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# Analysis of an Outbreak of Non-Phage-Typeable Methicillin-Resistant *Staphylococcus aureus* by Using a Randomly Amplified Polymorphic DNA Assay

A. TAMBIC, E. G. M. POWER,* H. TALSANIA, R. M. ANTHONY, AND G. L. FRENCH

*Department of Microbiology, UMDS of Guy's and St. Thomas's Hospitals, London SE1 7EH, United Kingdom*

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**A cluster of methicillin-resistant *Staphylococcus aureus* (MRSA) infections among patients on an intensive care unit (ICU) was detected by routine infection control surveillance. In the period from 5 January to 22 June 1995, 10 patients on the ICU and a further 6 patients (5 on one ward that had received colonized patients transferred from the ICU) were affected by MRSA strains with the same antibiotic susceptibility patterns. Seven (44%) of these 16 colonized patients developed MRSA bacteremia. MRSA isolates with the same characteristics were also found on the hands of one member of the ICU staff. The isolates were untypeable by phage typing, but 15 of 17 outbreak strains analyzed genetically had identical randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) profiles. A single strain of MRSA that was nontypeable by phage typing and that was isolated on the ICU on 1 January and six nontypeable and epidemiologically unrelated MRSA isolates all had RAPD profiles distinct from that of the outbreak strain. Implementation of strict infection control measures stopped the further spread of MRSA on the ICU, the affected general ward, and seven other wards that received MRSA carriers from the ICU. Although nontypeable by phage typing and not previously recognized as an epidemic strain, this strain of MRSA was readily transmissible and highly virulent. RAPD typing was found to be a simple, rapid, and effective method for the epidemiological investigation of this outbreak, and performance of typing by this method was simpler and less time-consuming than that of typing by PFGE. RAPD typing may have more general application for the study of *S. aureus* infections in hospitals.**

Until the mid-1970s, methicillin-resistant *Staphylococcus aureus* (MRSA) strains usually occurred sporadically, were usually resistant only to beta-lactam antibiotics, and were not a serious clinical problem. Today, MRSA strains have become resistant to a wide range of other antibiotics and are a major cause of nosocomial infections throughout the world (3, 5). A variety of typing techniques are available to help determine the source and transmission routes of MRSA strains within a hospital (16, 27), with the most common technique being bacteriophage typing (17). However, some MRSA strains may be nontypeable by this method, and the limitations of phenotypic typing have stimulated the development of DNA-based techniques. Plasmid profile analysis was the first genotypic method used in epidemiological studies of *S. aureus* (11, 13, 27). Chromosomal DNA has been analyzed by a variety of techniques, including conventional restriction enzyme analysis, restriction fragment length polymorphism analysis, ribotyping, pulsed-field gel electrophoresis (PFGE), and PCR-based methods.

Randomly amplified polymorphic DNA (RAPD) assays use short primers with an arbitrary sequence to amplify genomic DNA in a low-stringency PCR. These primers randomly hybridize with chromosomal sequences that vary among different strains and that produce different amplification products. These products can be separated by gel electrophoresis to produce fingerprints or patterns characteristic of different epidemiological types. The method is attractive because it is simple to perform and, theoretically, can be applied to any organism (18). In this paper we describe the use of RAPD analysis to investigate a cluster of MRSA infections detected by routine infection control surveillance on the intensive care unit (ICU) at St. Thomas's Hospital.

* Corresponding author. Mailing address: Department of Microbiology, UMDS of Guy's and St. Thomas's Hospitals, London SE1 7EH, United Kingdom. Phone: 44 171 922 8385. Fax: 44 171 928 0730. E-mail: e.power@umds.ac.uk.

