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Analysis of methicillin-resistant *Staphylococcus aureus* by IS*1181* profiling

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SUMMARY

Variation in the genomic location and copy number of the insertion element IS*1181* in methicillin-resistant *Staphylococcus aureus* (MRSA) was investigated. Sixty-three isolates representing the Jevons type strain (NCTC 10442), phage-propagating strains, and epidemic strains were examined. A PCR amplicon of the insertion element was used to probe genomic restriction endonuclease digests. *Hin*dIII genomic digests gave 25 distinct IS*1181* patterns, while *Eco*RI digests gave 20 patterns. EMRSA-01, -02, -04, -06, -07, -09, -10, -11, -13 and -14 contained the element but could not be subtyped by profiling it. EMRSA-16 did not contain IS*1181*, consistent with a unique evolutionary origin for this major UK epidemic strain. Marked heterogeneity was observed among isolates of EMRSA-03. Each EMRSA-03 strain examined gave a unique pattern, thereby allowing subtyping of an important epidemic phage type for the purposes of hospital cross-infection control.

INTRODUCTION

Staphylococcus aureus is a major cause of nosocomial infection [1]. Within the UK, 16 epidemic methicillin-resistant *Staphylococcus aureus* variants (EMRSA) have been reported [2], the predominant ones being EMRSA-03, EMRSA-15 and EMRSA-16 [3]. Whereas the incidence of EMRSA-15 and EMRSA-16 has continued to increase annually, that of EMRSA-03 remains unchanged [3].

The continuing evolution of antibiotic resistance in *S. aureus* is now of major concern for hospital cross-infection control. Resistance to methicillin was first observed soon after its introduction in 1960 [4]. Recent reports also suggest the emergence of strains resistant to vancomycin, one of the few remaining clinically effective antibiotics [5].

Typing is an important part of the control of MRSA, helping the source of the infection to be traced and thus control measures to be implemented. Over the past 40 years, the method of choice has been bacteriophage typing, a phenotypic technique whereby strains are classified according to their susceptibility to a set of internationally agreed or experimental bacteriophages [6]. Though much information has been gained, some isolates are now phage non-typable or then phage type imperfectly.

Recently, genetic typing based on pulsed-field gel electrophoresis (PFGE) has been applied to strains of *S. aureus* [7, 8]. PFGE compares favourably with other techniques [9–11], but is laborious, and generates patterns which can be difficult to interpret [12].

The identification of microorganisms, using mobile genetic elements such as insertion sequences and transposons has been successfully applied to *Mycobacterium* spp. and *Salmonella* spp. [13]. A number of staphylococcal mobile elements have been characterized, including the insertion sequences IS*256* [14, 15] and IS*257* [16], and the transposons Tn*4001* [17,

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Table 1. *Strains of* Staphylococcus aureus *studied: phenotypic characters and IS1181 profile*

