An epidemiological assessment of coagulase-negative staphylococci from an intensive care unit

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Summary. Detection of an unusual combination of four resistance markers among coagulase-negative staphylococci (CNS) isolated in the same intensive care unit led to the undertaking of an epidemiological assessment. Seventeen CNS isolates from the same unit and 38 epidemiologically unrelated Staphylococcus epidermidis isolates were typed by eight methods, including analysis of immunoblot patterns and hybridisation patterns (HP) obtained with three probes. The probes comprised plasmids carrying the genes encoding 16S rRNA (pBA2), aacA-aphD (pSF815A), and aacA-aphD with part of IS256 (pIP1307). Immunoblot patterns and HP with pIP1307 indicated that 14 of the 17 CNS isolates from the same unit resulted from the spread of an epidemic strain.

Introduction

Coagulase-negative staphylococci (CNS) are found commonly on the body surfaces of man and animals. Staphylococcus epidermidis is the most prevalent species in normal skin flora, and also the most prevalent causative agent of opportunistic infections associated with implanted medical devices. The ubiquitous nature of CNS has posed problems in deciding the significance of an individual isolate as a causative agent of infection. Assessment is based on the clinical condition of the patient and the presence of closely related isolates in subsequent specimens taken from the patient. Outbreaks of nosocomial infections caused by epidemic strains have been reported, but conclusive evidence has been established rarely. Indeed, it is difficult to conclude that any two isolates are closely related because of phenotypic variations often observed among CNS and the limitations of the traditional methods used to type CNS. These methods include biotyping, drug resistance phenotyping, phage-typing and serotyping. Various molecular methods, such as analysis of plasmid content, cellular DNA restriction patterns, restriction polymorphism of the rRNA gene region, electrophoretic profiles of the cellular proteins, immunoblotting profiles and analysis of cellular fatty acids, have been utilised in an attempt to overcome these problems. This paper contains an epidemiological assessment of CNS isolated in the intensive care unit of a Parisian hospital. Of the 198 CNS isolated in this unit over a 5-month period in 1989, 37 (19%) had numerous common antibiotic resistance markers, including resistance to pefloxacin, rifampicin, fusidic acid and fosfomycin. This combination was unusual in comparison with the normal resistance phenotypes encountered among CNS isolated in other French hospitals and sent to our reference centre. Since these four antibiotics were in common therapeutic use in the intensive care unit, the prevalence of such CNS could result from dissemination of an epidemic strain of CNS or from selection of resistance in unrelated isolates. Of the 37 CNS isolates from the same unit, 17 were typed by eight methods, including hybridisation with three probes previously described for typing S. aureus and immunoblotting with a rabbit antiserum raised against a S. epidermidis strain. In addition to the 17 isolates from the same unit, 38 S. epidermidis isolates from different geographical areas were also included in this study to evaluate the discriminatory power of the new typing methods.

Materials and methods

Bacterial strains and plasmids

The CNS studied consisted of 38 S. epidermidis isolates originating from different geographical areas (10 isolates from 10 states in the USA and 28 isolates from 11 French cities) and 17 isolates obtained between 6 Jan. 1989 and 18 May 1989 in an intensive care unit in Paris. The 17 latter isolates were resistant to pefloxacin, rifampicin, fusidic acid and fosfomycin.
and were isolated from blood cultures and intravascular catheters of 17 patients with clinical evidence of infection. For 12 of these patients, at least two CNS resistant to the four antibiotics were isolated from two independent blood samples.