## MATERIALS AND METHODS

**MRSA surveillance and control measures.** Surveys for MRSA isolates were performed daily through the pathology laboratory computer system. The index patient (patient 1) was admitted to the ICU on 2 January 1995, and he was found to be MRSA positive on 5 January (Table 1; Fig. 1). Another patient (patient A) colonized with an MRSA strain with a susceptibility pattern different from that of the strain colonizing patient 1 had been on the ICU on 3 January, and an MRSA strain with a pattern similar to that of the strain colonizing patient 1 was detected on 30 January (patient 2). Patients in the ICU were screened for MRSA carriage by taking swabs from the anterior nares, throat, axilla, groin, and perineum. MRSA strains were isolated from wound swabs, blood cultures, sputum, and MRSA screen swabs. All the staff in the ICU were screened on two occasions by taking swabs of their hands, anterior nares, and any cutaneous lesions. By 21 March another nine patients on the same ward had been infected or colonized with MRSA strains with the same antibiotic susceptibility pattern as the index strain (resistance to penicillin, gentamicin, amoxicillin-clavulanate, erythromycin, methicillin, clindamycin, rifampin, and neomycin and susceptibility to tetracycline, co-trimoxazole, fusidic acid, mupirocin, and vancomycin). By 22 June 1995, another five patients (patients 10, 12, 15, 16, and 17) on another ward (general surgical ward P) had been affected by MRSA strains with the same antibiotic susceptibility patterns as the index strain. In addition, a similar strain was isolated from a patient (patient 6) on cardiothoracic surgical ward D on 15 February.

Once the outbreak was recognized, hospital wards were visited by members of the infection control team. Initially, efforts were concentrated on the ICU, but later other wards receiving patient transfers from the ICU were targeted. Although it was hospital policy to implement the control measures for MRSA described in the guidelines produced by the Working Party of the British Society for Antimicrobial Chemotherapy and the Hospital Infection Society (20), it was obvious that these guidelines were not always being followed. The policy was circulated and explained to staff and was strictly applied. Patients and staff on wards with MRSA were screened for carriage; carriers were isolated or sent home and were treated with mupirocin nasal ointment and chlorhexidine baths. Carriers remained in isolation or were excluded until three screens showed that

TABLE 1. Characteristics of patients and MRSA isolates

| Patient | Ward | Site of isolation | Date of isolation of first MRSA isolate (day.mo.yr) | Susceptibility pattern[a] | RAPD type |
|---|---|---|---|---|---|
| A | ICU | Wound | 03.01.1995 | P,G,A,E,M,N,TMP | A |
| 1 | ICU | Blood | 05.01.1995 | P,G,A,E,M,C,R,N | B |
| 2 | ICU | Wound | 30.01.1995 | P,G,A,E,M,C,R,N | B |
| 3 | ICU | Blood | 02.02.1995 | P,G,A,E,M,C,R,N | B |
| 4 | ICU | Sputum | 08.02.1995 | P,G,A,E,M,C,R,N | ND[b] |
| 5 | ICU | Wound | 09.02.1995 | P,G,A,E,M,C,R,N | B |
| 6 | Ward D | Wound | 15.02.1995 | P,G,A,E,M,C,R,N | B |
| 7 | ICU | Blood | 19.02.1995 | P,G,A,E,M,C,R,N | B |
| 8[c] | ICU | Screen | 19.02.1995 | P,G,A,E,M,C,R,N | B |
| 9 | ICU | Blood | 07.03.1995 | P,G,A,E,M,C,R,N | B |
| 10 | Ward P | Wound | 15.03.1995 | P,G,A,E,M,C,R,N | B |
| 11 | ICU | Blood | 17.03.1995 | P,G,A,E,M,C,R,N | B |
| 12 | Ward P | Wound | 19.03.1995 | P,G,A,E,M,C,R,N | B |
| 13 | ICU | Wound | 20.03.1995 | P,G,A,E,M,C,R,N | B |
| 14 | ICU | Blood | 21.03.1995 | P,G,A,E,M,C,R,N | B |
| 15 | Ward P | Wound | 10.05.1995 | P,G,A,E,M,C,R,N | ND |
| 16 | Ward P | Wound | 12.05.1995 | P,G,A,E,M,C,R,N | B |
| 17 | Ward P | Blood | 22.06.1995 | P,G,A,E,M,C,R,N | B |
| 18 | Unrelated[d] | | | P,G,A,E,M,C,R,N,Tc | C |
| 19 | Unrelated | | | P,G,A,E,M,C,R,N,Tc | D |
| 20 | Unrelated | | | P,G,A,E,M,C,R,N,Tc | E |
| 21 | Unrelated | | | P,G,A,E,M,N,Mu | F |
| 22 | Unrelated | | | P,G,A,E,M,N,Tc,FA | G |
| 23 | Unrelated | | | P,G,A,E,M,C,R,N,Tc | H |

[a] Resistant to penicillin (P), gentamicin (G), amoxicillin-clavulanate (A), erythromycin (E), methicillin (M), clindamycin (C), rifampin (R), neomycin (N), trimethoprim (TMP), mupirocin (Mu), fusidic acid (FA), or tetracycline (Tc).
[b] ND, not done.
[c] Patient 8 was an ICU nurse.
[d] Unrelated isolates were obtained from other hospitals.

they were clear of MRSA. Hand washing with chlorhexidine disinfectants by staff after caring for each patient was strictly enforced. Regular visits to the wards ensured that the policy was being followed, and laboratory surveillance for MRSA isolates continued. No further MRSA strains with the same susceptibility pattern as the index strain were isolated from any other patient from 18 June to 1 August 1995.