Strain (isolate)	Phage type	Supplementary phages	Coagulase type†	Toxin‡ production	Urease§ production	Protein A production	IS*1181* Profile	
							*Hin*dIII	*Eco*RI
EMRSA*-01 (0)	85+88A/932	620+/622/617/626/630	4	A	++	±	H22	E19
EMRSA-02 (331)	80/85/90/932	616/617/622/626/630	7	A	−	++	H22	E19
EMRSA-03 (1)	80/85/90/932	616/617/622/626/630	7	A	−	++	H22	E19
EMRSA-03 (1)	75/83A/932	618+/620+/623+/629+	1	-ve	−	++	H7	E7
EMRSA+03 (12)	75/83A/932	618+/620+/623+/629+	1	-ve	−	++	H5	E5
EMRSA-03 (19)	75/83A/932	618+/620+/623+/629+	1	-ve	++	H10	E10	
EMRSA-03 (20)	75/83A/932	618+/620+/623+/629+	1	-ve	−	++	H6	E6
EMRSA-03 (52)	75/83A/932	618+/620+/623+/629+	ND‖	-ve	−	++	H11	E11
EMRSA-04 (506)	85/90/932	623+	ND	A	+	++	H19	E19
EMRSA-05 (372)	77/84	618/620	ND	A, B & C	++	+	H24	E24
EMRSA-06 (486)	90/932	NT¶	A	−	++	H19	E19	
EMRSA-07 (063)	84inh	NT	ND	A & C	+	++	H19	E19
EMRSA-08 (597)	83A/83C/932	620+/617/622/630	ND	-ve	++	++	H25	E25
EMRSA-09 (203)	77/84/932	620+/622+626/630	ND	-ve	++	+	H22	E19
EMRSA-10 (037)	77/83A/29/75/85	626+/617/618/622/630	ND	A & B	−	+	H19	E19
EMRSA-11 (695)	84	617/618/620/622	ND	A	++	++	H22	E19
EMRSA-12 (258)	75/83A/83C/932	617/622/629	ND	-ve	−	++	H19	E19
EMRSA-12 (607)	75/83A/83C/932	617/622/629	ND	-ve	−	++	H21	E19
EMRSA-13 (409)	29/83C/932	620+/629/630	ND	-ve	++	++	H22	E19
EMRSA-14 (587)	29+/6/47/54/90/932	629/630	ND	-ve	−	+	H22	E19
EMRSA-15 (2, 3, 4, 17, 22, 28)	75+	NT	2	C	−	++	NT	NT
EMRSA-15 (5)	75+	NT	4	C	−	++	NT	NT
EMRSA-15 (16-Airedale)	75+	NT	6	-ve	−	++	NT	NT
EMRSA-15 (18)	75+	NT	2	C	−	++	H15	ND
EMRSA-15 (29)	75+	NT	2	C	−	++	H15	ND
EMRSA-15 (30)	75+	NT	5	C	−	++	H21	ND
EMRSA-16 (6, 7, 8, 14, 15, 24, 25, 26, 27)	29inh/52inh/75/77/83A	618±	3	A & TSST-1	++	±	NT	NT
EMRSA-16 (K-06)	29inh/52inh/75/77/83A	618±	ND	A & TSST-1	++	±	NT	NT
PS**42E	42E	NT	9	-ve	++	ND	H13	E13
PS 71	3C/55/71	NT	10	TSST-1	++	ND	H14	E14
PS 06	6/47/53/54/75/83A	616/617/620/622/623/626	ND	-ve	++	ND	H21	E19
PS 29	29	NT	ND	C & TSST-1	++	ND	H16	ND
PS-53	53/54/75/77/84/85	617/622	ND	A & B	++	ND	H19	E19
PS 75	53/75/77/84/85	618/626	ND	B	++	ND	H21	E19
PS 94	94/96	NT	ND	B	++	ND	NT	NT

PS 95	95	NT	ND	B	++	ND	NT	NT
PS 96	94/96	NT	ND	B	++	ND	NT	NT
(96/32425)	(III+79)	ND	7	ND	ND	ND	H22	E19
(96/32446)	(I+III)	ND	11	ND	ND	ND	H19	E9
(96/32456)	(I+III)	ND	12	ND	ND	ND	H2	ND
(96/31195)	54/77/83A/932	ND	7	ND	ND	ND	H4	E4
(96/31276)	85/83C	ND	7	ND	ND	ND	H21	E19
(96/20684)	85/81/91/83C	ND	6	ND	ND	ND	H8	E8
(96/12628)	47/54/75/77	ND	8	ND	ND	ND	H20	E20
(96/31885)	I+95/92	ND	8	ND	ND	ND	H21	E19
NCTC 10442[T]	47/53/54/75/77/84/85	616/617/618/622/623/625/626/629/630	5	B	ND	++	H23	E23
(QC-7 France)	77/84	ND	ND	ND	ND	ND	H17	E17
(212 Spain)	29/77/84/932	NT	ND	A	++	±	H18	E18
(94/14013 Germany)	54	625±	8	ND	ND	ND	H12	E12
BM3121	NT	NT	ND	ND	ND	ND	H1	E1

* EMRSA, Epidemic methicillin-resistant *Staphylococcus aureus*; † personal communication (J. V. Hookey); ‡ Toxin A, B, C and/or Toxic Shock Syndrome Toxin-1 (TSST-1), -ve, no toxin produced; § urease-negative, ± weak positive, + positive, ++ strong positive; ‖ ND, not done; ¶ NT non-typable; ** methicillin-sensitive propagating strains (PS) for the international phage set.

18], Tn*554* [19, 20], and Tn*4002* [21]. These are either inserted within, or flank, antibiotic resistance genes [22]. In contrast, one recently described element, IS*1181*, is not associated with antibiotic resistance genes, and has been shown by Southern blot analysis to be present in multiple copies (up to 8) in some European isolates of *S. aureus* [23].

In this study we examined the genomic distribution and copy number of IS*1181*, and assessed its potential use as a genetic typing system for the differentiation of predominant UK epidemic strains of methicillin-resistant *S. aureus*.

