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

SIEGLINDE WALCHER-SALESSE, C. MONZON-MORENO, SYLVIE AUBERT and NÉVINE EL SOLH*

Centre National de Référence pour les Staphylocoques, Laboratoire des Staphylocoques et des Streptocoques, Institut Pasteur, 75724 Paris Cedex 15, France

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.¹ *Staphylococcus epidermidis* is the most prevalent species in normal skin flora,¹ and also the most prevalent causative agent of opportunist infections associated with implanted medical devices.^{2–11} 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.^{12–17} Indeed, it is difficult to conclude that any two isolates are closely related because of phenotypic variations often observed among CNS^{16, 18–20} and the limitations of the traditional methods used to type CNS.^{21–24} These methods include biotyping,^{15, 25–29} drug resistance phenotyping,^{15, 25, 27, 30, 31} phage-typing^{27, 29, 32–38} and serotyping.³⁹ Various molecular methods, such as analysis of plasmid content,^{25, 27, 30, 31, 40–44} cellular DNA restriction patterns,^{45, 46} restriction polymorphism of the rRNA gene region,^{47–51} electrophoretic profiles of the cellular proteins,^{29, 31, 50, 52–56} immunoblotting profiles^{46, 52, 56} and analysis of cellular fatty acids,⁵⁷ 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*⁵⁸ 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,

Received 29 April 1991; accepted 8 July 1991.

*Correspondence should be sent to Dr N. El Solh.

and were isolated from blood cultures and intra-vascular 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.⁵⁹⁻⁶¹ 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 *Alu*I 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 IS256.⁵⁸

Species identification of staphylococci

Biochemical identification was as described previously,⁴⁸ and included use of the ID 32 Staph gallery (API System, La Balme Les Grottes, France).⁶² Genomic identification involved hybridisation of *Hind*III and *Eco*RI digests of bacterial DNA, separated by electrophoresis, with plasmid pBA2. Detection in the hybridisation patterns (HP) of the species-specific core of hybridising bands^{48, 49} allowed a precise species assignment.

Susceptibility to antimicrobial agents

Susceptibility to antimicrobial agents was determined with a disk diffusion assay⁶³ and commercially available antibiotic disks (Diagnostics 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), propamidine isethionate (200 µg) or cetyltrimethylammonium bromide (10 µg).

Serotyping

The isolates were typed by slide agglutination of formalin-killed strains as described previously.³⁹ 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 15305T); Cohnii (DSM 20260); and Xylosus (DSM 20266T). Immunisation of rabbits and preparation of the

absorbed hyperimmune antisera were as described previously.³⁹

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 AOAC (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,⁶⁴ 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 Lait, 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 diaminobenzidine 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.⁴⁸ Plasmid DNA

from *E. coli* strains was isolated and purified in a CsCl-EtBr gradient.⁶⁵ CNS strains were screened for plasmid DNA with the rapid procedure described by Kado and Liu.⁶⁶

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) 0.8% w/v gels in Tris/borate buffer.⁶⁵ Bacteriophage λ DNA (Biolabs, New England, MA, USA) digested with *Bgl*II, a 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.

DNA blotting and hybridisation. DNA was transferred from agarose gels to nitrocellulose filters with the bidirectional method described by Smith and Summers.⁶⁷

Plasmids pBA2, pSF815A and pIP1307 were used as probes. Purified plasmid DNA was labelled with [α^{32} P] 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.* 10^9 cpm/ μ g of DNA. Hybridisation was performed under stringent conditions as described previously.⁴⁸ 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,⁶⁸ 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.^{48, 49} 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 I3) 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 I. Characteristics of the 17 CNS strains isolated from 17 patients in the same intensive care unit

