Rapid cost-effective subtyping of meticillin-resistant \textit{Staphylococcus aureus} by denaturing HPLC

F. Jury,\textsuperscript{1} M. Al-Mahrous,\textsuperscript{2} M. Apostolou,\textsuperscript{2} S. Sandiford,\textsuperscript{2} A. Fox,\textsuperscript{3} W. Ollier\textsuperscript{1} and M. Upton\textsuperscript{2}

Correspondence
M. Upton
m.upton@manchester.ac.uk

\textsuperscript{1}Centre for Integrated Genomic Medical Research, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

\textsuperscript{2}Division of Laboratory and Regenerative Medicine, Faculty of Medical and Human Sciences, University of Manchester, Clinical Sciences Building 1, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, UK

\textsuperscript{3}Health Protection Agency (HPA) North West Laboratory, Manchester Medical Microbiology Partnership, Clinical Sciences Building 2, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, UK

The importance of meticillin-resistant \textit{Staphylococcus aureus} (MRSA) in hospital-acquired infection is widely acknowledged. The UK government has stated that MRSA bloodstream infection rates will have to be halved by 2008. Such radical improvements will require advances on several fronts. Screening for MRSA in high-risk patients on arrival at hospital allows isolation of carriers and reduces transmission to staff and other patients. Concurrent subtyping of MRSA could also inform outbreak investigations and long-term epidemiological studies. The variability within the staphylococcal protein A, or \textit{spa}A, gene-repeat region can be used as a marker of short- and long-term genetic variation. A novel application is described of denaturing HPLC (DHPLC) for rapid, inexpensive characterization of \textit{spa}A gene amplification products, without the need for DNA sequence determination. The method allowed rapid and precise sizing of \textit{spa}A gene-repeat regions from 99 \textit{S. aureus} strains and was combined with heteroduplex analysis, using reference PCR products, to indicate the \textit{spa} type of the test isolate. The method allowed subtyping of strains in less than 5 h from receipt of a primary isolation plate. When applied to an outbreak that occurred during this study, the authors were able to demonstrate relatedness of the isolates more than 5 days before results were received from a reference laboratory. If combined with direct amplification from swabs, DHPLC analysis of \textit{spa}A gene variation could prove extremely valuable in outbreak investigation and MRSA surveillance.

INTRODUCTION

Meticillin-resistant \textit{Staphylococcus aureus} (MRSA) is a significant cause of hospital- and community-acquired disease globally. Between June 1999 and September 2002, the reported incidence of nosocomial MRSA infection in the UK was 41.5\%, more than twice the average for Europe as a whole, and the UK was one of only four European countries showing a statistically significant rise in numbers of infections (Tiemersma \textit{et al.}, 2004).

In the UK, detection of MRSA in patient samples with associated confirmatory susceptibility testing takes up to 48 h. Full characterization (e.g. phage typing or PFGE) usually requires up to 1 week, with clear implications for cross-infection control and outbreak identification.

Early identification of MRSA carriers would reduce staffing and financial burdens on hospitals by reducing unnecessary isolation of non-carriers and preventing carriers transmitting MRSA. A 'search and destroy' screening approach is known to have led to the very low levels of hospital-acquired MRSA (HA-MRSA) infections and transmission rates in Dutch hospitals (Wagenvoort, 2000).

Sequence-based approaches, most notably multilocus sequence typing (MLST; Enright \textit{et al.}, 2000), are...
revolutionizing microbial typing, but analysis of multiple loci becomes time-consuming and is expensive.

Protein A of S. aureus, encoded by the spa gene, is an example of a variable-number tandem-repeat locus (van Belkum et al., 1998; Versalovic et al., 1991). The approach of determining the DNA sequence of the repeat units and their succession within the X region, or spa typing, is becoming widely accepted as a valuable method for MRSA subtyping (Harmsen et al., 2003; Oliveira et al., 2001; Shopsin et al., 1999; Walker et al., 1998). Importantly, it has recently been suggested that the spa gene has potential for use as a single marker in studies of both short- and long-term epidemiology (Koren et al., 2004).