MATERIALS AND METHODS

Bacterial strains

Sixty-three strains were studied (Table 1). They included EMRSA 1–16, lytic groups, propagating strains (PS) used for the international phage set, non-epidemic sporadic isolates, selected European isolates and the Jevons strain (NCTC 10442). They included duplicate isolates (96/32446, 331, 19 and 12) and a duplicate positive control strain BM3121 [23].

Bacterial cultures were grown aerobically on 5% horse blood agar plates at 37 °C overnight. The isolation of chromosomal DNA was carried out according to manufacturer's instructions (Tri™ Reagent; Sigma), with modifications. Briefly, cells were suspended in TE glucose (2 mM Tris HCl, pH 8·0; 10 mM EDTA, pH 8·0; 1% w/v D-glucose), and lysed at 37 °C with 50 μl lysozyme (50 mg/ml TE glucose; Sigma) and 100 μl lysostaphin (50 mg/ml TE glucose; Sigma). One hundred μl Tri™ reagent (Sigma), were added, followed by 20 μl of chloroform. The suspension was centrifuged (12000 ***g***) for 15 min, and the supernatant discarded. Thirty μl of 100% ethanol were added, the sample centrifuged (12000 ***g***) for 10 min and the supernatant discarded. The resulting pellet was washed twice with 0·1 M sodium citrate followed by 75% ethanol. The pellet was resuspended in tissue culture grade water (Sigma) and centrifuged (12000 ***g***) for 10 min. The DNA-containing supernatant was transferred to a fresh tube and the concentration estimated by electrophoresis in a 1% agarose gel.

Preparation of labelled IS*1181* probe and Southern blots

The IS*1181* sequence element from *S. aureus* strain BM3121 [26] was amplified by PCR and labelled *in situ* with digoxigenin-11-dUTP (Boehringer–

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Mannheim) according to manufacturer's instructions. The PCR primers were 5′-CTAACGTATAAGCCT-TCGGCC-3′ (nt 564–584), and 5′-GGCGGCCAGT-CCATTATTGGGC-3′ (nt 1766–1745) [23]. PCR was performed on a Perkin–Elmer 480 DNA thermal cycler, at 95 °C for 5 min, followed by 95 °C for 40 s, 50 °C for 25 s, and 72 °C for 3 min for 30 cycles.

Approximately 1·5 μg DNA was digested with 15 U *Hin*dIII (Boehringer–Mannheim) or 15 U *Eco*RI (Boehringer–Mannheim) at 37 °C for 3 h. Samples were electrophoresed overnight at 30 V through a 0·7% agarose gel prepared with 1 × TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8·0). Digoxigenin-labelled lambda DNA cleaved with *Hin*dIII (Boehringer–Mannheim) served as a size marker. DNA fragments were blotted by standard procedures [27] onto Hybond-N nylon membranes (0·45 μm pore size; Amersham).

The denatured digoxigenin labelled *S. aureus* BM3121 IS*1181* probe was hybridized to the membrane bound DNA fragments at 68 °C overnight in 7·5 ml hybridization solution (5 × SSC, 1% blocking reagent [Boehringer–Mannheim], 0·1% *N*-lauroyl-sarcosine, 0·02% SDS) supplemented with 150 μl denatured herring sperm (10 mg/ml; Sigma) Membranes were washed twice in 2 × SSC, 0·1% SDS for 5 min at 25 °C, and twice in 0·1 × SSC, 0·1% SDS at 60 °C for 15 min. The hybridized filters were incubated in anti-digoxigenin-AP-Fab fragments (Boehringer–Mannheim) and developed using the substrates NBT (nitrobluetetrazolium, 75 mg/ml in 70% dimethyl formamide; Sigma) and BCIP (5′-bromo-4-chloro-3-indolyl-phosphate, 50 mg/ml; Sigma).

Analysis of results

Southern blots were scanned with an Epson GT-8000 scanner, and analysed by the GelCompar software (Applied Maths, Kortrijk, Belgium). Cluster analysis was performed using the Jaccard similarity coefficient, and the unweighted pair group method using arithmetic averages (UPGMA).