Date of sampling (1989) Day/month	Strain no.	Species	Drug resistance pattern	Serotype	Immunoblot pattern	Plasmid profile	Hybridisation pattern with				
							pBA2		pSF815A		pIP 1307
							HindIII	EcoRI	HindIII	EcoRI	
6/1	BM9886	<i>S. epidermidis</i>	TcSgAMLSTpSuPRIFaFo	52186,Epi	II	P4	H20	E21	H701	E718	H423
24/1	BM9872	<i>S. epidermidis</i>	SgAMLSTpSuPRIFaFo	N.T.	II	P2	H20	E21	H701	E718	H420
24/1	BM9885	<i>S. epidermidis</i>	MLSTpSuPRIFaFo	N.T.	II	P1	H20	E21	H701	E718	H421
6/2	BM9877	<i>S. epidermidis</i>	MLSTpSuPRIFaFoCd	52186,Epi	I2	P5	H20	E21	H701	E721	H429
7/2	BM9875	<i>S. epidermidis</i>	SgAMLS SuPRIFaFo	N.T.	II	P3	H20	E21	H701	E718	H420
3/3	BM9871	<i>S. epidermidis</i>	SgAMLSTpSuPRIFaFo	N.T.	II	P2	H21	E21	H701	E718	H428
10/3	BM9869	<i>S. epidermidis</i>	MLSTpSuPRIFaFo	N.T.	II	P2	H20	E21	H701	E718	H420
25/3	BM9868	<i>S. epidermidis</i>	SgAMLSTpSuPRIFaFo	N.T.	II	P2	H20	E21	H701	E718	H420
25/3	BM9881	<i>S. epidermidis</i>	Tc MLSTpSuPRIFaFo	N.T.	II	P1	H20	E21	H701	E718	H420
28/3	BM9870	<i>S. epidermidis</i>	MLSTpSuPRIFaFoCd	N.T.	II	P2	H20	E46	H701	E718	H420
30/3	BM9880	<i>S. haemolyticus</i>	MLSTp PRIFaFoCd	Hae	I3	P6	H32	E30	H701	E720	H426
5/4	BM9879	<i>S. haemolyticus</i>	MLS SuPRIFaFoCd	Hae	I3	P6	H32	E30	H701	E720	H426
17/4	BM9867	<i>S. epidermidis</i>	SgAMLSTpSuPRIFaFoCd	52186,Epi	II	P2	H20	E21	H701	E718	H424
2/5	BM9873	<i>S. epidermidis</i>	MLSTpSuPRIFaFoCd	N.T.	II	P2	H20	E46	H701	E718	H420
12/5	BM9884	<i>S. epidermidis</i>	TcSgAMLS SuPRIFaFo	N.T.	II	P1	H20	E21	H701	E718	H420
13/5	BM9874	<i>S. epidermidis</i>	MLSTpSuPRIFaFoCd	N.T.	II	P2	H20	E46	H701	E718	H422
18/5	BM9882	<i>S. epidermidis</i>	Tc MLSTpSuPRIFaFoCd	N.T.	II	P1	H21	E46	H701	E718	H422

Resistance markers: Af, acriflavine; As, sodium arsenate; Ca, cetyltrimethyl-ammonium bromide; Cd, cadmium acetate; Cm, chloramphenicol; Eb, ethidium bromide; Fa, fusidic acid; Fo, fosfomicin; 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, sulphphonamide; Tc, tetracycline; Tp, trimethoprim; Tm, tobramycin.

Serotypes: Epi, *Epidermidis*; Hae, *Haemolyticus*; N.T., non-typable.

Fig. 1. Whole cell polypeptide profiles (A), and immunoblot patterns (B), obtained with a rabbit hyperimmune antiserum raised against *S. epidermidis* strain ATCC 14990T, for eight of the CNS isolates listed in table I. Lane 1, molecular size standards; 2, BM9871; 3, BM9885; 4, BM9886; 5, BM9867; 6, BM9882; 7, BM9873; 8, BM9877; 9, BM9879.

hybridisation occurred with between four and nine *Hind*III fragments and between three and six *Eco*RI fragments. At least two independent restriction enzyme digestions and hybridisation experiments were performed to ensure that the HP were reproducible.

Fig. 2. Examples of the six plasmid profiles detected among the 17 CNS isolates included in the study.

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 *Eco*RI HP detected among the 15 *S. epidermidis* isolates, whereas HP H426 showed 18.1–36.3% similarity with the seven *Hind*III HP detected among these latter isolates. The H429-E429 HP of the *S. epidermidis* isolate BM9877 (table I) also showed low percentages of similarity with the HP of the other *S. epidermidis* isolates (15.3–25% with the *Hind*III HP, but no similarity with the *Eco*RI HP). In contrast, higher percentages of similarity were observed among the HP of the 14 other *S. epidermidis* isolates (66.6–88.8% for *Hind*III HP and 66.6–99% for the *Eco*RI 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).

Fig. 3. Schematic representation of the hybridisation patterns obtained between *HindIII* and *EcoRI* digests of total cellular DNA and α - ^{32}P 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,^{48,49} 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 with 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.⁶⁸ 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 *Hind*III HP. When recalculated after eliminating the *Hind*III HP consisting of a single 2.5-kb *Hind*III 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.⁵⁸ 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,⁵⁸ within *S. epider-*

Table II. Summary of the characteristics of the 53 *S. epidermidis* isolates studied

Typing method	Characteristics of			
	38 unrelated isolates		15 isolates from the same unit	
	Number of typable isolates	Number of distinct types	Number of typable isolates	Number of distinct types
Drug resistance pattern	38	38	15	12
Serotype	27	3	3	1
Immunoblot with antiserum against ATCC 14990T	38	30	15	2
HP between pBA2 and				
- <i>Hind</i> III digests	38	9	15	2
- <i>Eco</i> RI digests	38	4	15	2
- <i>Eco</i> RI + <i>Hind</i> III digests	38	11	15	4
HP between pSF815A and				
- <i>Hind</i> III digests	19	1	15	1
- <i>Eco</i> RI digests	19	15	15	2
HP between pIP1307 and				
- <i>Hind</i> III digests	19	12	15	7
- <i>Eco</i> RI digests	19	19	15	4

Fig. 5. Autoradiographs of cellular DNA from 19 epidemiologically unrelated *S. epidermidis* isolates, digested with *Hind*III (A) or *Eco*RI (B), and probed with labelled pIP1307 (A and B) or pSF815A (C). The 2.5-kb *Hind*III band marked with an asterisk in (A) hybridised also with pSF815A.

