst*), leukocidin E (*lukE*), staphylokinase (*sak*) and a serine protease-like protein, *splB*. The genes were chosen because they are known or potential virulence factors. Primers used to amplify each of these genes by PCR were as listed in an earlier report [5]. PCR products were labelled with horseradish peroxidase and hybridised to Southern blot membranes carrying *ClaI*-digested and electrophoresed whole genomic DNA from test isolates. Binding of the PCR probe was detected by enhanced chemiluminescence (Amersham). The size of the *ClaI* fragment carrying the gene in question was determined by comparing band sizes to a standard λ DNA *HindIII* digest [5].

Phage typing

Isolates were kindly phage typed by the Staphylococcal Reference Laboratory.

Results and discussion

Two core gene profiles were identified, with 18 isolates showing one pattern and 25 isolates the other. Phage typing of the first 18 isolates identified 16 as EMRSA-15 and the remaining two were non-typable. These 18 isolates have been called EMRSA-15. Of the 25 isolates with the second core gene profile, 24 were identified by phage typing as EMRSA-16, and one was non-typable. These 25 isolates have been called EMRSA-16. This is illustrated in Table 1, and the two core gene profiles are shown in Table 2. As only two patterns were identified, it is possible that the core genome approach is not very discriminatory. However, a previous study with MSSA [5] and the current study of EMRSA strains (see below) show that this is not the case. Rather the results reflect the dominance of these two clonal types. Therefore, it is proposed that these RFLP patterns could define EMRSA-15 and EMRSA-16 clonal types. MLST would also provide a suitable definition [6, 7].

The core genomes of both EMRSA-15 and EMRSA-16 were highly conserved, but the accessory gene carriage within each of these two groups showed variation. This variation is highlighted in Table 1. Some EMRSA-15 isolates had lost *sec* and *sak* genes, which are carried on pathogenicity islands and phages, respectively. The variation in *sbi* (IgG-binding protein) patterns could be due to the loss of a second copy of the *sbi* gene. As expected, *hly* showed variation because it is an integration site for bacteriophages, including phages carrying *sak*. Amongst the EMRSA-16 isolates, one had lost *tst*, which is carried on a pathogenicity island. Capsule type (*cap8*) had been lost or altered in one isolate, which could account for its increased resistance to phage lysis (Table 1). PM79 had an altered *hly* profile, possibly indicating that a phage that confers immunity to lysis by group III phages (including 75 and 77) had integrated into this gene. Therefore, variation in accessory gene carriage probably accounts for differences in phage profile or PFGE profile typically seen within each of these clonal types. Furthermore, loss of putative virulence genes, such as *sec* and *sak*, from strains still able to cause infection might indicate that these genes have a limited role in infection. On the other hand, the loss of genes only from isolates not associated with disease, such as *tst* in a single nasal isolate, might support their importance in pathogenesis.

Notably, EMRSA-16 had an identical core genome profile to the MSSA *agr* class III cluster from a previous study (Table 2) [5]. Similar results have been reported previously [6]. Furthermore, the accessory

gene carriage pattern of most EMRSA-16 isolates was identical to one of the MSSA isolates of *agr* class III, PM116, including the *hly* RFLP (data not shown) [5]. This suggests that these isolates are very closely related. It could mean that EMRSA-16 emerged as a variant of an MSSA *agr* class III strain that acquired methicillin resistance. Methicillin resistance is due to carriage of the *mec* gene cluster on a genetic element called *SCCmec* [14], and the acquisition of *SCCmec* by an MSSA strain *in vivo* during treatment has been documented [8]. Alternatively, *agr* class III strains could be variants of EMRSA-16 that have lost *SCCmec*. *SCCmec* is unstable in some isolates during storage in the laboratory [15; unpublished observations], so this might also occur in the hospital environment. It is also possible that movement of this element in and out of these strains occurs frequently. These results are also interesting in light of the ongoing discussion about whether MRSA are less virulent than MSSA. The data suggest that there are few differences between some strains of MRSA and MSSA.