*Escherichia coli* strains used were SF8, SF815A and BM3887, harbouring plasmids pBA2, pSF815A and pIP1307 respectively. Plasmid pBA2 consists of a 2.3-kb *Bacillus subtilis* DNA fragment encoding 16S rRNA inserted in the *Hind*III site of pBR322. Plasmid pSF815A consists of pUC8 with a 1.5-kb *Ahu* insert carrying the *Enterococcus faecalis aacA-aphD* gene specifying the bifunctional enzyme AAC6'-APH2' that can modify gentamicin, kanamycin and related aminoglycosides. Plasmid pIP1307 consists of pBR322 with a 2.1-kb *Hind*III insert carrying the *aacA-aphD* gene from a methicillin-resistant *S. aureus* strain isolated in France. This insert is derived, by deletion, from the 2.5-kb *Hind*III internal fragment of *Tn4001*, and contains, in addition to the *aacA-aphD* gene, a 297-bp sequence from *IS256*.

**Species identification of staphylococci**

Biochemical identification was as described previously, and included use of the ID 32 Staph gallery (API System, La Balme Les Grottes, France). Genomic identification involved hybridisation of *Hind*III and *EcoR*I digests of bacterial DNA, separated by electrophoresis, with plasmid pBA2. Detection in the hybridisation patterns (HP) of the species-specific core of hybridising bands allowed a precise species assignment.

**Susceptibility to antimicrobial agents**

Susceptibility to antimicrobial agents was determined with a disk diffusion assay and commercially available antibiotic disks (Diagnostica Pasteur, Marne La Coquette, France). Additional disks prepared in our laboratory contained 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), propanolol isethionate (200 μg) or cetyltrimethylammonium bromide (10 μg).

**Serotyping**

The isolates were typed by slide agglutination of formalin-killed strains as described previously. The following absorbed rabbit antisera, raised against the CNS strains in parentheses, were used: 52186 (*S. epidermidis* 52186); 52260 (*S. epidermidis* 52260); Epidermidis (ATCC 14990T); Hominis (ATCC 27844T); Warneri (ATCC 27836T); Haemolyticus (DSM 20263T); Simulans (ATCC 27848T); Capitis (ATCC 27840T); Saprophyticus (ATCC 15050T); Cohiii (DSM 20260); and Xylosus (DSM 20266T). Immunisation of rabbits and preparation of the absorbed hyperimmune antisera were as described previously.

**Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of bacterial proteins**

CNS isolates were grown overnight at 37°C in 10 ml of medium containing (g/L): synthetic broth AGAC (Difco) 17, casaminoacids (Difco) 17, yeast extract (Difco) 5, glucose 5. The resulting culture was centrifuged at 5000 g for 15 min at 4°C. The cell pellet was suspended and washed three times in 5 ml of NaCl 0.85% w/v. The washed pellet was resuspended in 1 ml of lysis solution (NaCl 0.85% w/v; lysostaphin, Sigma 100 μg/ml, and incubated at 37°C for 30 min. Samples, containing an average of 500 μg of protein, were solubilised by mixing in 4:1 proportions with 0.25 M Tris HCl, pH 6.8, containing sodium dodecyl sulphate (SDS) 12.5% w/v, glycerol 50% w/v, dithiothreitol 7.7% w/v, bromophenol blue sodium salt (Serva, Heidelberg, Germany) 0.005% w/v, and boiled for 5 min before application to SDS-PAGE gels.

SDS-PAGE was performed as described by Laemmli, with an acrylamide 3.5% w/v stacking gel over an acrylamide 10% w/v separating gel. High and low molecular mass standards (BioRad Laboratories) were run as markers on each gel. The gels were stained with a solution containing Coomassie Brilliant Blue R250 (Sigma) 0.1% w/v, ethanol 50% v/v, acetic acid 10% v/v, and destained with a solution containing ethanol 10% v/v and acetic acid 7% v/v.