**Strain identification.** All *S. aureus* isolates were identified by routine laboratory procedures. Gram-positive, catalase-positive cocci were tested for mannitol fermentation on salt mannitol agar plates, clumping factor was detected by using the Staphaurex kit (Wellcome Diagnostics), and organisms were confirmed to be *S. aureus* by the tube coagulase test.

**Antibiotic susceptibility testing.** Antibiotic susceptibility testing was performed by the disk diffusion method for methicillin, penicillin, gentamicin, amoxicillin-clavulanate, erythromycin, clindamycin, rifampin, tetracycline, cotrimoxazole, fusidic acid, mupirocin, neomycin, and vancomycin according to British Society for Antimicrobial Chemotherapy guidelines (30).

**Bacteriophage typing.** Bacteriophage susceptibility testing was kindly performed by the Central Public Health Laboratory, Colindale, London, United Kingdom, with the international set of *S. aureus* typing phages.

**DNA isolation.** DNA was isolated as described previously (1), except that the pelleted bacterial cells were first treated with 50 μl of lysostaphin (50 μg/ml) and 100 μl of lysozyme (2 mg/ml) (Sigma Chemical Co.) for 1 h at 37°C.

**Typing by RAPD analysis.** We used four primers that in pilot experiments had shown a good ability to discriminate *S. aureus* strains for typing purposes. The nucleotide sequences of the primers are listed in Table 1. For each sample we used 200 μM (each) nucleotide (Sigma), 5 μl of 10× reaction buffer, 500 nM primer, 50 ng of template DNA, and 2 U of *Taq* XL DNA polymerase (Northumbria Biochemicals Ltd., Cramlington, United Kingdom), and the mixture was made up to 50 μl with molecular biology-grade water. This mixture was overlaid with 50 μl of mineral oil. RAPD cycling parameters were 94°C for 5 s, 34°C for 30 s, and 72°C for 1 min for 35 cycles, with a final extension of 72°C for 5 min. Reactions were carried out in a Hybaid Thermal Reactor (Hybaid, Teddington, United Kingdom). The products were electrophoresed on 2% agarose gels in 0.5× TBE (Tris, borate, EDTA) buffer. Synthetic PCR molecular size markers (Cambridge Bioscience Ltd., Cambridge, United Kingdom) were included in each gel. The gels were run for 5 to 6 h at 60 V to allow for the complete separation of the bands. DNA profiles were visualized with UV light after ethidium bromide staining and were photographed with a red filter and Polaroid 667 film. Analyses were performed with the two individual primers EP017 and EP007 alone and with the combinations EP015 + KAY1 and EP007 + KAY1 (Table 2).

**Computer analysis of RAPD fingerprints.** Polaroid photographs of the gels were scanned and saved as 256-color TIF files. These images were normalized, a similarity matrix was produced by the Dice coefficient, and a dendrogram was constructed from the resulting data by the unweighted pair group method with arithmetic means; these analyses were performed by the GelCompar computer program (Applied Maths, Kortrijk, Belgium) (26).

**PFGE typing.** The isolates were also subjected to typing by PFGE. Briefly, 5 to 10 colonies of an overnight growth from a Columbia blood agar plate incubated at 37°C were harvested into 100 μl of 1 M NaCl–10 mM EDTA (pH 7.5), and 3 μl of 1 mg of lysostaphin per ml was added. The preparation was mixed with an equal volume of 1% low-melting-point agarose at 50°C, allowed to set in a mold, and incubated at 37°C for 4 h in 1 ml of 1 M NaCl–10 mM EDTA (pH 7.5). The liquid was removed and replaced with 1 ml of 0.5 M EDTA (pH 7.5) containing 1 mg of proteinase K (Boehringer Mannheim) and 1% *N*-laurylsarcosine (Sigma), and then the mixture was incubated at 50°C for 18 h. The plugs were then washed and digested with the restriction enzyme *Sma*I as described previously (14). Pulsed-field gel electrophoresis was carried out on a Bio-Rad Dr II system on a 1% agarose gel in 0.5× TBE at 14°C for 24 h with a ramped pulse time of from 19 to 100 s. DNA profiles were visualized as described above.

