where multiple cultures were done, as in cases no. 4, 5 and 6, this did not greatly assist in establishing a diagnosis. Overall, of 28 cultures taken, 14 were negative and only 9 of the remainder grew the shunt colonising strain.

Most cases of shunt colonisation are due to *Staphylococcus epidermidis*. Using the ASET test, the titre of antibody to this organism can be shown to rise in a predictable manner with age in uninfected patients, and to rise to very high levels in those with colonised VA shunts (6). The antigenic complex used in the ASET is common to most species of coagulase-negative staphylococci, and this is shown by the reaction to *Staphylococcus capitis* in case no. 4. The test can be used diagnostically but it can also be used as a screening test in the first six months after surgery (7). In our experience all cases of VA shunt colonisation are detectable serologically in this period, although it is possible that some may take longer to show seroconversion.

The CRP level is rarely raised in uncomplicated *Staphylococcus epidermidis* VA shunt colonisation, and a high CRP level found long after surgery is often indicative of immune complex disease although this must be confirmed by demonstration of depressed C3c and C4 fractions. Anaemia, refractory to iron therapy, is almost always a feature of VA shunt colonisation (2, 6), even in the absence of shunt nephritis, and is a clinically useful finding.

Colonisation due to *Staphylococcus epidermidis* can be diagnosed reliably in chronic cases using the ASET test and if this is done early, immune complex nephritis can be avoided (6).


### Acknowledgements

We are indebted to Dr Gillian Hunt, Department of Urology, Addenbrooke's Hospital, Cambridge, for helpful criticism of drafts of this paper. We are also grateful to those colleagues who sent us case material and assisted in data collection. The work was supported by grants from the Association for Spina Bifida and Hydrocephalus and the Wade Charitable Trust.


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# Comparison of Four Genotyping Assays for Epidemiological Study of Methicillin-Resistant *Staphylococcus aureus*

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**Twenty-six methicillin-resistant *Staphylococcus aureus* strains were genetically differentiated by interrepeat PCR and the results compared with those of ribotyping, pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA analysis obtained in a previous study for the same strains. The comparison showed that the PCR-mediated assays were as discriminatory as PFGE, whereas ribotyping was the least powerful**

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**genotyping method. Due to the ease of performance, PCR fingerprinting may become the method of choice for establishing clonal relationships among *Staphylococcus aureus* isolates.**

Genetic analysis of microorganisms provides a means for determination of relationships between bacterial isolates (1). In the case of *Staphylococcus aureus*, a frequently encountered nosocomial pathogen, molecular genetic methods have been used intensely to investigate the spread or persistence of a given strain (2). In particular methicillin-resistant *Staphylococcus aureus* (MRSA) has been studied in detail (3). It has been suggested that the MecA gene was introduced only once from its original source into *Staphylococcus aureus* (4). On the other hand, MecA is clearly present in a variety of different genetic backgrounds, strongly suggesting horizontal transfer of this gene between staphylococcal chromosomal lineages (5). From MRSA typing studies it is obvious that the method used to determine clonality (or not) is of major importance for the epidemiological conclusions drawn. For this reason several comparisons of methods for typing MRSA have been made (6–10).

In the present paper we compare the performance of four genotyping assays for determination of clonality in a French collection of 26 MRSA isolates selected because of differing pulsed-field gel electrophoresis (PFGE) macrorestriction patterns. The genetic interrelationships of these isolates had previously been determined by ribotyping and random amplified polymorphic DNA (RAPD) analysis. In the present study interrepeat polymerase chain reaction (PCR) assays were performed in addition and the results of all methods compared.

***Materials and Methods.*** Twenty-six MRSA strains were used for the study. MRSA were identified as facultatively anaerobic gram-positive cocci producing coagulase and acetoin, but not β-galactosidase. Resistance to methicillin was considered relevant when the inhibition zone for 5 μg oxacillin disks was < 20 mm (6). Strains were stored in brain heart infusion medium containing 10 % horse serum and 10 % glycerol. The procedures and results of ribotyping, PFGE and RAPD analysis have been described elsewhere (6, 7). Interrepeat PCR was performed as described previously for a set of Dutch MRSA strains (11). For PCR, primers homologous to enterobacterial repetitive intergenic consensus sequences ERIC1 and ERIC2 (12), primer BG2 and the MecA2 consensus primer were applied in four different assays. All amplicons were analysed by electrophoresis through 1–2 % agarose gels cast in 40 mM Tris acetate-2 mM EDTA buffer (pH 7.5) containing ethidium bromide. Gels were photographed using Polaroid Polapan films. On the basis of the different DNA band patterns, strains could be divided into various PCR fingerprint groups.