For immunoblotting, the separated proteins from unstained gels were transferred on to nitrocellulose membranes in 25 mM Tris, 192 mM glycine buffer, pH 8.3, containing methanol 20% v/v, at 25°C with a current of 0.5 A applied for 45 min in a LKB Transblot cell. After transfer, the membranes were washed three times with 0.5 M Tris, 0.1 M NaCl, pH 8. Free protein-binding sites were then saturated by incubation at 37°C for 60 min in the same buffer supplemented with low-fat milk powder 1% w/v (Regilait écrémé, France). The membranes were washed with the same buffer and then incubated at 37°C for 60 min with unabsorbed rabbit antiserum raised against *S. epidermidis* ATCC 14990T added to the blocking buffer to a final 100-fold dilution. The membranes were washed four times with blocking buffer and then incubated at 37°C for a further 75 min with horseradish peroxidase-linked goat anti-rabbit IgG (BioRad) diluted 1000-fold in blocking buffer. After 10 washes in blocking buffer, and a further five washes in the same buffer without milk, the membranes were stained with a solution containing diamino-benzidine tetrahydrochloride (Sigma) 0.05% w/v and H₂O₂ 0.01% v/v in 0.1 M Tris HCl, pH 7.6.

**DNA isolation and analysis**

Total cellular DNA was isolated from CNS strains and purified as described previously. Plasmid DNA
from *E. coli* strains was isolated and purified in a CsCl–EtBr gradient. CNS strains were screened for plasmid DNA with the rapid procedure described by Kado and Liu.

Restriction endonucleases *EcoRI* and *HindIII* (Amersham International, Little Chalfont, Bucks) were used according to the manufacturer's instructions. Electrophoresis of digested DNA was carried out in agarose (Sigma) buffer. Bacteriophage λ DNA (Biolabs, New England, MA, USA) digested with *BglII*, a 1-kb DNA ladder (Bethesda Research Laboratories, Inc., Cockeysville, MD, USA) and the Raoul I ladder (Appigene, Strasbourg, France) were used as molecular size markers.

DNA blotting and hybridisation. DNA was transferred from agarose gels to nitrocellulose filters with the bidirectional method described by Smith and Summers.

Plasmids pBA2, pSF815A and pIP1307 were used as probes. Purified plasmid DNA was labelled with [*32P*]dCTP (3000 Ci/mmol; Amersham International) with the Amersham Multiprime DNA labelling system as recommended by the manufacturer. The specific activity of the probes was c. 10⁹ cpm/µg of DNA. Hybridisation was performed under stringent conditions as described previously. The blots were exposed to Fuji RX film at −70°C, with intensifying screens, for between 24 h and several days.

Comparison of hybridisation patterns (HP). The average similarity between any two HP was assessed by the coefficient of Dice, calculated as follows:

\[
\text{Similarity (\%) = } \frac{\text{number of matching bands} \times 2}{\text{total number of bands}} \times 100
\]

Results

Characteristics of the 17 CNS isolates from the same unit

The isolates were speciated by determining their phenotypic characteristics and by analysis of the *HindIII* and *EcoRI* cellular DNA fragments that hybridised with the pBA2 probe. The hybridisation patterns of the 17 isolates were compared with those known to be produced by different staphylococcal species. Of the 17 isolates, 15 carried the *S. epidermidis*-specific core of common bands hybridising with pBA2; two isolates carried the *S. haemolyticus*-specific core of common bands. A good correlation was observed between the results obtained by phenotypic and genotypic speciation methods. Susceptibility to 27 antimicrobial agents was determined. All 17 isolates shared resistance to 10 antimicrobial agents (table I).

The isolates were typed with 11 different absorbed rabbit antisera raised against CNS. Three of the 15 *S. epidermidis* isolates reacted with antisera 52186 and Epidermidis; the other 12 strains were non-typable. The two *S. haemolyticus* isolates reacted with the absorbed sera prepared against the *S. haemolyticus* type strain, reinforcing the assignment to this species established on the basis of phenotypic and genotypic characteristics.

