

Usefulness of three probes in typing isolates of methicillin-resistant *Staphylococcus aureus* (MRSA)

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Summary. Fifty-nine epidemiologically unrelated methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from different geographical areas and 23 phage-type 77 MRSA isolates from France were investigated. Cellular DNA, digested with restriction endonucleases *Eco*RI or *Hind*III, was probed with plasmids carrying the gene encoding 16S rRNA (pBA2), the gene *aacA-aphD* (pSF815A) and the gene *aacA-aphD* plus part of IS256 (pIP1307). When probed with pBA2, most of the unrelated isolates displayed the same hybridisation pattern. A greater diversity in patterns was detected in gentamicin-resistant strains with the two other probes. The most accurate fingerprinting of these isolates was obtained with the probe pIP1307. Moreover, this probe appeared to be useful for tracing the phage-type 77 epidemic MRSA isolates widespread in French hospitals.

Introduction

Outbreaks of nosocomial infections attributable to methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been reported worldwide.^{1–14} Epidemiological assessment of each outbreak involves the typing of strains. Molecular methods for typing MRSA strains include analysis of plasmid content,^{15–22} cellular DNA restriction endonuclease patterns,^{23–27} electrophoretic profiles of the cellular proteins,^{28–31} immunoblotting profiles^{23, 24, 29, 32} and esterase electrophoretic profiles.³³ The numerous reports describing these methods reflect the limits of the more traditional typing methods, mainly consisting of drug-resistance phenotyping,^{6, 14, 34} phage-typing^{21, 35, 36} and serotyping.^{37–39} These limits were encountered particularly in typing the MRSA strains isolated in France that were found to have many properties in common.^{16, 33, 34, 40, 41}

Since December 1984, phage-type 77 MRSA strains have been isolated in numerous French hospitals. Additional typing methods are required to confirm that these isolates are closely related because the number of phage reactions was limited and because some of the reactions were weak and not reproducible. Among the numerous approaches that have been proposed to differentiate closely related prokaryotic organisms, cellular DNA analysis was chosen for the present analysis because, by this method, the potential pitfalls associated with the variable expression of phenotype can be avoided.

The aim of this study was to evaluate the usefulness of three DNA probes for typing MRSA strains and

for tracing the gentamicin-resistant phage-type 77 MRSA isolates that are widespread in French hospitals. Epidemiologically unrelated MRSA isolates, as well as phage-type 77 French MRSA isolates, were probed with three plasmids, pBA2⁴², pSF815A⁴³ and pIP1307⁴⁴ carrying, respectively, the *Bacillus subtilis* gene encoding 16S rRNA, the *aacA-aphD* gene encoding kanamycin-gentamicin resistance and isolated from a strain of *Enterococcus faecalis*, and the gene *aacA-aphD* and a 0.3-kb fraction of IS256 isolated from a strain of *S. aureus*.

Materials and methods

Bacterial strains and plasmids

One hundred and two clinical isolates of *S. aureus* were studied: (i) 59 MRSA isolates from different geographical areas (22 isolates from 18 different locations in the USA and Canada, 13 isolates from France and 24 isolates from 12 other European countries); (ii) 23 MRSA isolates with similar phage-types isolated over a 3-year period (1984–1987) in seven French hospitals, of which five were located in Paris (table I); and (iii) 20 epidemiologically unrelated methicillin-susceptible *S. aureus* (MSSA) strains consisting of 17 isolates from hospitals located in several French cities and strains ATCC12600, ATCC65380 (American Type Culture Collection) and RN450.⁴⁵

The *E. coli* strains used were HVC45,⁴⁶ SF8,⁴² SF815A⁴³ and BM3887,⁴⁴ harbouring plasmids pBR322,⁴⁷ pBA2,⁴² pSF815A⁴³ and pIP1307,⁴⁴ respectively. Plasmid pBA2 contains a 2.3-kb *B. subtilis* DNA fragment encoding 16S rRNA inserted in the *Hind*III site of plasmid pBR322. Plasmid pSF815A consists of plasmid pUC8 plus a 1.5-kb *Alu*I insert

carrying the *Ent. faecalis* gene *aacA-aphD* specifying the bifunctional resistance enzyme AAC6'-APH2" that inactivates gentamicin, kanamycin and related aminoglycosides. Plasmid pIP1307 consists of plasmid pBR322 with a 2.1-kb *HindIII* insert isolated from the cellular DNA of a French MRSA isolate and carrying the gene *aacA-aphD*. This insert was shown to be derived, by deletion, from the 2.5-kb *HindIII* internal fragment of transposon Tn4001 and to contain, in addition to the gene *aacA-aphD*, a 297-bp sequence from strain IS256 (this study).