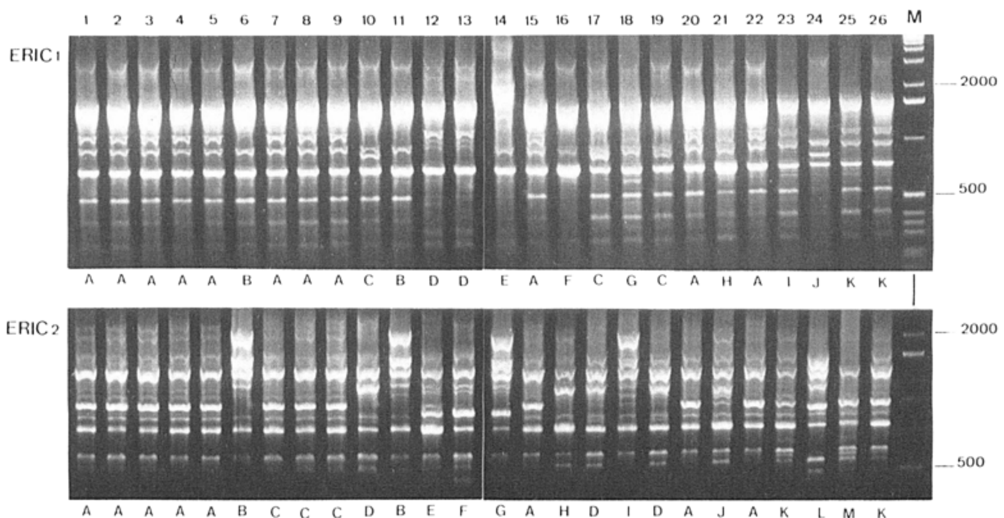


**Figure 1:** Example of the PCR fingerprints generated by the application of the repetitive motif primers ERIC1 (top panel) and ERIC2 (lower panel). The upper row gives the sample numbers (1–26) whereas below the respective panels the codes, as shown in Table 1, are given. The lanes on the right contain molecular markers in basepairs.