Whole cell polypeptide profiles were determined for the 17 isolates. The profiles of eight of these isolates are shown in fig. 1A. The comparative analysis of these profiles was difficult because of the large number of bands in each profile. Moreover, despite the use of standardised experimental conditions, the bands yielding distinguishable profiles within *S. epidermidis* were limited in number and not always reproducible. In contrast, the profiles of the *S. haemolyticus* isolates (e.g. fig. 1A, lane 9) were always distinguishable from those of the *S. epidermidis* isolates (fig. 1A, lanes 1–8).

Immunoblot patterns obtained with antisera raised against *S. epidermidis* strain ATCC 14990T revealed two clearly distinct patterns, I1 and I2, among the 15 *S. epidermidis* isolates. Although loaded with similar amounts of total cellular proteins, pattern I1 (fig. 1B, lanes 1–7) was characterised by a limited number of antigens reacting with the antiserum, whereas pattern I2 (fig. 1B, lane 8) consisted of a large number of bands. The patterns (pattern 13) of the two *S. haemolyticus* isolates (e.g., fig. 1B, lane 9) were identical, but were easily distinguished from patterns I1 and I2 (fig. 1B, lanes 1–8).

The six distinct plasmid profiles detected among the 17 isolates are shown in fig. 2. The distribution of these profiles among the isolates is shown in table I. Profile P2 was predominant and was found in eight of the *S. epidermidis* isolates. Profile P1, with four bands in common with profile P2, was found in four *S. epidermidis* isolates. Three other profiles, P3, P4 and P5, occurred once only. The two *S. haemolyticus* isolates both had a profile, P6, which contained a single extrachromosomal DNA band that migrated in the same position as one of the bands of profiles P1, P2, P3 and P5 (fig. 2).

In addition to their use for speciation, the determination of HP between *HindIII* and *EcoRI* digests and pBA2 allowed four types to be distinguished among the 15 *S. epidermidis* isolates: H20–E21 (10 isolates), H20–E46 (three isolates), H21–E21 (one isolate) and H21–E46 (one isolate). The two *S. haemolyticus* isolates both had the pattern H32–E30.

When the *HindIII* and *EcoRI* cellular DNA digests were hybridised with the pSF815A probe carrying the *aacA*-aphD gene, homology was detected with a 2.5-kb *HindIII* fragment in all the isolates (fig. 3). In 14 of the 15 *S. epidermidis* isolates, hybridisation was detected with an 18-kb *EcoRI* fragment (fig. 3), while in BM9877 (table I) hybridisation occurred with a 25-kb *EcoRI* fragment (fig. 3). In the two *S. haemolyticus* isolates, hybridisation occurred with a 9.2-kb *EcoRI* fragment (fig. 3).