A disadvantage of spa typing is the need to sequence the amplified region for full strain characterization. Denaturing HPLC (DHPLC) is a powerful technique that can be used for the separation and quantification of nucleic acids. The approach was developed for the detection of genetic mutation in humans, but has more recently been applied to bacterial typing (Domann et al., 2003; Evans et al., 2004; Hurtle et al., 2002, 2003) and examination of mixed microbial communities (Barlaan et al., 2005; Domann et al., 2003). DHPLC has also been used to analyse MLST PCR products, which allowed changes in meningococcal populations to be tracked following a vaccination programme in Israel (Shlush et al., 2002).

We have used DHPLC to detect the number of repeat units and degree of DNA sequence variation within the spa gene of S. aureus isolates in less than 5 hours, and suggest that DHPLC can be used as a rapid and cheap alternative to spa typing by conventional methods. The nomenclature adopted in this report is based on that used in the Ridom SpaServer (http://www.ridom.de/spaserver/), as this is publicly available and regularly updated.

METHODS

Bacterial strains. A total of 99 isolates were examined (Table 1). Four isolates were identified as meticillin-sensitive Staphylococcus aureus (MSSA). Isolates were predominantly blood culture and screening isolates recovered at UK National Health Service (NHS) bacteriology laboratories in the North-West of England between 1996 and 2005, and reflect the populations of MRSA circulating in the area. The collection also included 13 MRSA strains representing MRSA spread in the community during the study. Phage typing was not carried out, or gave indeterminate results for 23 isolates.

Amplification of spa gene fragments. Genomic DNA was extracted from cultured isolates using Qiamp DNA extraction kits (Qiagen) as per manufacturer’s instructions, with the inclusion of lysostaphin in the lysis buffer. Aliquots (1 µl) of genomic DNA were used as template in PCRs to amplify the X region of the spa gene using previously published primers [F3, 5′-GAACACGTAAAGGC- TTCCATCC-3′ (Koren et al., 2004); 1517R, 5′-GCTTGTGAATGT- CATTCTACG-3′ (Harmsen et al., 2003)]. PCR was carried out using Accuqyme Master Mix (Bioline) as per manufacturer’s instructions using the following cycling conditions: 95°C for 5 min; 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min; and a final extension step of 7 min at 72°C.

DNA sequence determination and analysis. PCR products were purified using PCR Cleanup Columns (Qiagen) as per manufacturer’s instructions, and 3–10 ng was used as template in cycle sequencing reactions with ABI BigDye version 1.1 (Applied Biosystems). Reaction products were analysed using an ABI Prism 3700 DNA analyser.

Collected sequence data were edited, and consensus sequences compiled, using the Staden software suite (http://sourceforge.net/projects/staden). Repeat units were identified using an in-house Microsoft Access database tool that recognized repeats of 24 nucleotides in length.

Information regarding the DNA sequence of individual repeats and the succession of repeat units was compared to data held at the Ridom SpaServer using the publicly accessible search feature (http://spaserver.ridom.de/).

DHPLC wave analysis for spa gene fragment sizing. DHPLC was carried out on the WAVE System 3500 HT DNA fragment analysis equipment (Transgenomic). PCR amplification analysis was carried out using BIOTAQ (Bioline) and reaction products did not require cleanup before DHPLC analysis. Five microlitres of each amplified sample were injected, one sample at a time, through a DNasep cartridge. Separation was performed sequentially on all samples within a batch at a constant temperature of 50°C to ensure non-denaturing conditions, and elution was at a flow rate of 0.75 ml min⁻¹ at a 1:43% acetonitrile gradient. Following DNA sequence analysis of MRSA isolates, a specific size standard was created from samples which contained 3, 5, 7, 9, 11, 13 and 15 repeat units. This size standard was then used to accurately determine the number of repeat units in unknown samples analysed subsequently.

Heteroduplex analysis. Equal amounts (by concentration) of a sample of unknown spa type were mixed with a reference sample of matching repeat unit length and known DNA sequence. A one-tenth volume of a 10× annealing buffer (100 mM Tris/HCl, pH 8.0, 1 mM EDTA, 1 M NaCl) was added to ensure heteroduplex formation of the previously purified products. The mixed products were screened for heteroduplexes by subjecting 20 µl of each mixed sample to a denaturation step (95°C, 5 min) and then a gradual annealing gradient of −1°C per 1:5 min to a final temperature of 23°C. The WAVE system model 3500HT was used to separate 5 µl of product through a 2% linear acetonitrile gradient, at an optimal temperature for partial denaturation of the reference sample. Samples were passed through the UV detector and analysed for evidence of heteroduplex peaks, against a reference homoduplex pattern. The standard buffers were prepared from concentrated triethylammonium acetate (TEAA; 100 ml) to give buffer A, 0.1 M TEAA, and buffer B, 0.1 M TEAA, 25% acetonitrile, pH 7. The oven temperature for optimal heteroduplex separation was determined using the WAVEMAKER version 4.1.0 software (Transgenomic). The temperature giving a 70–80% helical fraction of the wild-type DNA was used.