RESULTS

Digoxigenin-11-dUTP was incorporated *in vitro* during the PCR amplification of the IS*1181* genetic element. As predicted from the sequence data [23] a single band of 1200 bp was seen on ethidium bromide-stained agarose gels, and was found to hybridize to the genome of the positive control strain BM3121. IS*1181* profiles were generated by probing genomic

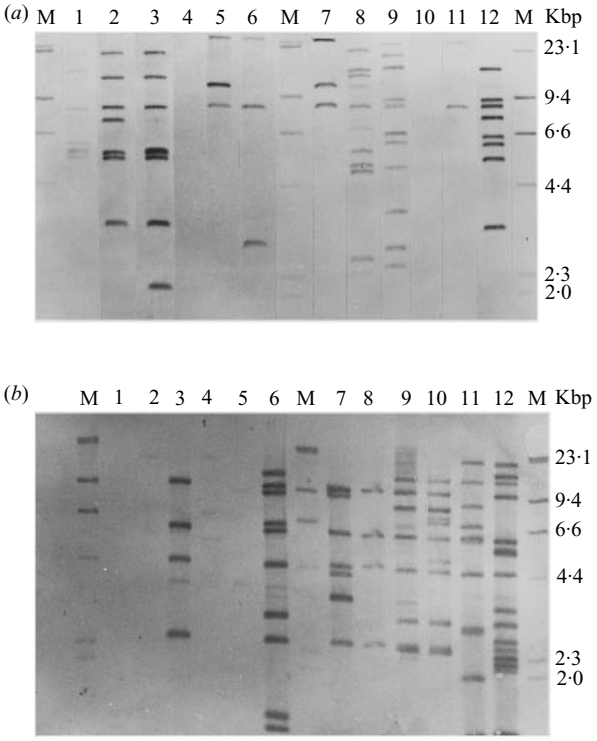

Fig. 1. (*a*) Insertion sequence IS*1181* *Hin*dIII profiles from representative MRSA isolates. Lane 1, EMRSA-03 (isolate 21); lane 2, EMRSA-03 (isolate 19); lane 3, EMRSA-03 (isolate 12); lane 4, EMRSA-15 (isolate 2); lane 5, EMRSA-01 (isolate 9); lane 6, NCTC 10442^T; lane 7, EMRSA-02 (isolate 331); lane 8, isolate 94/14013; lane 9, PS 42E; lane 10, EMRSA-16 (isolate 6); lane 11, EMRSA-12 (isolate 607); lane 12, isolate 96/32446. Lanes marked M, *Hin*dIII-digested lambda DNA marker – digoxigenin-labelled. (*b*) Inseration sequence IS*1181* *Eco*EI profiles from representative MRSA isolates. Lane 1, EMRSA-15 (isolate 2); lane 2, EMRSA-16 (isolate K-06); lane 3, PS 75; lane 4, PS 94; lane 5, PS 96; lane 6, EMRSA-05 (isolate 372); lane 7, EMRSA-08 (isolate 597); lane 8, EMRSA-09 (isolate 203), lane 9, isolate QC-7; lane 10, isolate 212; lane 11, isolate 96/12628; lane 12, BM3121. Lanes marked M, *Hin*dIII-digested lambda DNA marker – digoxigenin-labelled.

Southern blots made with either *Hin*dIII, or *Eco*RI with the digoxigenin-labelled probe (Fig. 1). These IS*1181* profiles were both stable and reproducible.

Phenotypic characteristics

Salient phenotypic and other characteristics of the 63 isolates of *S. aureus* examined are provided in Table 1.

Genetic characteristics

*IS*1181 Hin*dIII profiles*

Of the 63 isolates of *S. aureus* examined, 42 contained IS*1181*. The IS*1181* *Hin*dIII profiles for these 42