## RESULTS

**Description of the outbreak.** Between 5 January and 21 March 1995, MRSA strains with the same antibiotic susceptibility patterns were isolated from 10 patients on the ICU. Six of these patients (60%) developed MRSA bacteremia. Among the ICU staff, MRSA strains with the same susceptibility patterns were found on the hands of one nurse during the first screening only.

The patient colonized with the index strain was admitted from the community with a history of having spent some hours in the Accident and Emergency Unit of another hospital 2 days

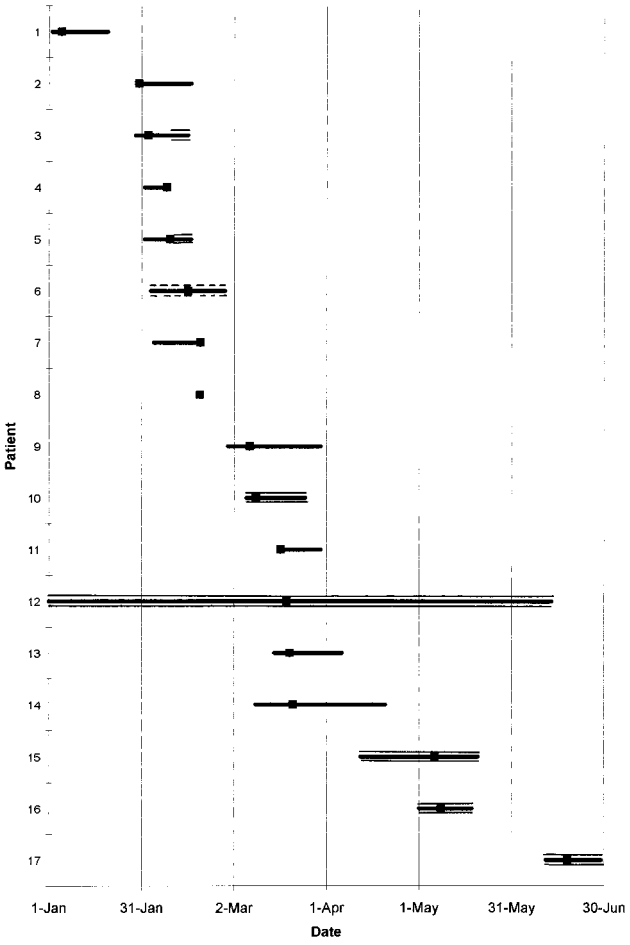


FIG. 1. Lengths of stay on the ward for patients infected or colonized with the epidemic strain of MRSA, as indicated by the groups of lines. The squares indicate the time of isolation of the first isolate of MRSA. The stay on the ICU is indicated by a single solid line, the stay on ward P is indicated by triple solid lines, and the stay on ward D is indicated by two dashed lines and one solid line.

previously. No MRSA strain with the same or similar antibiotic susceptibility pattern had been isolated on the ICU during the previous 2 months. The most recent previous isolate of MRSA (from patient A; Table 1) on the ICU had an antibiotic susceptibility pattern quite different from that of the index strain, but a strain (from patient 6; Table 1) with the same susceptibility pattern as the strains involved in the ICU outbreak was isolated concurrently on another ward (ward D) on 15 February 1995. No MRSA-positive patients from the ICU had recently been transferred to that ward.

All the ICU patients involved in the outbreak had overlapping stays on that ward (Fig. 1). Two of them died while in the ICU; one was transferred to two other wards before returning to the ICU, where he died; the other seven patients were transferred to seven different wards. Two patients were transferred to general surgical ward P and were still found to be MRSA carriers after transfer. Another five patients on ward P were subsequently colonized or infected with an MRSA strain with the same susceptibility pattern as the outbreak strain (Fig. 1; Table 1). One of these patients developed MRSA bacteremia.

Following strict implementation of the British guidelines for the control of MRSA (20) on the ICU and the other affected wards, the outbreak was brought under control, and no further isolates of the outbreak strain were found.