With pIP1307 as a probe, eight *HindIII* and five *EcoRI* HP were detected (fig. 3). With each isolate,
<table>
<thead>
<tr>
<th>Date of sampling (1989)</th>
<th>Strain no.</th>
<th>Species</th>
<th>Drug resistance pattern</th>
<th>Serotype</th>
<th>Immunoblot pattern</th>
<th>Plasmid profile</th>
<th>Hybridisation pattern with pSF815A</th>
<th>pIP 1307</th>
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<td>52186, Epi</td>
<td>I1 P4</td>
<td></td>
<td>H20 E21 H701 E718</td>
<td>H423</td>
</tr>
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<td>S. epidermidis</td>
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<td>SgAMLS Su P F Fa Fo As</td>
<td>EbAfCaPi</td>
<td>N.T.</td>
<td>P2</td>
<td>H20 E21 H701 E718</td>
<td>H420</td>
</tr>
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<td>24/1 BM9885</td>
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<td>MLSTspUP F Fa Fo As</td>
<td>EbAfCaPi</td>
<td>N.T.</td>
<td>P1</td>
<td>H20 E21 H701 E718</td>
<td>H421</td>
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<td>MLSTspUP F Fa FoCd As</td>
<td>HgEbAfCaPi</td>
<td>52186, Epi</td>
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<td>H20 E21 H701 E721</td>
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<td>H420</td>
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<td>II P2</td>
<td></td>
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<td>H420</td>
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<td>MLSTspUP F Fa Fo As</td>
<td>N.T.</td>
<td>II P2</td>
<td></td>
<td>H20 E21 H701 E718</td>
<td>H420</td>
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<td>H420</td>
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<td>MLSTspUP F FaFoCd As</td>
<td>HgEbAfCaPi</td>
<td>N.T.</td>
<td>II P2</td>
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<tr>
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<td>MLSTp P F FaFoCd Hg</td>
<td>Hae</td>
<td>I3 P6</td>
<td></td>
<td>H32 E30 H701 E720</td>
<td>H426</td>
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<td>MLSTp UP F FaFoCd Hg</td>
<td>Hae</td>
<td>I3 P6</td>
<td></td>
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<td>17/4 BM9867</td>
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<td>SgAMLSTpUP F Fa FoCd As</td>
<td>HgEbAfCaPi</td>
<td>52186, Epi</td>
<td>I2 P2</td>
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<td>TcS gAMLST SuP F Fa Fo As</td>
<td>N.T.</td>
<td>II P1</td>
<td></td>
<td>H20 E21 H701 E718</td>
<td>H420</td>
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<tr>
<td>13/5 BM9874</td>
<td>S. epidermidis</td>
<td>PcOx SpKm TmGm</td>
<td>MLSTspUP F Fa FoCd As</td>
<td>HgAfCaPi</td>
<td>N.T.</td>
<td>II P2</td>
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<td>TcMLSTspUP F Fa FoCd As</td>
<td>HgAfCaPi</td>
<td>N.T.</td>
<td>II P1</td>
<td>H21 E46 H701 E718</td>
<td>H422</td>
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</table>

Resistance markers: Af, acriflavine; As, sodium arsenate; Ca, cetrimethylammonium bromide; Cd, cadmium acetate; Cl, chloramphenicol; Eb, ethidium bromide; Fa, fusidic acid; Fo, fosfomycin; Gm, gentamicin; Hg, mercuric nitrate; Km, kanamycin; MLS, macrolides-lincosamides-Streptogramin B; Nm, neomycin; Ox, oxacillin; Pc, penicillinase production; Pf, pefloxacin; Pi, propamidine isothionate; Rf, rifampicin; SgA, streptogramin A; Sp, Spectinomycin; Sm, streptomycin; Su, sulphonamide; Tc, tetracycline; Tp, trimethoprim; Tm, tobramycin.

Serotypes: Epi, Epidermidis; Hae, Haemolyticus; N.T., non-typable.
hybridisation occurred with between four and nine HindIII fragments and between three and six EcoRI fragments. At least two independent restriction enzyme digestions and hybridisation experiments were performed to ensure that the HP were reproducible.

The two *S. haemolyticus* isolates had the same HP, H426–E428 (fig. 3). These HP were compared with those of the *S. epidermidis* isolates by calculating the Dice coefficients. HP E428 showed no similarity with the four EcoRI HP detected among the 15 *S. epidermidis* isolates, whereas HP H426 showed 18.1–36.3% similarity with the seven HindIII HP detected among these latter isolates. The H429–E429 HP of the *S. epidermidis* isolate BM9877 (table 1) also showed low percentages of similarity with the HP of the other *S. epidermidis* isolates (15.3–25% with the HindIII HP, but no similarity with the EcoRI HP). In contrast, higher percentages of similarity were observed among the HP of the 14 other *S. epidermidis* isolates (66.6–88.8% for HindIII HP and 66.6–99% for the EcoRI HP).

**Characteristics of the 38 *S. epidermidis* isolates originating from different geographical areas**

These isolates were included in the study to evaluate the discriminatory power of the new typing methods used to compare the 17 isolates from the same unit.