RESULTS AND DISCUSSION

Reduction of HA-MRSA infection is a major priority across the globe. Improved hospital hygiene will go some way to achieving targets for reduced infection rates (Hota, 2004), but preventing the import of MRSA into hospitals is a key component of any infection control strategy (Ayliffe et al., 1998). Screening of new, high-risk patients that are likely to be carrying MRSA allows isolation of colonized patients (Wernitz et al., 2005a, b). In a recent active screening study
carried out in Germany, isolation of all positive patients led to a predicted reduction of 48% in HA-MRSA infections (Wernitz et al., 2005a).

Being able to carry out such screening rapidly greatly reduces the cost of inappropriate isolation of non-carriers and, more significantly, ensures reduced exposure of hospital staff and patients to MRSA in colonized individuals.

Numerous approaches have recently been suggested as useful for MRSA screening, but generally they do not give any information regarding type or subtype of the isolates.

Table 1. Representatives of the spa gene-repeat variants (based on the nomenclature used in the Ridom SpaServer, http://www.ridom.de/spaserver/) identified in a collection of 99 S. aureus isolates, including isolates from across North West England and pandemic clones

Entries in bold type indicate sequences newly discovered in the present study.

<table>
<thead>
<tr>
<th>Location*</th>
<th>DHPLC repeat no.</th>
<th>Sequence repeat no.</th>
<th>Repeat organization</th>
<th>Phage types (EMRSA types) found†</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>3</td>
<td>3</td>
<td>t777</td>
<td>r26r23r17</td>
<td>MSSA</td>
</tr>
<tr>
<td>NW</td>
<td>4</td>
<td>4</td>
<td>t1304</td>
<td>r26r23r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>5</td>
<td>5</td>
<td>t662</td>
<td>r11r17r34r22r25</td>
<td>29, 52, 52A</td>
</tr>
<tr>
<td>NW</td>
<td>5</td>
<td>5</td>
<td>t803</td>
<td>r07r23r2r12r23</td>
<td>MSSA</td>
</tr>
<tr>
<td>NW</td>
<td>6</td>
<td>6</td>
<td>t880</td>
<td>r09r02r16r34r34</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>6</td>
<td>6</td>
<td>t838</td>
<td>r08r16r34r13r16r34</td>
<td>26, 52, 52A</td>
</tr>
<tr>
<td>NW</td>
<td>6</td>
<td>6</td>
<td>t883</td>
<td>r26r23r17r25r16r28</td>
<td>75 (15)</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>6</td>
<td>t030</td>
<td>r15r12r16r24r24</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>6</td>
<td>6</td>
<td>t897</td>
<td>r07r23r12r21r17r34</td>
<td>MSSA</td>
</tr>
<tr>
<td>P</td>
<td>7</td>
<td>7</td>
<td>t037</td>
<td>r15r12r16r02r25r17r24</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>7</td>
<td>7</td>
<td>t190</td>
<td>r11r17r34r24r34r22r25</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>7</td>
<td>7</td>
<td>t1149</td>
<td>r08r16r34r24r34r17r17</td>
<td>MSSA</td>
</tr>
<tr>
<td>P</td>
<td>9</td>
<td>9</td>
<td>t038</td>
<td>r08r39r34r13r17r34r16r34</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>10</td>
<td>10</td>
<td>t015</td>
<td>r08r16r216r34r13r17r34r16r34</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>10</td>
<td>10</td>
<td>t002</td>
<td>r26r23r17r34r17r20r17r12r17r16</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>10</td>
<td>10</td>
<td>t008</td>
<td>r11r19r212r17r34r24r34r22r25</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>10</td>
<td>10</td>
<td>t012</td>
<td>r15r12r16r25r16r02r25r17r24r24</td>
<td>75, 77, 83A (16)</td>
</tr>
<tr>
<td>NW, P</td>
<td>11</td>
<td>11</td>
<td>t018</td>
<td>r15r12r16r02r16r02r25r17r24r24</td>
<td>75, 83A (16)</td>
</tr>
<tr>
<td>P</td>
<td>11</td>
<td>11</td>
<td>t214</td>
<td>r26r23r17r34r17r20r17r12r17r16r16</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>11</td>
<td>11</td>
<td>t910</td>
<td>r26r23r13r23r23r31r29r17r25r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>13</td>
<td>13</td>
<td>t379</td>
<td>r26r23r13r23r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>13</td>
<td>13</td>
<td>t906</td>
<td>r07r23r13r29r17r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>15</td>
<td>15</td>
<td>t022</td>
<td>r26r23r13r23r31r29r17r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW</td>
<td>15</td>
<td>15</td>
<td>t025</td>
<td>r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28</td>
<td>(Distinct)</td>
</tr>
<tr>
<td>NW</td>
<td>15</td>
<td>15</td>
<td>t1148</td>
<td>r26r23r23r13r31r29r17r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
<tr>
<td>NW, P</td>
<td>16</td>
<td>16</td>
<td>t032</td>
<td>r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
</tbody>
</table>