Susceptibility to antimicrobial agents

Susceptibility to antimicrobial agents was determined by a disk diffusion assay as described previously³⁴ with disks containing: penicillin G, oxacillin, streptomycin, neomycin, gentamicin, chloramphenicol, tetracycline, minocycline, erythromycin, lincomycin, sulphonamide, trimethoprim, pefloxacin, rifampicin, fusidic acid, fosfomycin, spectinomycin, and vancomycin (Diagnostics Pasteur, Marne La Coquette, France). Disks were prepared with streptogramin A 20 µg, streptogramin B 40 µg, cadmium acetate 0.2 µmol, mercuric nitrate 0.2 µmol, sodium arsenate 2 µmol, ethidium bromide 200 µg, acriflavine 200 µg, propamidine isethionate 200 µg and cetyltrimethylammonium bromide 10 µg.

Detection of antibiotic-inactivating enzymes

Enzymes inactivating penicillin G and streptogramins A and B were detected as described previously.³⁴

Phage-typing

Isolates were phage-typed by the standard method of Blair and Williams³⁵ with the international set of 23 phages used at Routine Test Dilution (RTD) and 100 × RTD. Typing was also performed after incubation of bacterial cultures at 56°C for 2 min.

Serotyping

Isolates were typed by slide agglutination^{38,39} of formalin-killed cells with 16 specific antisera (I, II, III, 6, 7, 9, 10, 14, 15, 16, 17, 18, 66438, 61218, 64043 and 64048).

DNA isolation and analysis

Total cellular DNA was isolated from the staphylococcal strains and purified as described previously.⁴⁴

A rapid procedure⁴⁸ was used to screen for plasmid DNA. A large-scale plasmid isolation technique, involving alkaline lysis⁴⁹ followed by purification on a caesium chloride-ethidium bromide gradient, was also used to obtain plasmids from the staphylococcal strains isolated in France. Plasmid DNA isolated from

E. coli strains was purified by centrifugation in a caesium chloride-ethidium bromide gradient.⁵⁰

The restriction endonucleases *AluI*, *AvaII*, *BglII*, *DraI*, *EcoRI*, *HincII*, *HindIII*, *Sau3A*, *ScaI*, *TaqI* (Amersham International, Little Chalfont) and *DdeI* (Biolabs, New England, MA, USA) were used according to the manufacturer's instructions. Electrophoresis of digested DNA was performed in agarose (Sigma) 0.8% w/v gels in Tris-borate buffer as described by Sambrook *et al.*⁴⁸ Bacteriophage λ DNA (Biolabs, New England, MA, USA) digested by *BglII*, 1-kb DNA ladder (Bethesda Research Laboratories, Inc., Cockeysville, MD, USA) and the Raoul I ladder (Appligene, Strasbourg, France) were used as molecular size markers.

Blotting and hybridisation

DNA was transferred from agarose gels to nitrocellulose filters by the bidirectional method described by Smith and Summers.⁵¹ Plasmids pBA2, pSF815A and pIP1307 were used as probes. Purified plasmid DNA was labelled with [α^{32} P] dCTP (3000 Ci/mmol; Amersham International) by the multiprime DNA labelling system (Amersham International) according to the manufacturer's instructions. The specific activity of the probes was *c.* 10⁹ cpm/µg of DNA. Hybridisation was done under stringent conditions as described previously.⁴⁴ The blots were exposed to Fuji RX films at -70°C with intensifying screens for 24 h up to several days.

Comparison of hybridisation patterns (HP)

The average similarity between any two HP was assessed by use of the coefficient of Dice,⁵² calculated as follows: Percentage similarity

$$(\%S) = \frac{\text{number of matching bands} \times 2}{\text{total number of bands}} \times 100$$

Results

Relevant characteristics of the MRSA strains

Phage-type, susceptibility to 27 antimicrobial agents, plasmid content determined by the rapid procedure, and serotype were determined for the 82 MRSA isolates used in this study.