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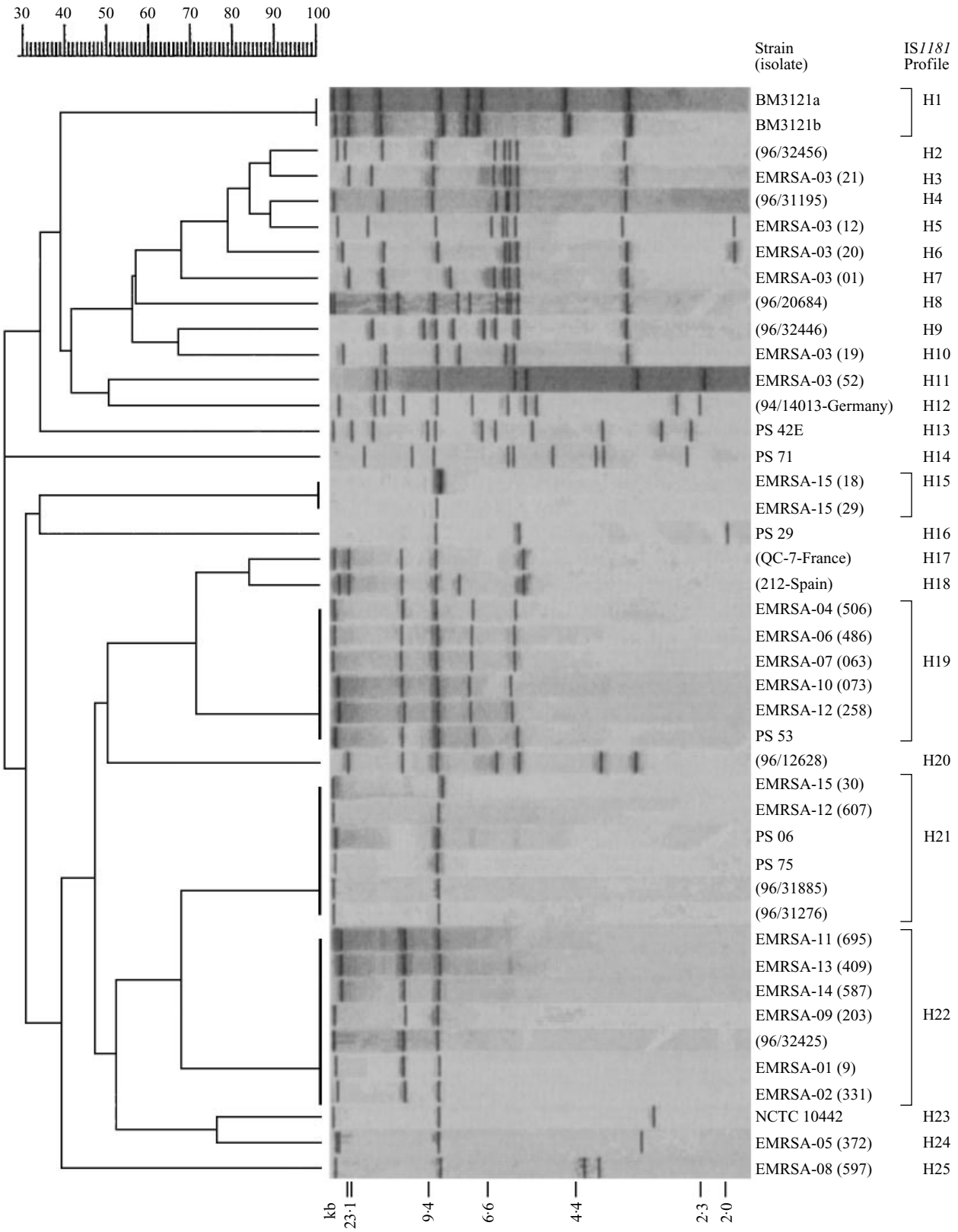

Fig. 2. Cluster analysis of *Hin*dIII IS*1181* profiles for representative MRSA isolates. Note top of gel (*ca.* 25 kb fragments) is adjacent to dendrogram.