*DhPLC* repeat no. | Sequence repeat no. | Repeat organization | Phage types (EMRSA types) found† | No. of isolates |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NW, P</td>
<td>11</td>
<td>t1148</td>
<td>r26r23r23r13r31r29r17r31r29r17r25r17r25r16r28</td>
<td>ND</td>
</tr>
</tbody>
</table>

*NW, North West England; P, pandemic isolate.
†An indication of the phage/EMRSA types identified for each spa type. ND, Not determined; NT, non-typable.

http://jmm.sgmjournals.org 1055
examined, information that is vital for effective cross-infection investigation and for long-term epidemiological purposes (Fang & Hedin, 2003; Francois et al., 2004; Huletsky et al., 2005; Levi et al., 2003; Oliveira & Lencastre, 2002; Warren et al., 2004; Zhang et al., 2004).

**Amplification of spa gene repeats and DNA sequence determination**

The X region of the staphylococcal protein A gene is composed of a succession of predominantly 24-nucleotide repeats (Frenay et al., 1994). A small number of repeat units of 21 and 27 nucleotides have now been described [Koreen et al., 2004; data held at the Ridom SpaServer (http://www.ridom.de/spaserver/)].

All isolates in the present study yielded a PCR product that could be used to estimate the number of repeats from migration on agarose gels (data not shown). DNA sequence analysis confirmed the number of repeat units and revealed the succession of individual repeats (Table 1). The number of spa repeats identified ranged from 3 to 16, although the majority were of 11 (30/99), 15 (14/99) or 16 (30/99) units in length (Table 1). This is not surprising in a group of isolates recovered from cases of infection, as higher numbers of repeat units in MRSA have been correlated with an increased propensity to cause infection (Frenay et al., 1994; Walker et al., 1998).

Of the isolates studied that had been phage typed, the majority were epidemic MRSA (EMRSA) types 15 (n=41) or 16 (n=27). EMRSA strains are particularly successful pathogens able to spread rapidly (Marple et al., 1986), and EMRSA types 15 and 16 cause the majority of nosocomial S. aureus infections in the UK (Ayliffe et al., 1998; Johnson et al., 2005). A clear correlation was observed between these EMRSA types and 15/16 or 11 repeats, respectively (Table 1). Of the 30 isolates that carried a spa gene with 11 repeat units, 28 were EMRSA type 16 (including two EMRSA 16 variant strains). The two remaining isolates in this group had not been phage typed. Two other EMRSA type 16 isolates were included in the study and both carried genes with 10 repeat units. Of note here is the observation that the EMRSA strains with 11 repeats only differed from those with 10 repeats in the duplication of the terminal repeat unit (r24) in the latter sequence type (Table 1). Such a difference implies the evolution of EMRSA 16 strains carrying 10 repeats into those with 11 repeats by slipped-strand mispairing (SSM), a phenomenon that has been documented before (Kahl et al., 2005). Examination of isolate recovery dates showed that the ancestral clone with 10 repeats is still circulating in the region (data not shown). The finding that strains with 11 repeats are predominant supports the above suggestion that longer repeat regions are correlated with increased pathogenicity (Frenay et al., 1994; Walker et al., 1998).