**Strain characteristics.** None of the 17 strains involved in the outbreak could be typed by phage typing (Table 1). They all had the same antibiotic susceptibility pattern. One of the 11 outbreak strains isolated from ICU patients and staff and 1 of 5 outbreak strains isolated from ward P patients were lost before RAPD and PFGE analyses could be performed. Fifteen of the remaining 16 isolates were indistinguishable by RAPD analysis when they were tested with the four different primers or primer combinations and by PFGE (Fig. 2 and 3), respectively. Strain A, which was nontypeable by phage typing and which had an antibiogram different from those of the outbreak strains, was isolated on the ICU on 1 January, before the outbreak, and had a RAPD pattern different from those of the outbreak strains. Six other strains of MRSA nontypeable by phage typing and epidemiologically unrelated to the outbreak described here also had RAPD profiles different from those of the outbreak strains (Fig. 4).

**Cluster analysis of RAPD results.** The results of cluster analysis of the RAPD results with GelCompar software are presented in Fig. 5. The 15 outbreak isolates included on the dendrogram fell into a single cluster at a similarity level of 76%. The isolate from patient A from the ICU was not part of the outbreak and did not cluster with any of the other isolates analyzed. The six epidemiologically unrelated isolates (isolates from patients 18 to 23) did not cluster with the outbreak strains. The bacteriophage lambda markers formed a cluster at a similarity level of 84%. The variation among the outbreak strains and the lambda markers detected by this analysis probably does not represent genuine differences but probably represents artifacts due to variations in the PCR, electrophoresis conditions, and subsequent manipulation of the images.

TABLE 2. Arbitrary primers used for RAPD typing

| Primer | Sequence | Source or reference |
|---|---|---|
| EP017 | 5′-TACACCCGTCAACATTGAGG-3′ | This study |
| EP015 | 5′-ACAACTGCTC-3′ | This study |
| KAY1 | 5′-AGCAGCCTGC-3′ | 29 |
| EP007 | 5′-AGCACGCTGTCAATCATGTA-3′ | This study |

## DISCUSSION

As a result of a routine infection control surveillance, a cluster of MRSA isolates with the same antibiotic susceptibility patterns was noticed on an ICU. Since the antibiogram may be poorly discriminatory (2, 22), the MRSA strains were phage typed, which is still a mainstay in the epidemiological analysis of *S. aureus* infections (7, 28). In this case, however, all the MRSA isolates involved in the outbreak were nontypeable by phage typing. They were therefore typed by RAPD analysis and PFGE and were found to have identical profiles by these two methods. Another MRSA strain isolated from an ICU patient just before this outbreak was also nontypeable by phage typing but had an antibiotic susceptibility pattern different from those of the outbreak isolates and a distinct RAPD fingerprint. Eleven other epidemiologically unrelated isolates of MRSA nontypeable by phage typing showed six distinct RAPD and PFGE patterns. On the basis of these DNA typing results, we concluded that 13 MRSA strains nontypeable by phage typing and 2 epidemiologically related strains that were also nontypeable by phage typing but that were lost before RAPD analysis could be performed were all part of a single hospital outbreak.