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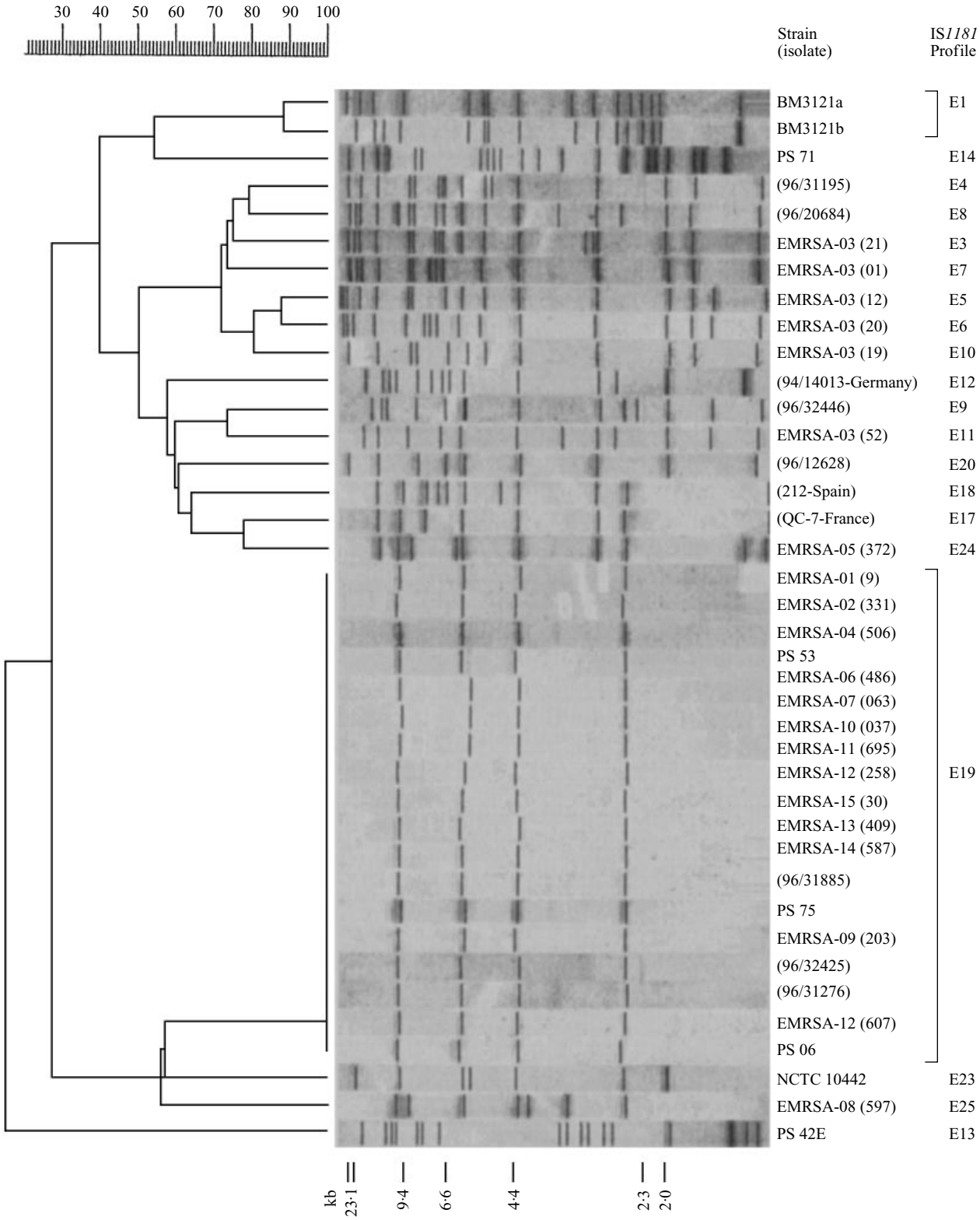

Fig. 3. Cluster analysis of *Eco*RI IS*1181* profiles for representative MRSA isolates. Note top of gel (*ca.* 25 kb fragments) is adjacent to dendrogram.

isolates are given in Figure 2. The homologous profile for strain *S. aureus* BM3121 gave 8 bands that ranged in size from 3 to *ca.* 25 kb. The *Hin*dIII profiles for strain BM3121 (Fig. 2) were reproducible with a variation in migration position between gels of $\pm 2{\cdot}5\,\%$. The number of bands for the *Hin*dIII IS*1181*

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digest varied from 1 (isolates 18, 19) to 12 (isolate 96/20684) and their sizes from 2 to 25 kb (Fig. 2). Also, duplicate isolates (96/32446, 331, 19, 12) run on separate gels gave the same profiles (data not shown). Fifty percent of isolates gave unique profiles (Fig. 2), but other profiles were shared by a number of different phage types (IS*1181* profiles H15, H19, H21 and H22, Fig. 2). The EMRSA-15, -18 and -29 isolates were epidemiologically related and gave the same profile, H15, no pattern being generated by *Eco*RI (Fig. 2). Isolates of EMRSA-03 were distinghished by the presence of three conserved bands at 3·5, 5 and 5·4 kb (Fig. 2). In contrast none of the EMRSA-16 isolates examined contained the IS*1181* element. A common 'core' fragment of approximately 9 kb was present in 98% of isolates.

*IS*1181 Eco*RI profiles*

Of the 42 isolates studied 88% gave readable IS*1181* *Eco*RI profiles (Fig. 3). Strain BM3121 gave 16 bands, varying in size from 0·7 to 25 kb. Duplicate profiles for strain BM3121 were 90% similar between gels (Fig. 3). The number of bands for *Eco*RI digests varied from 4 to 21 (isolate PS71), and their sizes from 0·5 to 25 kb (Fig. 3). A conserved profile E19 was found in almost 50% of the isolates.

DISCUSSION

Bacteriophage typing has been the method of choice for typing *S. aureus* [6]. However, some isolates are now non-typable, or type only with 100 × routine test dilution (RTD) of phage. Alternatives have therefore been proposed based on moleculr methods [25, 26]. Pulsed-field gel electrophoresis (PFGE), has been found to give good discrimination between epidemic strains of *S. aureus* [7, 8], and has replaced bacteriophage typing in some centres [27]. However, PFGE is time-consuming, the patterns can be difficult to interpret [12], and there is no agreed standardization of patterns between investigators [28].