The 59 MRSA isolates originating from different geographical areas exhibited phage-types distinguishable by at least three strong reactions at 100 × RTD and when the bacterial cultures were incubated for 2 min at 56°C. These isolates were also distinguishable by their antimicrobial-resistance phenotypes. Therefore, they are considered to be unrelated. Although having distinct drug-resistance phenotypes, the 13 French MRSA had eight resistance markers in common (resistance to penicillin G, streptomycin,

neomycin, tetracycline, minocycline, sulphonamides, cadmium acetate and sodium arsenate). Each of the unrelated MRSA isolates, except the French ones, could be distinguished by the plasmid(s) it harboured. In contrast, the 13 French unrelated isolates could not be differentiated on the basis of their plasmid content. One strain lacked plasmid DNA and the other 12 were divided into only three plasmid profiles. They all carried the same 22-kb plasmid, pIP1066,¹⁶ encoding resistance to penicillin G, cadmium acetate and sodium arsenate. Nine of these isolates had an additional cryptic plasmid, pIP1067,¹⁶ and one isolate had two other cryptic plasmids, pIP1067 and pIP1068.¹⁶ The North American isolates, when serotyped with 16 antisera, were found to belong to nine serotypes: serotypes I (five isolates), I-66438 (three isolates), I-III (three isolates), I-II (one isolate), I-III-66438 (six isolates), III (one isolate), III-66438 (one isolate), 10 (one isolate) and 14 (one isolate), whereas the 37 European isolates belonged to serotypes III (16 isolates) and 18 (21 isolates).

The 23 MRSA isolates sampled over a 3-year period in seven French hospitals (table I) were susceptible to phage 77 at RTD. When the phage was used at 100 × RTD, and the bacterial cultures incubated at 56°C for 2 min, susceptibility to additional phages was revealed: 29, 54, 75 and/or 84. All the phage-type 77 French MRSA isolates produced penicillinase and were resistant to 16 antimicrobial agents: streptomycin, spectinomycin, gentamicin, tetracycline, minocycline, erythromycin, lincomycin, streptogramin B, sulphonamides, rifampicin, cadmium acetate, mercuric nitrate, ethidium bromide, acriflavine, propamidine isethionate and cetyltrimethylammonium bromide. Twenty-one isolates were also resistant to pefloxacin, eight isolates were resistant to fosfomycin

and four isolates were resistant to one of the following agents: chloramphenicol, trimethoprim, neomycin or sodium arsenate. These isolates could not be distinguished, on the basis of plasmid content, from the unrelated French MRSA isolates. They all harboured plasmid pIP1066¹⁶ and nine of them also harboured plasmid pIP1067.¹⁶ The 23 isolates belonged to serotype 18.

Hybridisation patterns (HP) obtained with probe pBA2

With plasmid pBA2 as probe, nineteen different HP were observed when the cellular DNA of the 102 *S. aureus* isolates was digested with *Eco*RI (labelled E) and nine HP when it was digested with *Hind*III (labelled H). No hybridisation was detected when the same cellular DNA was probed with plasmid pBR322. The schematic representation of the *Eco*RI and *Hind*III HP is shown in fig. 1. The frequency with which each pattern occurred within each group of strains is reported in table II. Sixty-six percent of the 59 unrelated MRSA isolates had the same *Eco*RI HP (E1) and 90% of these had the same *Hind*III HP (H1). Thus, despite their different geographical origins, most of the unrelated MRSA isolates displayed similarly localised rRNA sequences. In contrast, these two patterns were uncommon among the 20 unrelated MSSA strains. Even though fewer MSSA strains were studied and despite the common French source of 17 of them, the diversity of their HP was greater than that observed among the unrelated MRSA isolates.

Among the 23 phage-type 77 French MRSA isolates, five *Eco*RI HP and three *Hind*III HP could be distinguished. As observed for the unrelated isolates, hybridisation patterns E1 and H1 (fig. 1) were predom-

Table I. Phage-types of 23 epidemic MRSA isolates from seven French hospitals over a 3-year period (1984–1987)

Hospital designation, city	Number of strains	Phage-type*	
		100 × RTD	RTD
A, Paris	2	54+ 77+ + 84+ +	77+ + or 77+ or 77+
	5	54+ + 77+ + 84+ +	
B, Paris	1	54+ 77+ + 84+ +	
C, Paris	1	54+ + 77+ + 84+ +	
B, Paris	3	77+ + 84+ +	
D, Toulouse	1	77+ + 84+ +	
E, Paris	1	77+ + 84+ +	
F, Nevers	1	77+ + 84+ +	
A, Paris	1	54+ + 77+ +	
C, Paris	1	54+ + 77+ +	
G, Paris	1	54+ + 77+ +	
A, Paris	1	29+ + 77+ + 84+ +	
F, Nevers	1	29+ + 77+ + 84+ +	
A, Paris	1	54+ + 75+ + 77+ + 84+ +	
C, Paris	1	54+ + 75+ + 77+ + 84+ +	
C, Paris	1	54+ + 75+ + 77+ + 84+ +	
			54+ 75+ 77+ +

* After incubation of the bacterial cultures at 56°C for 2 min. The phage reactions underlined were not detected without incubation at 56°C.