Compared with other EMRSA in the UK, EMRSA-15 and EMRSA-16 are more successful at surviving, colonising or spreading in the hospital environment, and this could be due to their distinct core background or to the carriage of unique accessory genes. The core gene profiles of hospital EMRSA-15 and EMRSA-16 isolates were not only different from each other, but were clearly different from other EMRSA strains (Table 2). EMRSA-3, the third most frequently isolated MRSA in the UK, was also unique. EMRSA-1, -4, -7, -9 and -11 were closely related to each other. EMRSA-2, -5, -6, -8, -10, -12, -13, -14 and -17 were similarly related to each other.

Accessory gene carriage between EMRSA-15 and EMRSA-16 compared with the other EMRSAs also varied. The most striking feature was that EMRSA-15, -16 and -3 all carried *seg* and *sei*, whereas none of the other isolates did. These two genes form part of a genetic element containing several enterotoxin genes known as *egc*, the enterotoxin nursery [16], that could play a role in virulence. Similarly, EMRSA-15 and EMRSA-16 did not carry *lukE* and *splB*, but the other EMRSA types did. The results are consistent with the previous report that isolates carrying *egc* are usually missing *lukE* and *splB* [5]. Clearly, *lukE* and *splB* are not essential for EMRSA strains to cause disease or become established in hospitals. EMRSA-15 and EMRSA-16 also had a unique *fntA* RFLP compared with other EMRSA types. All these factors might influence the dominance of EMRSA-15 and EMRSA-16 in hospitals.

The results of this study resolve some of the confusion surrounding the types of MRSA found in UK hospitals. They are predominantly from two distinct lineages that are essentially conserved, and some are related to

Table 1. EMRSA-15 and EMRSA-16 variants

Strain no.	Source	<i>tst</i>	<i>sec</i>	<i>sak</i>	<i>cap5</i>	<i>cap8</i>	<i>hlb</i>	<i>sbi</i>	Phage type
EMRSA-15									
PM25	Blood culture	–	9.4	19	4	–	12 + 19	6 + 14	75
PM28	Blood culture	–	9.4	19	4	–	12 + 19	6 + 14	75
PM31	Blood culture	–	–	19	4	–	12 + 19	6 + 14	75
PM32	Blood culture	–	–	19	4	–	12 + 19	6 + 14	75
PM41	Blood culture	–	–	19	4	–	12 + 19	6 + 14	75
PM56	Blood culture	–	–	–	4	–	4	6 + 14	75
PM60	Nose swab (screen)	–	–	19	4	–	12 + 19	6 + 14	75
PM61	Sputum	–	9.4	19	4	–	12 + 19	6 + 14	75
PM62	Sputum	–	9.4	19	4	–	12 + 19	6 + 14	75
PM77	Blood culture	–	9.4	19	4	–	4 + 12	6 + 14	75
PM78	Blood culture	–	9.4	–	4	–	4	14	75
PM84	Wound swab	–	9.4	19	4	–	12 + 19	6 + 14	75
PM86	Groin swab (screen)	–	9.4	19	4	–	12 + 19	6 + 14	75
PM104	Wound swab	–	–	19	4	–	12 + 19	6 + 14	75
PM112	Wound swab	–	9.4	19	4	–	12 + 19	6 + 14	75
PM136	Wound swab	–	9.4	19	4	–	12 + 19	6 + 14	Non-typable
PM145	Nose swab (screen)	–	9.4	19	4	–	12 + 19	6 + 14	Non-typable
EMRSA-16									
PM5	Sputum	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 77
PM6	Sputum	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 75, 77, 88A, 932
PM7	Sputum	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM11	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM17	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, <u>75</u> , <u>77</u>
PM22	Sputum	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 75, 77, 88A, 932
PM27	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u> , <u>83A</u>
PM30	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 79, <u>75</u> , <u>77</u> , <u>83A</u> , <u>83C</u>
PM33	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM36	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM38	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	<u>29</u> , <u>52</u> , 52A, <u>79</u> , 80, <u>75</u> , <u>77</u> , <u>83A</u> , <u>85</u> , <u>83C</u> , <u>932</u> , <u>95</u>
PM42	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u> , <u>83A</u> , <u>83C</u>
PM53	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	<u>29</u> , <u>52</u> , 77, 932, <u>95</u>
PM59	Nose swab (screen)	–	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM64	Sputum	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 75, 77
PM66	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 75, 77, 83A, 83C
PM67	Blood culture	16	–	7.7	–	–	6.7 + 7.7	6	Non-typable
PM69	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	75
PM73	Blood culture	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 75, 77, 83A, 83C
PM79	Blood culture	16	–	7.7	–	0.8 + 14	7.7 + 12	6	29, 52
PM85	Wound swab	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u> , 83C
PM87	Drain fluid	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>
PM88	Nose swab (screen)	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 75, 77, 83C
PM91	Wound swab	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, 52, 80, <u>42E</u> , <u>47</u> , <u>77</u> , <u>83A</u> , <u>83C</u> , <u>81</u>
PM92	Catheter specimen of urine	16	–	7.7	–	0.8 + 14	6.7 + 7.7	6	29, <u>52</u> , <u>75</u> , <u>77</u>