Typing based on mobile elements such as insertion sequences has been successfully applied to *Mycobacterium* spp. and *Salmonella* spp. [13], and typing of *Staphylococcus aureus* has been attempted with the insertion sequences IS*256* [14, 15], IS*257* [16], and the transposons Tn*4001* [17, 18], Tn*554* [19, 20], and Tn*4002* [21]. These elements, however, either contain or flank antibiotic resistance determinants. In contrast, a novel insertion sequence, IS*1181*, recently described by Derbise and colleagues [23], is not associated with antibiotic resistance determinants and is unrelated to any mobile elements so far described in staphylococci. IS*1181* is thought to transpose independently and, as yet, no preferred target site has been found [23]. These features make IS*1181* a good candidate for IS typing of *S. aureus*, independent of drug-resistant phenotypes. Derbise and colleagues [23] evaluated the prevalence of IS*1181* in 52 (predominantly French) isolates of MRSA IS*1181* was found in 79% of strains, indicating that it may be generally suitable for the analysis of MRSA.

In this report IS*1181* analysis of *S. aureus* was carried out with genomic DNA digested with either *Hin*dIII or *Eco*RI. These enzymes were chosen since they have cutting sites outside (*Hin*dIII) and within (*Eco*RI) this element [23]. *Hin*dIII profiles gave better strain discrimination than *Eco*RI profiles (cf. Figs. 2, 3). The copy number of IS*1181* ranged from 1 to 12, while the copy number for French isolates [23] was 2–8. A similar profile H21 (two fragments at *ca.* 25 and 9·0 kb; Fig. 2), could be equated to profile H6 for some French isolates [23]. Fifty percent of our UK isolates gave unique patterns, indicating that IS*1181* profiling may be useful in the discrimination of some MRSA strains. However, isolates for phage types EMRSA-01, -02, -04, -05, -06, -07, -09, -10, -11, -12, -13, -14 gave similar profiles (Fig. 2), suggesting that IS*1181* insertion sites are conserved across a broad range of EMRSA phage types. None of ten EMRSA-16 contained the element. The reason for its apparent absence in this important, currently highly prevalent, phage type is not clear, but the data suggest that EMRSA-16 has a distinct evolutionary origin from other EMRSA strains. This is being investigated further in our laboratory.

Epidemiologically unrelated isolates of EMRSA-03 exhibited marked heterogeneity with respect to their IS*1181* profiles. All had unique *Hin*dIII and *Eco*RI profiles that were distinct from other epidemic phage types. Copy numbers varied from 7 (isolates 19, 52) to 9 (isolate 20). The high level of heterogeneity within the EMRSA-03 isolates compared to other EMRSA phage types may suggest a higher level of IS*1181* transposition in this lineage, which is distinguishable by the presence of three common IS*1181* *Hin*dIII bands (Fig. 2). Of 8 non-epidemic sporadic isolates (non-EMRSA phage types; Table 1), 4 (96/32456, 96/31195, 96/20684, 96/32446) displayed similar profiles to the EMRSA-03 isolates. Although these isolates were diverse with respect to phage type and coagulase gene type, 3 out of 4 contained the 3

common *Hin*dIII bands characteristic of the EMRSA-03 lineage and the other contained 2 of these bands. Since similarities in the number and position of insertion sequences in isolates may indicate a common ancestry [30], there may be a common evolutionary origin between EMRSA-03 and these isolates. IS*1181* profiling will be useful for discrimination between unrelated EMRSA-03 isolates and a significant adjunct to phage typing in investigations where EMRSA-03 is implicated.