RTD, Routine Test Dilution; 100 × RTD, phage at 100-fold higher concentration.

+ +, ≥ 50 plaque-forming units (pfu); +, 20–50 pfu; ±, < 20 pfu.

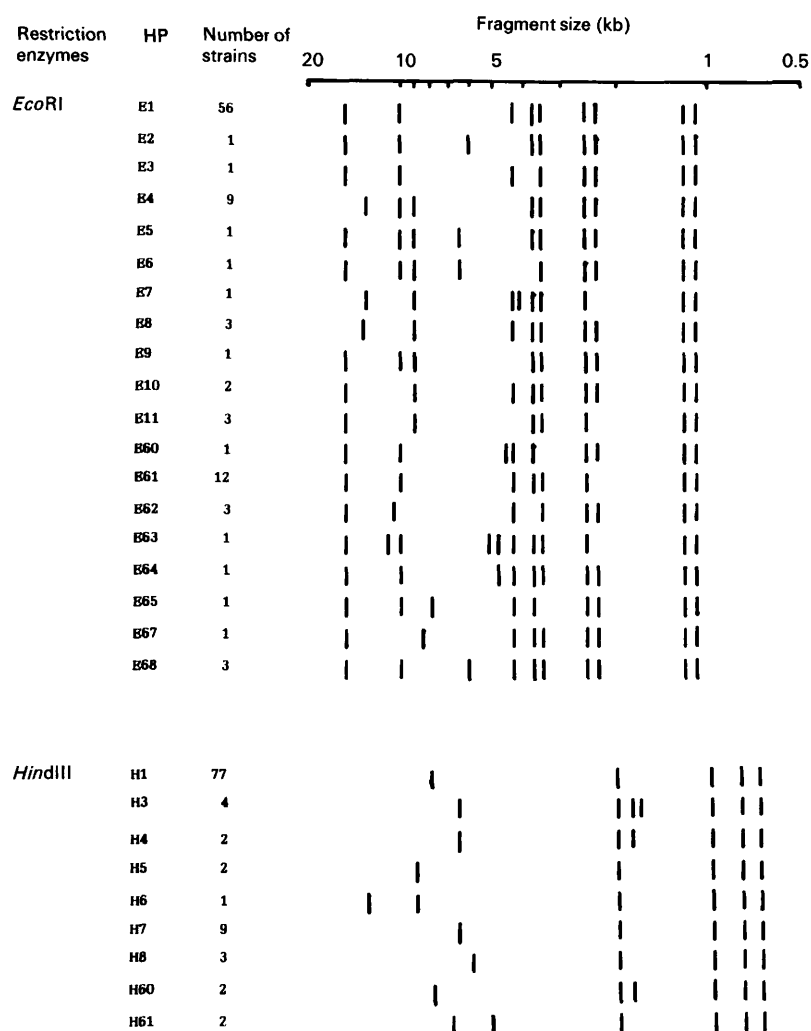


Fig. 1. Schematic representation of the 19 *EcoRI* and nine *HindIII* HP detected in the 102 *S. aureus* strains analysed with α^{32} P-labelled pBA2 as a probe.

inant; they occurred in 15 and 21 isolates, respectively (table II).

Hybridisation patterns obtained with probe pSF815A

In the isolates resistant to gentamicin (36 of the 59 unrelated MRSA isolates and all of the 23 phage-type 77 French isolates), restriction fragments that hybridised with probe pSF815A were detected on a 2.5 kb-*HindIII* fragment (results not shown) and on *EcoRI* fragments of various sizes (fig. 2). Fourteen distinct *EcoRI* HP were observed among the 36 unrelated gentamicin-resistant MRSA isolates. Twelve of these patterns consisted of a single hybridising band and the two others consisted of two bands. Each of the 23 phage-type 77 French MRSA isolates carried a single *EcoRI* fragment of 8.5 kb that hybridised with probe pSF815A, as did six unrelated MRSA isolates, including three French isolates.

None of the strains which were susceptible to gentamicin (23 of the 59 unrelated MRSA isolates and the 20 MSSA) carried detectable nucleotide sequences that hybridised with the probe pSF815A. Moreover,

as has been previously reported,⁴⁴ none of the plasmids harboured by the French MRSA, pIP1066, pIP1067 and pIP1068, hybridised with this probe.