Isolates, their source, accessory gene carriage and phage type are listed. Only accessory genes showing variation are listed; please refer to the text for a description of each gene. Specific gene variants are highlighted by boxes. Accessory gene data are presented as the size of the *Cla*I fragment encoding the gene in kb, as determined by Southern blotting and comparison to a λ DNA *Hind*III ladder. –, indicates that the gene was not found; +, indicates that more than two *Cla*I fragments hybridised to the probe, suggesting a *Cla*I cut site within the gene or two copies of the gene. Phage typing was kindly performed by PHLS Staphylococcal Reference Laboratory, Colindale, London, underlined phages showed a strong reaction whilst the rest were weak or inhibitory. See Table 2 for core gene profiles of EMRSA-15 and EMRSA-16.

Table 2. Core gene and accessory gene profiles of MRSA

Strain type	Strain no.	Core genes							Accessory genes									
		<i>rnaIII</i> (<i>agr</i> class)	<i>sarA</i>	<i>ssp</i>	<i>spa</i>	<i>icaA</i>	<i>fnbA</i>	<i>hlg</i>	<i>lukE</i>	<i>splB</i>	<i>seg</i>	<i>sei</i>	<i>tst</i>	<i>sak</i>	<i>sea</i>	<i>cna</i>	<i>cap5</i>	<i>cap8</i>
EMRSA-15	PM60	2.3 + 12 (I)	21	6.5	6.6	10	3.8	2.1 + 6 + 20	–	–	8.2	8.2	–	19	–	6	4	–
EMRSA-16	PM64	2.3 + 14 (III)	9.5	7 + 20	7.9	10	3.8	2.1 + 6	–	–	5.8	5.8	16	7.7	7.7 + 8.5	17	–	0.8 + 14
EMRSA-3	M307	2.3 + 9 (II)	21	7	9.4	8	3.8	2.1 + 6	3.8	9.4	5.3	4	–	9	9	–	4	–
EMRSA-1	NCTC11939	2 + 12 (I)	21	9	6.6	10	2.8	2.1 + 6	24	24	–	–	–	6.7	2.5 + 6.7	9.4	–	0.8 + 14
EMRSA-4	M211	2.3 + 12 (I)	21	9	6.6	10	2.8	2.1 + 6	24	24	–	–	–	6.7	2.5 + 6.7	9.4	–	0.8 + 14
EMRSA-7	M063	2.3 + 12 (I)	21	9	6.6	10	2.8	2.1 + 6	24	24	–	–	–	6.7	2.5 + 6.7	9.4	–	0.8 + 14
EMRSA-9	M203	2.3 + 12 (I)	21	9	6.6	10	2.8	2.1 + 6	20	8.9	–	–	–	19	–	9.4	–	0.8 + 14
EMRSA-11	M695	2.3 + 12 (I)	21	9	6.6	6	2.8	2.1 + 6	24	24	–	–	–	6.5	2.5 + 6.5	9.4	–	0.8 + 14
EMRSA-2	NCTC12232	2.3 + 12 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	6.7	2.5 + 6.7	–	4	–
EMRSA-5	M527	2.3 + 7.5 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	6.7	2.5 + 6.7	–	4	–
EMRSA-6	M406	2.3 + 12 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	6.7	2.5 + 6.7	–	4	–
EMRSA-8	M597	2.3 + 14 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	23	–	–	4	–
EMRSA-10	M103	2.3 + 12 (I)	21	7	9.4	8	2.8	2.1 + 6	6	17	–	–	–	7.7	7.7 + 8.5	–	4	–
EMRSA-12	M258	2.3 + 12 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	19	–	–	4	–
EMRSA-13	M274	2.3 + 12 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	19	–	–	4	–
EMRSA-14	M585	2.3 + 12 (I)	21	9	9.4	8	2.8	2.1 + 6	6	17	–	–	–	19	–	–	4	–
EMRSA-17	99ST2758	2.5 + 10 (I)	21	9	9.4	8	2.8	2.1 + 6	5.5	17	–	–	–	6.7	7.7 + 8.5	–	4	–
MSSA <i>agr</i> III	PM116	2.3 + 14 (III)	9.5	7 + 20	7.9	10	3.8	2.1 + 6	–	–	5.8	5.8	16	ND	7.7 + 8.5	17	–	0.8 + 14