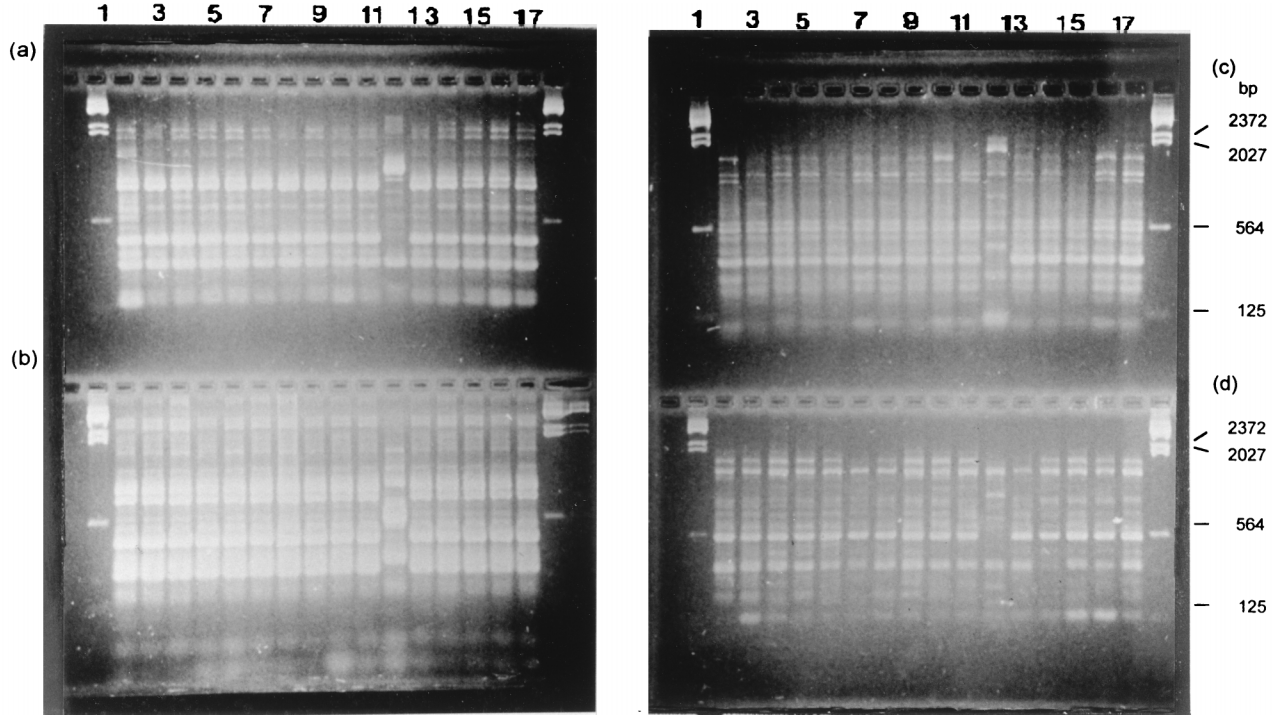


FIG. 2. RAPD fingerprints of MRSA isolates produced by primer EP007 (a), primers EP007 + KAY1 (b), primers EP015 + KAY1 (c), and primer EP017 (d). Lanes 1 and 18, bacteriophage lambda *Hin*dIII molecular size markers; lanes 2 to 4, patient isolates 1 to 3, respectively; lanes 5 to 11, patient isolates 5 to 11, respectively; lane 12, patient A isolate; lanes 13 to 15, patient isolates 12 to 14, respectively; lanes 16 and 17, patient isolates 16 and 17, respectively. Patient isolate numbers correspond to the patient numbers in Table 1.

The patient colonized with the index strain was admitted from the community with a history of a brief stay in another hospital 2 days prior to admission to St. Thomas's Hospital, and the other hospital may have been the source of the outbreak strain. However, an MRSA strain with the same antibiotic susceptibility pattern and RAPD profile as the index strain was isolated on another ward (ward D) on 15 February, but that ward had no history of a recent patient transfer from the ICU. It is thus possible that the outbreak strain was introduced into the ICU from ward D via a transfer of an unrecognized MRSA carrier prior to identification of the index strain. Coello et al. (6) showed that a substantial number of asymptomatic MRSA carriers can be missed if screening is not done.

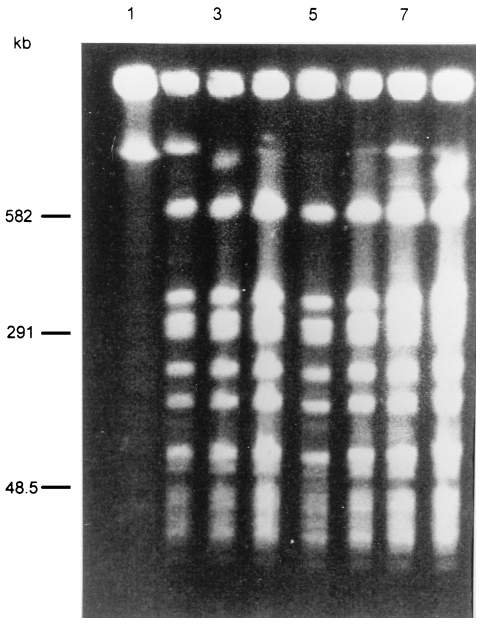


FIG. 3. PFGE fingerprints of MRSA isolates. Lane 1, bacteriophage lambda concatemers; lanes 2 to 4, patient isolates 1 to 3, respectively; lanes 5 to 8, patient isolates 5 to 8, respectively. Patient isolate numbers correspond to the patient numbers in Table 1.