Mapping of the insert carried by the probe pIP1307

As reported previously,⁴⁴ plasmid pIP1307 consists of pBR322 containing a 2.1-kb *HindIII* insert carrying the gene *aacA-aphD* isolated from the chromosomal DNA of a French MRSA isolate. The recognition sites for the restriction enzymes *AluI*, *AvaII*, *DdeI*, *DraI*, *HincII*, *Sau3A*, *ScaI* and *TaqI* were mapped in the 2.1-kb insert of plasmid pIP1307 and the map was compared to that of Tn4001,^{53, 54} deduced from the corresponding nucleotide sequence. This comparison suggested that the 2.1-kb insert of plasmid pIP1307 was derived from the internal 2.5-kb *HindIII* fragment of Tn4001 by a deletion of 0.4 kb. This deletion was located between the initiation codon of the gene *aacA-aphD* (nt 700) and the *AvaII* site (nt 193) upstream from this codon. Thus, the 2.1-kb insert contained the *aacA-aphD* gene and at least one intact copy of the 297-bp sequence of IS256 located within the internal *HindIII* fragment of Tn4001, downstream from the resistance gene.⁴⁴

Table II. *EcoRI* and *HindIII* hybridisation patterns (HP) of cellular DNA of 102 *S. aureus* strains probed with pBA2

Relevant characteristics of the isolates	Restriction endonuclease	HP (number of isolates belonging to each HP)
<i>Epidemiologically-unrelated strains</i> 13 French MRSA isolates	<u><i>EcoRI</i></u> <u><i>HindIII</i></u>	<u>E1(9)</u> ; E3(1); E60(1); E61(2) <u>H1(12)</u> ; H60(1)
24 MRSA isolates from 11 European cities except France	<u><i>EcoRI</i></u> <u><i>HindIII</i></u>	<u>E1(15)</u> ; E11(2); E61(7) <u>H1(22)</u> ; H7(1); H61(1)
22 MRSA isolates from 18 North-American states	<u><i>EcoRI</i></u> <u><i>HindIII</i></u>	<u>E1(15)</u> ; E4(1); E8(1); E65(1); E67(1); E68(3) <u>H1(19)</u> ; H5(1); H7(2)
20 MSSA strains including 17 from France and three from culture collections	<u><i>EcoRI</i></u> <u><i>HindIII</i></u>	<u>E1(2)</u> ; E2(1); E4(8); E5(1); E6(1); E7(1); E8(2); E9(1); E10(2); E11(1) <u>H1(3)</u> ; H3(4); H4(2); H5(1); H6(1); H7(6); H8(3)
23 phage-type 77 French MRSA	<u><i>EcoRI</i></u> <u><i>HindIII</i></u>	<u>E1(15)</u> ; E61(3); E62(3); E63(1); E64(1) <u>H1(21)</u> ; H60(1); H61(1)

^a The hybridisation patterns underlined were predominant, accounting for > 62% of the isolates analysed.

Hybridisation patterns obtained with probe pIP1307

None of the strains which were susceptible to gentamicin carried nucleotide sequences detectable by hybridisation with plasmid pIP1307. In each of the gentamicin-resistant MRSA isolates, the nucleotide sequences hybridising with plasmid pIP1307 were located on one of the 12 *EcoRI* fragments and on 1-14 *HindIII* fragments. At least two unrelated hybridisation experiments were done to ensure that the HP is reproducible. The schematic representation of the 42 *EcoRI* HP detected in the 59 gentamicin-resistant MRSA isolates analysed including 36 unrelated MRSA is shown in fig. 2. The *EcoRI* HP of 20 French MRSA isolates are shown in fig. 3. Among the 36 unrelated MRSA isolates studied, 32 *EcoRI* HP (fig. 2, HP 11-42) and 28 *HindIII* HP (results not shown) were detected. Each of the unrelated MRSA isolates carrying more than one *EcoRI* or *HindIII* fragment and hybridising with plasmid pIP1307 was characterised by its own *EcoRI* HP or *HindIII* HP.

In the 23 phage-type 77 French MRSA isolates, 10 *EcoRI* HP were detected (fig. 2, HP 1-10; fig. 3, HP 1-10). These patterns consisted of 8-12 *EcoRI* fragments hybridising with plasmid pIP1307, of which seven were common to all the HP. Seven *HindIII* HP were observed in the cellular DNA of these isolates (results not shown). Each *HindIII* HP consisted of 11-14 bands, of which nine were common to all the HP.