**Table 1:** Differentiation of 26 French MRSA strains by four genetic typing methods.

| Strain no.[a] | PFGE type *Sma*I[b] | Ribotype[c] | | | Ribotype overall | RAPD[c] | | | RADP overall | Interrepeat PCR[c,d] | | | | Interrepeat PCR overall |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Eco*RI | *Cla*I | *Hind*III | | Primer 2 | Primer 3 | Primer 5 | | E1 | E2 | E1E2 | mecA2 BG2 | |
| 1 | I | E1 | C1 | H1 | I | A | A | A | I | A | A | A | A | I |
| 2 | II | E2 | C1 | H1 | II | B | B | A | II | A | A | A | A | I |
| 3 | III | E1 | C1 | H1 | I | B | C | A | III | A | A | B | B | II |
| 4 | IV | E1 | C1 | H1 | I | B | D | B | IV | A | A | A | C | III |
| 5 | V | E1 | C1 | H1 | I | C | E | A | V | A | A | A | A | I |
| 6 | VI | E5 | C4 | H4 | III | D | F | C | VI | B | B | C | D | IV |
| 7 | VII | E2 | C2 | H2 | IV | C | C | C | VII | A | C | A | C | V |
| 8 | VIII | E1 | C1 | H1 | I | B | D | D | VIII | A | C | A | E | VI |
| 9 | IX | E1 | C1 | H1 | I | B | D | D | VIII | A | C | A | C | V |
| 10 | X | E1 | C1 | H1 | I | E | G | E | IX | C | D | D | E | VII |
| 11 | XI | E2 | C2 | H2 | IV | F | H | F | X | B | B | E | D | VIII |
| 12 | XII | E1 | C1 | H1 | I | G | I | D | XI | D | E | F | F | IX |
| 13 | XIII | E4 | C4 | H4 | V | H | J | G | XII | D | F | F | – | X |
| 14 | XIV | E4 | C1 | H1 | VI | I | K | H | XIII | E | G | G | F | XI |
| 15 | XV | E6 | C1 | H1 | VII | J | L | I | XIV | A | A | A | C | III |
| 16 | XVI | E2 | C2 | H2 | IV | K | M | J | XV | F | H | H | A | XII |
| 17 | XVII | E1 | C1 | H1 | I | L | N | K | XVI | C | D | I | C | XIII |
| 18 | XVIII | E1 | C1 | H1 | I | M | O | C | XVII | G | I | J | – | XIV |
| 19 | XIX | E3 | C3 | H3 | VIII | N | N | L | XIII | C | D | D | G | XV |
| 20 | XX | E1 | C1 | H1 | I | B | P | C | XIX | A | A | A | A | I |
| 21 | XXI | E1 | C1 | H1 | I | O | Q | J | XX | H | J | K | H | XVI |
| 22 | XXII | E1 | C1 | H1 | I | G | R | F | XXI | A | A | A | A | I |
| 23 | XXIII | E2 | C3 | H3 | IX | B | E | M | XXII | I | K | L | – | XVII |
| 24 | XXIV | E1 | C1 | H1 | I | P | S | N | XXIII | J | L | H | I | XVIII |
| 25 | XXV | E1 | C1 | H1 | I | N | T | O | XXIV | K | M | L | – | XIX |
| 26 | XXVI | E3 | C2 | H2 | X | Q | – | J | XXV | K | K | A | A | XX |
| No. of types[e] | 26 | 6 | 4 | 4 | 10 | 17 | 20 | 15 | 25 | 11 | 13 | 12 | 9 | 20 |

[a] The strain numbers correspond to those published previously (6, 7).
[b] Isolates were selected on the basis of differing PFGE DNA fingerprints (column 2, type I to XXVI).
[c] Ribotyping was performed using three different restriction enzymes, whereas the two PCR methods were performed using three (RAPD) and four (IR PCR) assays, respectively.
[d] E1, E2, mecA2 and BG2 identify the ERIC1 primer, ERIC2 primer, methicillin resistance complex specific primer MecA2 and the arbitrary primer BG2, respectively.
[e] Overall scores give the combined discrimination of the respective assays.
A small horizontal line indicates that no data were available.

***Results and Discussion.*** Figure 1 gives a representative example of the results obtained by interrepeat PCR. Prior to performing interrepeat PCR with all 26 strains, assay reproducibility was determined by testing 20 colonies each of strains 1, 5 and 10. For all colonies of one strain, all PCR fingerprints were unequivocally identical (results not shown). As such, interrepeat PCR appears to be an experimentally reliable DNA typing assay. The results obtained in the four interrepeat PCR assays, summarized in an overall type given a roman number, are presented in Table 1, together with the previously published results of PFGE, ribotyping and RAPD analysis for the 26 French MRSA isolates (6, 7).

From Table 1 several conclusions can be drawn. Since the strains were selected on the basis of their differing PFGE patterns, it is obvious that this assay was the most discriminative. It should be emphasized, however, that using restriction enzymes other than *SmaI* for the same collection of strains, a lower degree of resolution may result. Ribotyping was clearly less discriminatory. Taken together with the fact that ribotyping is also technically more demanding, PFGE and both types of PCR, are to be preferred to ribotyping. Comparison of PFGE and PCR revealed that a single PCR assay did not achieve the same degree of resolution as PFGE. Whereas PFGE distinguished all isolates, PCR identified between 9 and 20 types per assay, its performance depending strongly on the choice of primer or primer combination. Performing PCR with different primers allows grouping of isolates, irrespective of strain origin or typing based on other tests. This was demonstrated by the fact that several strains which were identical in one or more of the PCR assays were not epidemiologically linked (results not shown). It thus appears that different genotypes of MRSA coexist (with geographically different frequencies). The difference between RAPD analysis and interrepeat PCR exists in the nature of the primers. Whereas RAPD analysis uses DNA oligonucleotides of arbitrary nucleotide order which average 6 to 10 nucleotides in length, interrepeat PCR employs longer oligonucleotides, the sequence of which is usually based on repetitive elements as found in prokaryotic genomes. On combination of PCR fingerprints, irrespective of the fact that individually RAPD analysis and interrepeat PCR displayed resolution lower than that of PFGE, all strains could be discriminated. By combining the RAPD overall score with the $MecA_2/BG_2$ PCR result, even strains no. 8 and 9 could be discriminated (Table 1).