Each of the 38 unrelated *S. epidermidis* isolates was characterised by its own drug resistance phenotype consisting of 2–19 resistance markers, but without any marker shared by all the isolates (results not shown).
<table>
<thead>
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<th>Restriction enzyme</th>
<th>Pattern</th>
<th>Number of strains</th>
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<tbody>
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<td>HindIII</td>
<td>H426</td>
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<tr>
<td></td>
<td>H429</td>
<td>1</td>
</tr>
<tr>
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<tr>
<td></td>
<td>H428</td>
<td>1</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>E427</td>
<td>1</td>
</tr>
</tbody>
</table>

**Fig. 3.** Schematic representation of the hybridisation patterns obtained between HindIII and EcoRI digests of total cellular DNA and α-32P labelled pIP1307. The bands marked with an arrow were those which hybridised also with the pSF815A probe.

Of the 38 unrelated *S. epidermidis* isolates, 27 reacted with either one or two of the three absorbed antisera raised against *S. epidermidis* strains. The typable isolates could be grouped into three serotypes: 52186-Epidermidis (22 isolates), 52186–52260 (three isolates) and 52186 (two isolates). None of the isolates reacted with the eight other absorbed antisera raised against strains belonging to other staphylococcal species.

Immunoblotting with the rabbit antiserum raised against *S. epidermidis* strain ATCC 14990T distinguished 30 immunoblot patterns among the 38 isolates analysed. The results obtained for eight of these isolates are shown in fig. 4B.

With the pBA2 probe, the 38 isolates were grouped into either nine or four types according to the HP obtained after cleavage of the cellular DNA with HindIII or EcoRI respectively. After cleavage with HindIII, two predominant HP were observed, H20 and H51, which accounted for 14 and 11 isolates respectively. The seven other HP observed were uncommon: H21 (one isolate), H45 (four isolates), H47 (one isolate), H50 (three isolates), H53 (one isolate), H55 (two isolates) and H56 (one isolate). After EcoRI cleavage, HP-E21 was predominant (34 isolates), whereas the other three HP observed—E45, E46 and E47—were rare (one, two and one isolates respectively). Combined use of HindIII and EcoRI allowed 11 HP to be distinguished among the 38 isolates: H20–E21 (13 isolates), H51–E21 (11 isolates), H45–E21 (four isolates), H50–E21 (three isolates), and a further seven combinations which accounted for one isolate each: H20–E45, H21–E46, H47–E21, H53–E21, H55–E21, H55–E46 and H56–E47. All these HP, which have been described previously, contained the *S. epidermidis*-specific core of common bands. These results confirmed the assignment to *S. epidermidis* based on phenotypic characteristics.

None of the 19 isolates susceptible to gentamicin carried detectable nucleotide sequences that hybridised with pSF815A or pIP1307. In the 19 other isolates which were resistant to gentamicin, hybridisation was detected between the pSF815A probe and a 2.5-kb HindIII fragment (fig. 5A), and EcoRI fragments of various sizes (fig. 5C) which identified 15 distinct EcoRI HP. With the pIP1307 probe, hybridisation was detected between one and 11 HindIII fragments (fig. 5A) and between one and seven EcoRI fragments (fig. 5B). Twelve distinct HindIII HP (fig. 5A) and 19 distinct EcoRI HP (fig. 5B) were detected among the 19 gentamicin-resistant isolates. The eight isolates with the same HindIII HP had a single HindIII fragment of 2.5 kb that hybridised with pIP1307 (fig. 5A). Each of the isolates with more than one HindIII fragment that hybridised with pIP1307 was characterised by its own HindIII HP. The HP were compared by calculating the Dice coefficient. The extreme values observed were 0–40% for the 19 EcoRI HP and 12.5–60% for...
Fig. 4. Whole cell polypeptide profiles (A) of eight epidemiologically unrelated isolates of *S. epidermidis*, and the immunoblot patterns (B) obtained with rabbit hyperimmune antisera raised against *S. epidermidis* ATCC 14990T.

the 12 HindIII HP. When recalculated after eliminating the HindIII HP consisting of a single 2.5-kb HindIII band, the extreme values were 12.5–50%.