Data for a representative of each EMRSA-1 to EMRSA-17 are shown, and are grouped into related clusters based on core gene data. MSSA *agr* class III isolate PM116 [5] is included to allow comparison to EMRSA-16. See text and a previous study [5] for a full description of genes. Data in the table refer to the size of the *Cla*I fragment encoding the gene in kb, as determined by Southern blotting and comparison to a λ DNA *Hind*III ladder. –, indicates that the gene was not found; +, indicates that more than two *Cla*I fragments hybridised to the probe, suggesting a *Cla*I cut site within the gene or two copies of the gene. *agr* class (shown in brackets) was determined according to Moore and Lindsay [5]. ND, not done.

MSSA found in hospitals. There is significant variation between related strains in their carriage of accessory genetic elements encoding virulence genes. The carriage of these genes might impact on the virulence of an isolate or its ability to survive in the hospital environment.

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References

1. Kuroda M, Ohta T, Uchiyama I *et al.* Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 2001; **357**: 1225–1240.
2. Fitzgerald JR, Sturdevant DE, Mackie SM, Gill SR, Musser JM. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proc Natl Acad Sci USA* 2001; **98**: 8821–8826.
3. Betley MJ, Mekalanos JJ. Staphylococcal enterotoxin A is encoded by phage. *Science* 1985; **229**: 185–187.
4. Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. *Mol Microbiol* 1998; **29**: 527–543.
5. Moore PCL, Lindsay JA. Genetic variation among hospital isolates of methicillin-sensitive *Staphylococcus aureus*: evidence for horizontal transfer of virulence genes. *J Clin Microbiol* 2001; **39**: 2760–2767.
6. Enright MC, Day NPJ, 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.
7. Day NPJ, Moore CE, Enright MC *et al.* A link between virulence and ecological abundance in natural populations of *Staphylococcus aureus*. *Science* 2001; **292**: 114–116.
8. Wielders CLC, Vriens MR, Brisse S *et al.* Evidence for in-vivo transfer of *mecA* DNA between strains of *Staphylococcus aureus*. *Lancet* 2001; **357**: 1674–1675.
9. Smeltzer MS, Hart ME, Iandolo JJ. The effect of lysogeny on the genomic organization of *Staphylococcus aureus*. *Gene* 1994; **138**: 51–57.
10. O'Neill GL, Murchan S, Gil-Setas A, Aucken HM. Identification and characterization of phage variants of a strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-15). *J Clin Microbiol* 2001; **39**: 1540–1548.
11. 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.
12. Ayliffe GA, Buckles A, Casewell MW *et al.* Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. *J Hosp Infect* 1998; **39**: 253–290.
13. Working Party of the British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. *J Antimicrob Chemother* 1991; **27** Suppl D: 1–48.
14. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2000; **44**: 1549–1555.
15. Inglis B, El-Adhami W, Stewart PR. Methicillin-sensitive and -resistant homologues of *Staphylococcus aureus* occur together among clinical isolates. *J Infect Dis* 1993; **167**: 323–328.
16. Jarraud S, Peyrat MA, Lim A *et al.* *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J Immunol* 2001; **166**: 669–677.