In the current collection, 44 isolates were found to have 15 or 16 repeat units. Of these, seven had not been phage typed or were non-typable (~ 70 % of phage non-typable strains are EMRSA type 15; A. Kearns, personal communication). The remaining isolates with 15 or 16 repeat units were EMRSA type 15. Four other EMRSA type 15 isolates had a range of repeat unit lengths (Table 1). Evidence of evolution through SSM is also apparent in these isolates.

Clearly, and in support of previous findings, spa typing was more discriminatory than phage typing and good congruence was observed between lineages described by each technique (Shopsin et al., 1999; Walker et al., 1998).

**Incidence of spa types**

Up-to-date information on the incidence and diversity of spa repeats can be obtained by accessing the Ridom SpaServer (http://www.ridom.de/spaserver/). In this database, repeat unit types are designated rN, rN+1 etc. in order of discovery. The repeats are organized into different combinations of repeat unit successions (Ridom spa types) and numbered tN, tN+1 etc. The results of the present study contrast with the data held on the Ridom SpaServer, which contains predominantly German isolates. Over half of the isolates were of three types, namely t018 (n=28 isolates), t032 (n=30) and t025 (n=11). These types were present in the Ridom SpaServer at prevalences of 1-02, 9-52 and 0-02 %, respectively. Type t003, the most prevalent in the Ridom database (16-07 %), was not found in our study.

The regional diversity of strains within Europe has been highlighted in this study. Global populations of MRSA are known to show regional variability against a background of a small number of clones with widespread distribution (Diekema et al., 2000; Enright et al., 2002). Regional variations in spa type have been reported elsewhere (Oliveira et al., 2001), and these would favour the DHPLC approach we propose (see below).

A number of isolates carried spa genes with previously unreported successions of known repeat units. These sequences were submitted to the server for addition to the database and designation of new repeat codes (Table 1).

**spa repeat unit sizing by DHPLC analysis**

In every case, the number of repeat units identified in each isolate by WAVE analysis matched exactly those determined by agarose gel electrophoresis and DNA sequence analysis (Table 1). Sizing of bands took less than 14 min per sample, and was greatly assisted by the construction of a size standard containing repeat units amplified from representative isolates (Fig. 1). It could be argued that this is not more time efficient than gel electrophoresis, but small sample numbers could be sized more rapidly by WAVE analysis than by electrophoresis, and large numbers (up to 192) could be batch-processed. Of note is the fact that results can be accessed in real time, as samples are being analysed.
Heteroduplex analysis for rapid strain subtyping

Each heteroduplex profile took 2.5 min per sample, including column regeneration and equilibration. Heteroduplexing the amplicons from test organisms with reference DNA generated either a homoduplex pattern indicating sequence homology or a heteroduplex profile (Fig. 2). Where the test and reference samples were of the same allele, a single homoduplex peak was observed (Fig. 2A). When a test sample with a variant allele was mixed with the reference sample, a heteroduplex profile was observed (Fig. 2B). Several different elution profiles were recorded for each size group tested for which more than one allele was observed.

The sensitivity of the WAVE technology has been reported to be close to 100% (Liu et al., 1997; O'Donovan et al., 1998), therefore a match to a single homoduplex reference peak is sufficient to confirm a spa type without the need for sequencing. Similar heteroduplex profiles can be observed with test samples of a fixed repeat unit length, but different DNA sequence, so at present it is not possible to assign particular heteroduplex profiles to individual spa types. We would therefore initially suggest sequencing of the spa gene of isolates giving a heteroduplex pattern, rather than exhaustive testing against a panel of reference DNA samples.

Demonstration of utility of DHPLC for outbreak investigation

During the study period, an outbreak of suspected multi-resistant MRSA occurred on the intensive care unit at a local hospital. Five isolates were subjected to the standard procedures used for outbreak strain characterization, being referred to the HPA reference laboratory, Colindale, UK.