The discriminatory power of PCR can be modulated by altering the choice of primer and annealing temperatures. This is advantageous for typing purposes. On the other hand, PCR typing may vary because of differences in the thermal cyclers used or the DNA quality or concentration (14). These disadvantages are offset by the fact that PCR is technically less demanding than PFGE and has prospects of wider use in diagnostic microbiology. PCR fingerprinting may become the procedure of choice for typing MRSA, especially when the optimal primers (or combinations thereof) have been determined in preliminary experiments. Moreover, the possibility of directly including whole bacterial cells in PCR assays should enhance the importance of PCR fingerprinting in microbiology (13).

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# Evaluation of a Semi-Automated 24-Hour Commercial System for Identification of *Enterobacteriaceae* and Other Gram-Negative Bacteria

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**A semi-automated commercial system (ID 32 E, bioMérieux) for 24-hour identification of *Enterobacteriaceae* and other gram-negative fermentative and nonfermentative bacteria encountered in diagnostic microbiology was evaluated. Overall, the system correctly identified 506 (91.5 %) of the 553 strains tested, 94 (17.0 %) strains requiring additional tests for complete identification. Six (1.1 %) strains were misidentified and 33 (6.0 %) strains were not identified. Eight (1.4 %) strains were not present in the database and were misidentified or not identified. The system is a suitable alternative to existing systems for the identification of *Enterobacteriaceae* and other gram-negative bacteria frequently encountered in clinical samples.**

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The number of known members of the family *Enterobacteriaceae* has increased to more than 140 species during the past 15 years (1, 2). Although well-represented species, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, are still responsible for most infections due to *Enterobacteriaceae*, other species can be recovered from all kinds of clinical specimens, including blood and cerebrospinal fluid (1). Consequently, O'Hara et al. (3) reevaluated the API 20E system for identification of *Enterobacteriaceae*. Unless supplementary tests were performed, they found a lower 24-hour accuracy than in earlier evaluations of this 20-year old system. Manufacturers have developed new rapid systems to identify *Enterobacteriaceae* species in the clinical laboratory (2, 4–7); however, these systems generally have not been evaluated for new species (4, 5, 7) or did not provide sufficiently accurate results (6). In the present study a new semi-automated system (ID 32 E, bioMérieux, France) for 24-hour identification of members of the family *Enterobacteriaceae* and other gram-negative bacteria frequently isolated from clinical samples was evaluated.

***Materials and Methods.*** A total of 553 strains of gram-negative bacteria were tested (Table 1) of which 468 were members of the family *Enterobacteriaceae*. The other strains were fermentative bacteria belonging to the genera *Aeromonas*, *Plesiomonas*, *Vibrio*, *Listonella* and *Pasteurella*, and nonfermentative bacteria belonging to the genera *Pseudomonas*, *Xanthomonas*, *Sphingomonas*, *Flavobacterium* and *Acinetobacter*. Most of the strains were of clinical origin with a distribution approximating that of strains likely to be routinely isolated in a clinical microbiology laboratory. Seventy-nine strains of environmental origin belonging to species that might be recovered from clinical samples and 41 reference strains were included in the study.

Most of the clinical strains commonly encountered in the laboratory had previously been identified with the API 20E system (bioMérieux) and supplementary tests if necessary (1, 4). *Klebsiella*, *Serratia*, *Aeromonas*, and nonfermentative gram-negative bacilli were identified by carbon substrate assimilation tests (8–11). Fastidious or rarely encountered species, especially those from the environment, had previously been identified by conventional methods (1, 12).

The ID 32 E system comprises 32 wells containing dehydrated reagents for the following biochemical tests: six conventional tests (lysine decarboxylase, ornithine decarboxylase, arginine dihy-