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REFERENCES

1. Haley RW. Methicillin-resistant *Staphylococcus aureus*: Do we just have to live with it? Ann Intern Med 1991; **114**: 162–4.
2. Cox RA, Conquest C, Mallagham C, Marples RR. A major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage type (EMRSA-16). J. Hosp Infect 1995; **29**: 87–106.
3. Anonymous. Epidermic methicillin-resistant *Staphylococcus aureus*. Commun Dis Rep 1997; **7**: 191.
4. Jevons MP. 'Celbenin'-resistant staphylococci. BMJ 1961; **1**: 124–5.
5. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 1997; **40**: 135–6.
6. Parker MT. Phage typing of *Staphylococcus aureus*. In: Norris JR, Ribbons DW, eds. Methods in microbiology 1972; **7B**: 1–28.
7. Ichiyama S, Ohta M, Shimokata K, Kato N, Takeuchi J. Genomic DNA fingerprinting by pulsed-field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1991; **29**: 2690–5.
8. Lindhart F, Ziebuhr W, Meyer P, Witte W, Hacke RJ. Pulsed-field gel electrophoresis of genomic restriction fragments as a tool for the epidemiological analysis of *Staphylococcus aureus* and coagulase negative staphylococci. FEMS Microbiol Lett 1992; **95**: 181–6.
9. Nada T, Ichiyama S, Osada Y, et al. Comparison of DNA fingerprinting by PFGE and PCR-RFLP of the coagulase gene to distinguish MRSA isolates. J Hosp Infect 1996; **32**: 305–17.
10. Prevost G, Jaulhac B, Piemont Y. DNA fingerprinting by pulsed-field gel electophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. J Clin Microbiol 1992; **30**: 967–73.
11. Sauliner P, Bourneix C, Prevost G, Andremont A. Random amplified polymorphic DNA assay is less discriminant than pulsed-field gel electrophoresis for typing strains of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1993; **31**: 982–5.
12. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
13. Stanley J, Saunders N. DNA insertion sequences and the molecular epidemiology of *Salmonella* and *Mycobacterium*. J Med Microbiol 1996; **45**: 236–51.
14. Dyke KGH, Aubert S, El Sohl N. Multiple copies of IS*256* in staphylococci. Plasmid 1992; **28**: 235–46.
15. Wei M-Q, Udo EE, Grubb WB. Typing of methicillin-resistant *Staphylococcus aureus* with IS*256*. FEMS Microbiol Lett 1992; **99**: 175–80.
16. Barberis-Maino L, Berger-Bachi B, Weber H, Beck WD, Kayser FH. IS*431*, a staphylococcal insertion sequence-like element related to IS*26* from *Proteus vulgaris*. Gene 1987; **59**: 107–13.
17. Gillespie MT, Lyon BR, Messerotti LJ, Skurray RA. Chromosome- and plasmid-mediated gentamicin resistance in *Staphylococcus aureus* encoded by Tn4001. J Med Microbiol 1987; **24**: 139–44.
18. Jordens JZ, Hall LMC. Chromosomally-encoded gentamicin resistance in 'epidemic' methicillin-resistant *Staphylococcus aureus*: detection with a synthetic oligonucleotide probe. J Antimicrob Chemother 1989; **23**: 327–34.
19. Chikramane SG, Matthews PR, Noble WC, Stewart PR, Dubin DT. Tn554 inserts in methicillin-resistant *Staphylococcus aureus* from Australia and England: comparison with an American methicillin-resistant group. J Gen Microbiol 1991; **137**: 1303–11.
20. Kreisworth BN, McGeer A, Kornblum J, et al. The use of variable gene probes to investigate a multihospital outbreak of MRSA. In: Novick RP, ed. Molecular biology of the staphylococci. New York: VCH Publishers, 1990: 521–30.
21. Skurray RA, Rouch DA, Lyon BR, et al. Multiresistant *Staphylococcus aureus*: genetics and evolution of epidemic Australian strains. J Antimicrob Chemother 1988; **21** Suppl C: 19–38.
22. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol Rev 1987; **51**: 88–134.
23. Derbise A, Dyke KGH, El Sohl N. Isolation and characterization of IS*1181*, an insertion sequence from *Staphylococcus aureus*. Plasmid 1994; **31**: 251–64.
24. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
25. Maslow JN, Mulligan ME, Arbeit RD. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. Clin Infect Dis 1993; **17**: 153–64.
26. Tenover FC, Arbeit R, Archer G, et al. Comparison of

traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J Clin Microbiol 1994; **32**: 407–15.
27. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J Clin Microbiol 1995; **33**: 551–5.
28. Cookson BD, Aparicio P, Deplano A, Struelens M, Goering R, Marples R. Inter-centre comparison of pulsed-field gel electrophoresis for the typing of methicillin-resistant *Staphylococcus aureus*. J Med Microbiol 1996; **44**: 179–84.
29. Richardson JF, Reith S. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. J Hosp Infect 1993; **25**: 45–52.
30. Sawyer SA, Dykhuizen DE, DuBose RF, et al. Distribution and abundance of insertion sequences among natural isolates of *Escherichia coli*. Genetics 1987; **115**: 51–63.