Table II summarises the characteristics of all 53 *S. epidermidis* isolates included in this study.

### Discussion

In addition to a precise speciation, eight typing methods have been used to type CNS isolates, including analysis of immunoblot patterns and determination of HP obtained with three probes used previously to type *S. aureus* isolates. The ability to discriminate between CNS strains by these latter methods was evaluated by examining 38 unrelated *S. epidermidis* isolates originating from different geographical areas. Analysis of immunoblot patterns showed that the use of unabsorbed antiserum raised against strain ATCC 14990T yielded the largest number of distinct types among the 38 unrelated *S. epidermidis* isolates (results not shown). The pBA2 probe allowed detection of re-arrangements occurring in the rRNA gene clusters or in the adjacent chromosomal sequences. In contrast with the results observed among *S. aureus* isolates, within *S. epider-

<table>
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midis isolates the use of HindIII allowed detection of a greater heterogeneity in HP than that detected following use of EcoRI. The patterns H20, H51 and E21 were predominant among the unrelated CNS isolates; therefore, only isolates having other patterns can be differentiated accurately with the pBA2 probe. Despite the low discriminatory power of this method, its use is recommended since it enables a precise speciation of CNS\(^{48,49}\) as a first step in typing.

With the two other probes, pSF815A and pIP1307, a greater diversity in patterns was observed among the unrelated S. epidermidis isolates. These two probes, which carry the gene aacA-aphD, appeared to be useful only for typing gentamicin-resistant isolates. In
each isolate, the gene \( aacA-aphD \) was located on a 2.5-kb HindIII fragment, suggesting that the isolates carry genetic elements identical or similar to \( Tn4001 \) or \( Tn4037 \); since the \( aacA-aphD \) gene of these transposons is also situated within a 2.5-kb HindIII internal fragment. In 13 of the unrelated isolates, several EcoRI and HindIII fragment patterns were obtained that hybridized with the pIP1307 probe. This probe contains, in addition to the \( aacA-aphD \) gene, part of IS256. Fragments hybridizing with pIP1307, but not with the \( aacA-aphD \) gene, carry sequences homologous to IS256, but such sequences were not detected in any of the gentamicin-susceptible unrelated S. epidermidis isolates that were tested. Recent studies have shown that the EcoRI and HindIII fragment patterns carrying these sequences also hybridized with a probe consisting of an amplified DNA sequence corresponding to the ORF390 of IS256 (K. Dyke and N. El Solh, unpublished observations). These results may reflect the presence of multiple copies of IS256 in the isolates carrying such sequences. Further studies are needed to verify this hypothesis. The preferential use of pIP1307 as a probe is recommended because of the generation of complex HP, thereby allowing easy fingerprinting, and because of the greater diversity of the HP obtained with pIP1307 had greater similarity (66-6-99\%) than any value detected between the HP of the unrelated S. epidermidis isolates. Within this group of 14 S. epidermidis isolates, strains BM9872 and BM9868 (table I) were indistinguishable from each other. Similarly, strains BM9870 and BM9873 were indistinguishable and, since they shared the same uncommon pBA2 HP (H2E046) and the same plasmid profile (P2), these strains were also related closely to strain BM9874.

In conclusion, the CNS strains isolated from the same unit that carried an unusual combination of drug resistance markers resulted mostly from the spread of an epidemic strain, but also from the occasional independent selection of unrelated isolates by the four drugs commonly used in the unit. Determination of drug resistance phenotypes was useful in indicating the possibility of epidemic spread of closely related isolates in the unit, but was not sufficient by itself to confirm this hypothesis. The use of several different typing methods was necessary for a proper epidemiological assessment. Speciation, analysis of immunoblot patterns, and determination of HP with pIP1307 as a probe were the methods that yielded the most conclusive results.

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