In the ICU outbreak reported here we found that the stays of infected or colonized patients in the ICU overlapped, and cross-infection presumably occurred by cross-infection from patient to patient. The fact that MRSA was found on the hands of one staff nurse supports the widely held view that the hands of personnel are the principal route of MRSA transmission (4).

It is clear that ICUs play a significant role in transferring MRSA isolates to other wards. It is more difficult to control the spread of MRSA in ICUs because patients are frequently subjected to invasive procedures and cohort nursing is not always possible. At the time of the outbreak, standard British infection control procedures (30) for MRSA were hospital policy but were not being strictly followed in all the wards. There was no further spread of infection on seven of eight wards which received MRSA carriers from the ICU, but on ward P, five patients acquired MRSA following the transfer from the ICU of two patients who were carriers of the outbreak strain. Following confirmation of an outbreak strain by phenotypic and genotypic characteristics, all wards were visited by members of the infection control team, and the infection control policies were explained and reinforced. Patient isolation, strict hand disinfection, use of barrier precautions, and nasal treatment of MRSA carriers with mupirocin ointment and chlorhexidine baths prevented the further spread of MRSA. Wards were

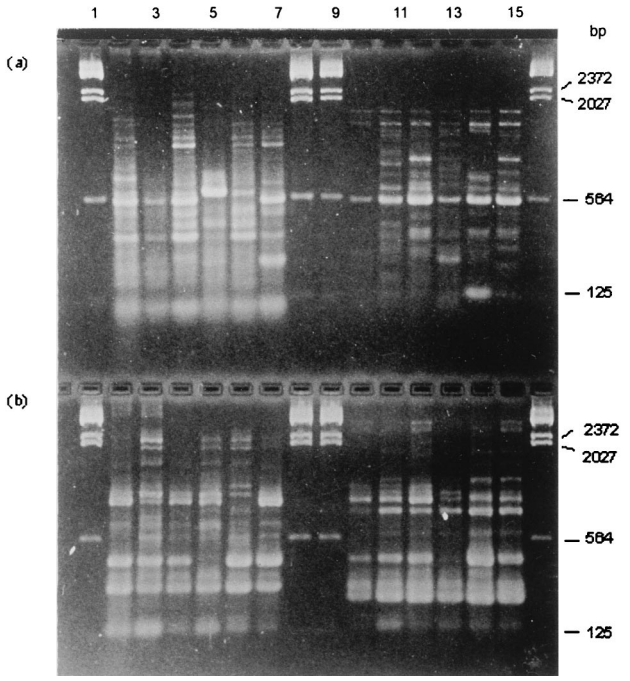


FIG. 4. RAPD fingerprints of unrelated MRSA isolates. (a) Lanes 1, 8, 9, and 16, bacteriophage lambda *Hin*dIII molecular size marker; lanes 2 to 7, patient isolates 18 to 23, respectively, typed with primer EP017; lanes 10 to 15, patient isolates 18 to 23 typed with primer combination EP015 and KAY1. (b) Lanes 1, 8, 9, and 16, bacteriophage lambda *Hin*dIII molecular size markers; lanes 2 to 7, patient isolates 18 to 23, respectively, typed with primer combination EP007 + KAY1; lanes 10 to 15, patient isolates 18 to 23 typed with primer EP007. Patient isolate numbers correspond to the patient numbers in Table 1.

subsequently visited at least twice weekly, and laboratory surveillance of MRSA isolates continued. Up to 1 August 1995, no further cases of infection with the outbreak strain were detected. Since then, only sporadic cases of the outbreak strain have been observed. The standard infection control procedures described above have been implemented, and we have experienced no more outbreaks with this strain.

It is apparent from this outbreak that this strain of MRSA nontypeable by phage typing had a high degree of transmissibility. It was also highly virulent, causing bacteremias in 7 of the 15 affected patients. Although six of these cases of bacteremia occurred in ICU patients and host factors were undoubtedly involved, we do not normally see such a high incidence of invasive *S. aureus* infection in our ICU. We believe that this strain of MRSA is an epidemic type which is not easily recognized because it is nontypeable by phage typing.