The *EcoRI* HP (fig. 2) were compared within that of each group of MRSA isolates and the Dice coefficient⁵² was calculated. The extreme values observed were 66.6-100% for the 23 phage-type 77 French isolates, 0-37.1% for the 10 unrelated French isolates, 0-36.3% for the 13 unrelated isolates from European cities outside of France, and 0-100% for the 13 unrelated North American isolates. The value obtained with the latter isolates was 100% because five of them had the same *EcoRI* HP pattern consisting of a single hybridising band. The extreme values for the

unrelated North American MRSA isolates could be recalculated by counting these five isolates as one; in this case, there were 13 distinct *EcoRI* HP and the extreme values were 0-50%.

Each of the *EcoRI* HP obtained for the phage-type 77 French MRSA isolates was compared with each of the *EcoRI* HP detected among the unrelated MRSA isolates (fig. 2). The extreme values observed were 0-58.8% with the 10 unrelated French isolates, 0-45.4% with the 13 unrelated European isolates except the French ones and 0-37.5% with the 13 unrelated North American isolates.

Discussion

The usefulness of three DNA probes for typing MRSA isolates and tracing epidemic MRSA strains widespread in French hospitals was evaluated in this study by analysing the HP obtained with these probes. *EcoRI* and *HindIII*-digested cellular DNA of unrelated MRSA isolates from different sources (Europe and North America), and of French MRSA isolates having identical or similar phage-types and numerous resistance markers in common were examined.

By the use of probe pBA2⁴² it was possible to detect re-arrangements occurring in the rRNA gene clusters or in the adjacent chromosomal sequences. In addition to its utility for species identification, this probe revealed diversity in patterns within most of the staphylococcal species investigated.⁵⁵ In this study, we showed that most of the MRSA isolates, whatever their sources, had the same *EcoRI* or *HindIII* HP. The most frequent patterns in the MRSA strains, E1 and H1, were shown to be uncommon among the MSSA isolates. These results suggested that many MRSA isolates may have evolved recently from a single ancestral strain, a hypothesis that has already been proposed for the MRSA strains isolated in Europe.⁵⁶ The number of patterns obtained with *EcoRI*-digested