Isolates were phage and PFGE typed and confirmed as EMRSA type 16 (n=4) and EMRSA type 15 (n=1) (Table 2). Data were returned to the bacteriology laboratory in Manchester 6 days after the outbreak was first suspected.

On the same day that the isolates were received in our laboratory, spa gene PCR was carried out, followed by DHPLC analysis. Within 5 hours total processing time from receipt of the isolates on primary isolation plates, it was possible to demonstrate a relationship between four of the isolates (all spa type t018 and thus probably EMRSA type...
Based on nomenclature used in the Ridom SpaServer (http://www.ridom.de/spaserver/).

We observed such a profile in the present study with a mixed PCR product (data not shown). The DHPLC apparatus can be used to separate individual peaks for subsequent heteroduplexing or DNA sequence determination.

By exploiting the ability of DHPLC to accurately size PCR amplicons and to differentiate variable DNA sequences through heteroduplex analysis, we have developed a novel method for subtyping MRSA in less than 5 h, without the need for costly DNA sequence analysis.

Capital costs for DHPLC apparatus are relatively high (€120 000), but they are comparable to those required for DNA sequence analysers, and consumable costs are minimal (~€0-60 per sample). Importantly, we envisage this technique having applicability to a range of other important nosocomial pathogens, some of which we are currently targeting. The technique is easy to carry out and can be adapted to a batch process format for high-throughput screening programmes. As such, DHPLC could form a core platform for rapid bacterial subtyping for surveillance and epidemiological purposes, including outbreak investigation.

**Proposed application of DHPLC analysis to active MRSA screening**

For widespread application of the DHPLC approach, we suggest that sequence-based methods are initially used to identify predominant lineages in a particular geographic region (Oliveira et al., 2001). Amplified **spa** gene products from these strains would then be used as ‘reference’ samples in DHPLC heteroduplex analysis of any products amplified from patient samples. A homoduplex pattern would indicate that a test isolate was the same as the reference. Heteroduplex traces would indicate that DNA sequence determination should be carried out. With the current strain collection, sequencing would not have been required for any isolate with 11 repeats (EMRSA type 16). For the two other predominant groups (15 and 16 repeats; EMRSA type 15), sequencing would only have been carried out on three occasions during analysis of 44 isolates.

A future improvement of the DHPLC protocol will be the performance of spa typing directly on material contained in patient swabs. The anterior nares, one of the usual screening sites for MRSA carriage, harbour numerous organisms. In the unlikely event that a person is carrying two or more different **S. aureus** strains, we are confident that the approach described here would allow subtyping of all strains yielding a PCR product. If multiple strains are present with different-length repeat regions, this will clearly be seen as multiple peaks with different retention times on a ‘sizing’ run. We observed such a profile in the present study with a

**Table 2.** Subtyping details for MRSA strains isolated as part of a suspected outbreak of multi-resistant **S. aureus** in North West England in spring 2005

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Repeat no.</th>
<th>Ridom spa type*</th>
<th>PFGE type†</th>
<th>Phage type†</th>
</tr>
</thead>
<tbody>
<tr>
<td>157</td>
<td>11</td>
<td>t018</td>
<td>EMRSA 16 new variant A</td>
<td>EMRSA 16</td>
</tr>
<tr>
<td>159</td>
<td>11</td>
<td>t018</td>
<td>EMRSA 16 new variant A</td>
<td>EMRSA 16</td>
</tr>
<tr>
<td>201</td>
<td>16</td>
<td>t032</td>
<td>EMRSA 15 variant B1</td>
<td>EMRSA 15</td>
</tr>
<tr>
<td>202</td>
<td>11</td>
<td>t018</td>
<td>EMRSA 16 new variant A</td>
<td>EMRSA 16</td>
</tr>
<tr>
<td>203</td>
<td>11</td>
<td>t018</td>
<td>EMRSA 16 new variant A</td>
<td>EMRSA 16</td>
</tr>
</tbody>
</table>

*Data supplied by Laboratory of HealthCare Associated Infection, HPA, Colindale, UK.

**ACKNOWLEDGEMENTS**

We are grateful to Dr Mark Enright for the kind provision of some of the isolates included in the present study. We would like to thank Dr Angela Kearns at the Laboratory of HealthCare Associated Infection, HPA, Colindale, UK, for carrying out phage typing on a number of isolates.

**REFERENCES**