Strict microbiological monitoring and epidemiological investigation are essential for controlling MRSA in hospitals (4, 5). The antibiogram provides useful information for routine surveillance (2, 4, 16, 22, 27), but in outbreaks suspected of being caused by MRSA, additional typing should be performed. Plasmid analysis was the first DNA-based method to be applied to *S. aureus*, and in some studies good discrimination was achieved by restriction endonuclease enzyme analysis of plasmid DNA (8, 9). However, the plasmid profiles of isolates are not necessarily consistent over time, because strains can spontaneously loose plasmids or acquire new ones (23, 27). Analysis of restriction fragment length polymorphisms (including ribotyping) of chromosomal DNA has frequently been used to type *S. aureus* strains (12, 15). The discriminatory power of this method depends on the restriction enzymes used (21). PFGE of chromosomal digests with infrequently cutting enzymes has been proposed as the method of choice for typing MRSA (19, 25), being a highly reproducible technique with good discriminatory power (7, 16), and we applied this technique to our isolates. RAPD analysis and PFGE gave similar results, but PFGE was both more time-consuming and technically demanding.

The RAPD assay, also called arbitrarily primed PCR, is rapid and technically simple. Different studies comparing the discriminatory power of RAPD analysis with those of other techniques have come to different conclusions. Van Belkum et al. (28) showed that RAPD analysis could discriminate 23 genotypes in a collection of 48 MRSA isolates, whereas only 13 different phage types could be distinguished. However, this high discriminatory power was achieved by using the combined DNA fingerprints obtained with several primers or primer pairs (four PCR tests). The same investigators found that some *S. aureus* strains had constant PFGE types but variable RAPD types, and vice versa, suggesting that additional resolution might be achieved by combined analyses (29). Saulnier et al. (24) reported that RAPD analysis was less discriminatory than PFGE in typing MRSA, but Hojo et al. (10) found that RAPD typing results correlated well with the results of PFGE. The variable RAPD typing results achieved by different workers may be due to their use of different primers.

In this study we analyzed MRSA isolates by four PCR tests using primers or primer pairs which had shown good discriminatory power in a pilot study. The method is relatively easy to perform, and even multiple tests with different primers are not too cumbersome. The fact that this technique can be used to type many other bacteria and yeasts (18, 31) makes it especially attractive for use in clinical laboratories. The major problem with RAPD analysis, as with all the other electrophoretic techniques, is the lack of standards for interpreting the results between laboratories.

Nevertheless, RAPD typing has proven to be valuable for determining the epidemiology of MRSA isolates. It is partic-

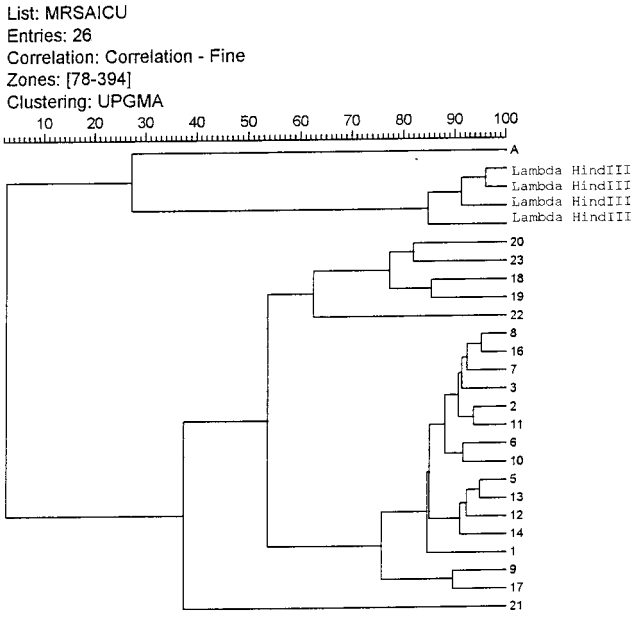


FIG. 5. GelCompar software analysis of epidemiologically related and unrelated MRSA isolates nontypeable by phage typing. Clustering was done with the unweighted pair group method with arithmetic averages (UPGMA) algorithm by using fine correlation on gel tracks. For interpretation of the results, see the text.

ularly useful, as in the outbreak described here, for typing isolates nontypeable by phage typing, because an apparent outbreak may in fact be due to different strains. The technique is easier and less time-consuming than PFGE and does not require pulsed-field equipment. The use of RAPD typing in the investigation of MRSA outbreaks should be further explored.

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