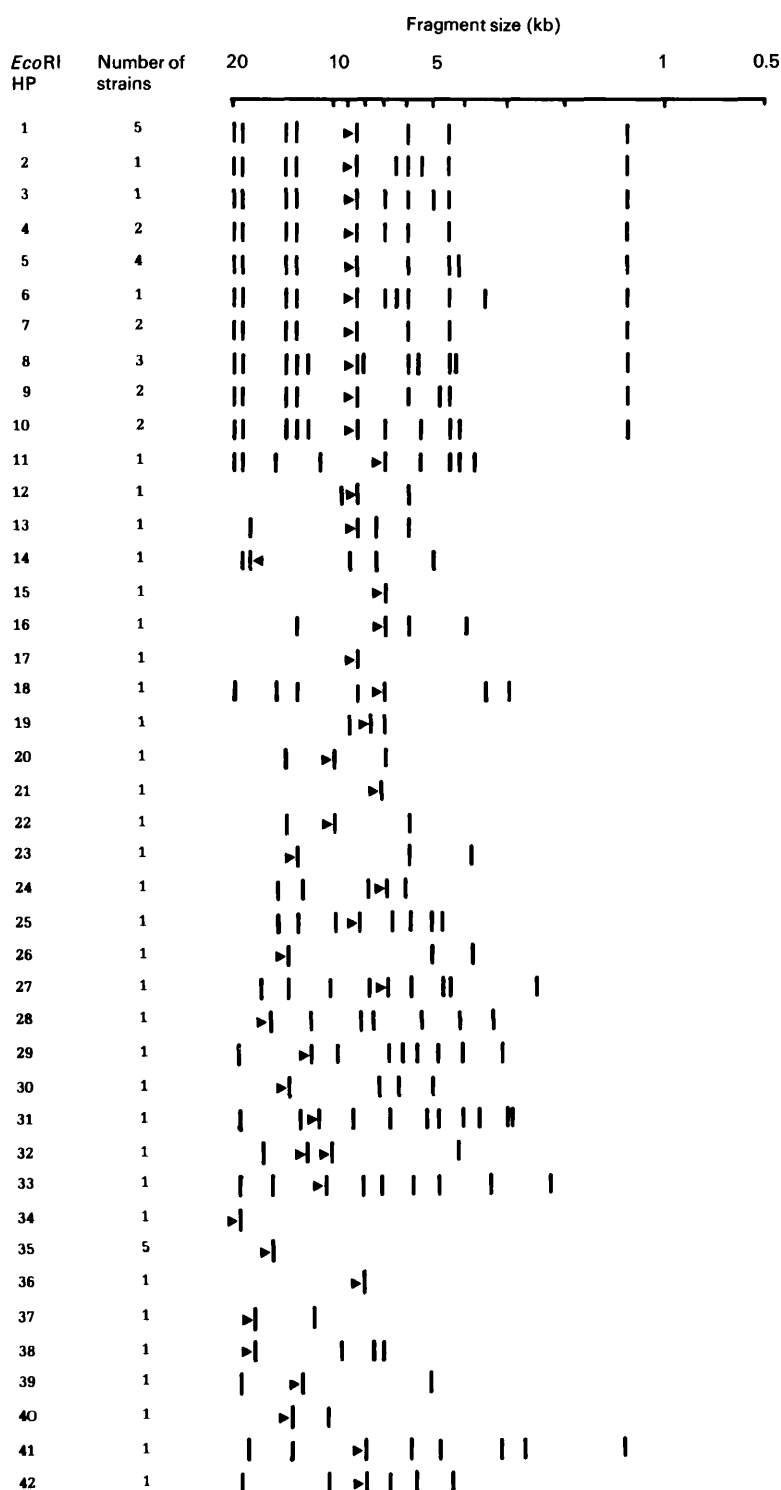


Fig. 2. Schematic representation of 42 *EcoRI* HP detected among 59 gentamicin-resistant MRSA isolates with α^{32} P-labelled pIP1307 as a probe. The bands marked with an arrow are those which also hybridised with probe pSF815A. HP 1–10 were obtained with the 23 phage-type 77 French isolates, HP 11–20 with the 10 French unrelated isolates, 21–33 with the 13 unrelated isolates from European cities outside France and 34–42 with the 13 North-American unrelated isolates.

DNA was greater than that obtained with *HindIII*-digested DNA. Therefore, the use of *EcoRI* instead of *HindIII* to digest cellular DNA is recommended for typing *S. aureus* isolates. However, only isolates having patterns other than E1 or H1 could be accurately differentiated with probe pBA2.

A greater diversity in patterns was observed with the two other probes, pSF815A⁴³ and pIP1307.⁴⁴

These two probes, which carry the gene *aacA-aphD*, could be used only for typing gentamicin-resistant isolates. The gene *aacA-aphD* was located on a 2.5-kb *HindIII* fragment indicating that these isolates may carry genetic element(s) identical or similar to the staphylococcal transposons, Tn4001^{53, 54} or Tn4031,⁵⁷ in which the *aacA-aphD* gene is situated on an internal 2.5-kb *HindIII* fragment. A single *EcoRI* restriction

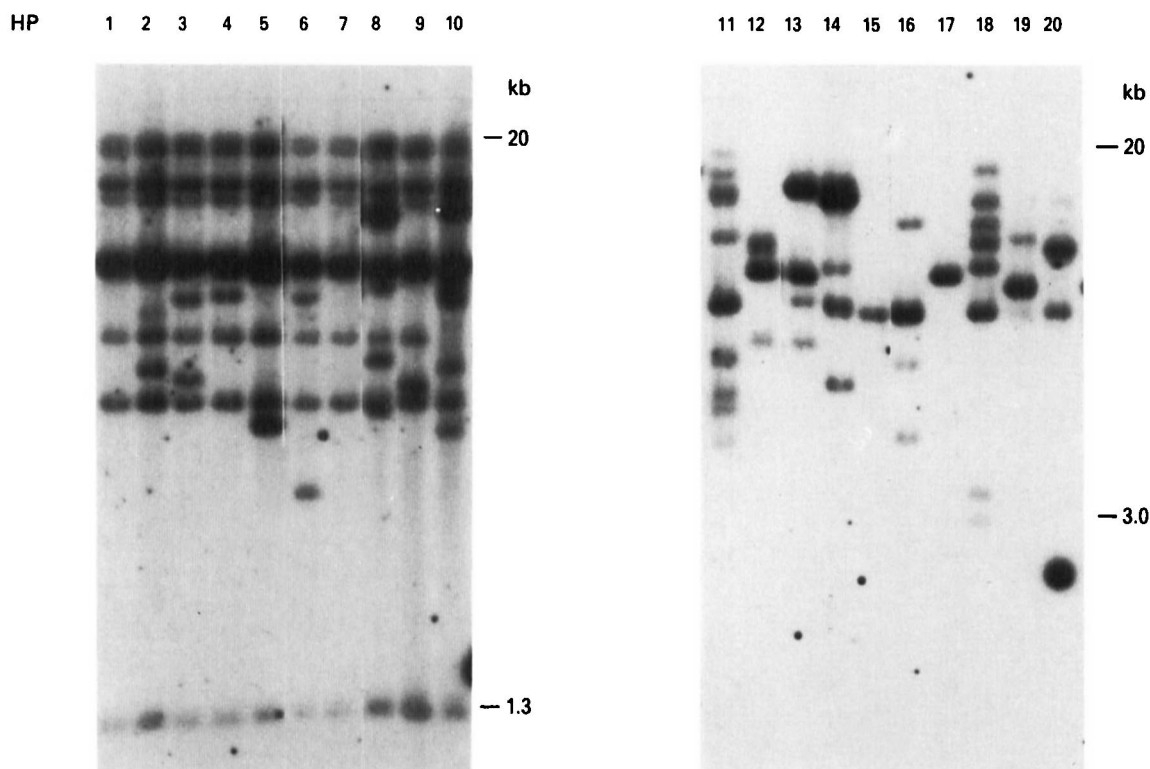


Fig. 3. Autoradiogram of *Eco*RI-digested cellular DNA of 20 French MRSA isolates separated by electrophoresis and probed with α^{32} P-labelled plasmid pIP1307. HP 1–10 were obtained with 10 phage-type 77 isolates and HP 11–20 with 10 unrelated isolates. The schematic representation of these HP is shown in fig. 2.

fragment hybridising with probe pSF815A, detected in 34 of the 36 unrelated MRSA isolates, suggests that a single copy of the gene *aacA-aphD* is present in these isolates. In most of the isolates, several *Eco*RI or *Hind*III hybridising fragments were detected with the probe pIP1307, which contain part of IS256 in addition to the gene *aacA-aphD*. Those fragments hybridising with probe pIP1307 but not with the *aacA-aphD* gene, probably carry sequences homologous to IS256. Further studies are needed to characterise the sequence hybridising with IS256 which were detected in gentamicin-resistant isolates but not in any of the gentamicin-susceptible isolates tested. It was easy to fingerprint the strains with the pIP1307 probe because numerous bands appeared in most of the HP. The diversity of patterns obtained with probe pIP1307 was much greater than that observed with probe pSF815A. With probe pIP1307, each of the unrelated isolates was characterised by its own HP, provided that the HP consisted of more than one band. The highest percentage of similarity detected among these latter HP was 50. Thus, the detection of isolates with identical HP consisting of more than one band or with HP sharing percentages of similarity higher than 50 would be good evidence that the strains were closely related. The hypothesis that isolates are closely related becomes more likely to be correct at the higher percentages of similarity.

The use of probe pIP1307, was the most effective for discriminating the phage-type 77 isolates from the epidemiologically unrelated MRSA isolates. With

probe pSF815A, a single *Eco*RI fragment of 8.5 kb was detected in all the phage-type 77 isolates. However, since a fragment of the same size was also detected in six of the 36 gentamicin-resistant unrelated MRSA isolates studied, the use of this probe alone is not enough to trace precisely the dissemination of phage-type 77 French MRSA isolates. In contrast, since the same seven *Eco*RI and nine *Hind*III fragments hybridising with the probe pIP1307 were detected in all phage-type 77 isolates, the use of this probe alone sufficed to trace the epidemic phage-type 77 strains widespread in the French hospitals. This typing method was particularly useful for epidemic isolates giving weak lytic reactions. However, if the isolates lack common bands, no conclusion concerning their relatedness can be formulated, because a single molecular re-arrangement occurring in an epidemic strain may cause a large modification of the HP. If such modifications take place, then the use of this probe will be limited.

The best typing methods are those by which one or more characteristics not commonly found in epidemiologically unrelated isolates can be detected. In epidemiological surveys, the presence of such characteristics in many strains studied suggests a close relatedness. Several methods are often required to detect uncommon markers to which we attribute a particular weight. To avoid applying numerous methods at the same time, we use successively the following techniques, reported here in decreasing order of priority, until we detect at least one such marker: drug

resistance phenotyping, phage-typing, hybridisation of *EcoRI*-digested cellular DNA with probes pIP1307 and pBA2 and plasmid content analysis. To establish the order of priority, we have taken into account the power of the methods in discriminating the French MRSA isolates as well as the convenience of the techniques and cost. Since North American isolates appear to display more diverse serotypes and plasmid

profiles than the French MRSA isolates, a greater priority may be attributed to these methods for typing the former isolates. An exchange of information with epidemiologists is required to facilitate the task of the microbiologists in epidemiological surveys.

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