midis isolates the use of *Hind*III allowed detection of a greater heterogeneity in HP than that detected following use of *Eco*RI. 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^{69, 70} or Tn4031⁷¹ 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* fragments were detected that hybridised with the pIP1307 probe. This probe contains, in addition to the *aacA-aphD* gene, part of IS256. Fragments hybridising 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* fragments carrying these sequences also hybridised 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 obtained when compared with pSF815A (19 compared with 15 *EcoRI* HP, and 12 compared with one *HindIII* HP among the 19 unrelated *S. epidermidis* isolates). Thus the detection of identical HP with pIP1307 as a probe would be good evidence that two strains are closely related.

Among the 17 CNS strains that were isolated in the same unit and shared 11 drug resistance markers, phenotypic as well as genotypic speciation methods separated two *S. haemolyticus* isolates from 15 *S. epidermidis* isolates. The two *S. haemolyticus* isolates were closely related by all typing methods tested.

Analysis of immunoblot patterns and percentages of similarity between the HP obtained with pIP1307 enabled a clear-cut distinction between BM9877 (table I) and the 14 other *S. epidermidis* isolates. The immunoblot patterns of these latter isolates were characterised by a limited number of bands and were not distinguishable reproducibly. In addition, the *aacA-aphD* gene carried by these 14 isolates was located on an *EcoRI* fragment of the same size, while 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 (H20–E46) 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 four 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.

We thank K. Dyke for criticism of the manuscript, Anne Burée, Annie Buu-Hoï and G. Ann Hébert for providing strains, and Odette Rouelland for secretarial assistance.

References

- Kloos WE, Schleifer KH. Genus IV. *Staphylococcus* Rosenbach 1884 18^{AL}, (Nom. Cons. Opin. 17 Jud. Comm. 1958. 153). In: Sneath PHA (ed) Bergey's Manual of systematic bacteriology, vol 2, Baltimore, Williams and Wilkins. 1986: 1013–1035.
- Archer GL, Armstrong BC. Alteration of staphylococcal flora in cardiac surgery patients receiving antibiotic prophylaxis. *J Infect Dis* 1983; **147**: 642–649.
- Bint AJ, Finch RG, Gokal R, Goldsmith HJ, Junor B, Oliver D. Diagnosis and management of peritonitis in continuous ambulatory peritoneal dialysis. (Report of a working party of the British Society for Antimicrobial Chemotherapy) *Lancet* 1987; **1**: 845–849.
- Bor DH, Rose RM, Modlin JF, Weintraub R, Friedland GH. Mediastinitis after cardiovascular surgery. *Rev Infect Dis* 1983; **5**: 885–897.
- Dismukes WE. Prosthetic valve endocarditis: factors influencing outcome and recommendations for therapy. In: Bisno AL (ed) Treatment of infective endocarditis. New York, Grune and Stratton. 1981: 167–191.
- Eisenberg ES, Ambalu M, Szylagi G, Aning V, Soeiro R. Colonization of skin and development of peritonitis due to coagulase-negative staphylococci in patients undergoing peritoneal dialysis. *J Infect Dis* 1987; **156**: 478–482.
- Gemmell CG. Pathogenicity of coagulase-negative staphylococci with respect to the nature of the host response. *Zentralbl Bakteriol Hyg A* 1987; **266**: 52–59.
- Karchmer AW, Archer GL, Dismukes WE. *Staphylococcus epidermidis* causing prosthetic valve endocarditis: microbiologic and clinical observations as guides to therapy. *Ann Intern Med* 1983; **98**: 447–455.
- Phillips I (ed). Focus on coagulase negative staphylococci. London, Royal Society of Medicine Services, International Congress and Symposium Series No. 151. 1989.
- Sidebottom DG, Freeman J, Platt R, Epstein MF, Goldmann DA. Fifteen-year experience with bloodstream isolates of coagulase-negative staphylococci in neonatal intensive care. *J Clin Microbiol* 1988; **26**: 713–718.
- Winston DJ, Dudnick DV, Chapin M, Ho WG, Gale RP, Martin WJ. Coagulase-negative staphylococcal bacteremia in patients receiving immunosuppressive therapy. *Arch Intern Med* 1983; **143**: 32–36.
- Archer GL, Vishniavsky N, Stiver HG. Plasmid pattern analysis of *Staphylococcus epidermidis* isolates from patients with prosthetic valve endocarditis. *Infect Immun* 1982; **35**: 627–632.
- Boyce JM, Potter-Bynoe G, Opal SM, Dziobek L, Medeiros AA. A common-source outbreak of *Staphylococcus epidermidis* infections among patients undergoing cardiac surgery. *J Infect Dis* 1990; **161**: 493–499.
- Christensen GD, Bisno AL, Parisi JT, McLaughlin B, Hester MG, Luther RW. Nosocomial septicemia due to multiply

- antibiotic-resistant *Staphylococcus epidermidis*. *Ann Intern Med* 1982; **96**: 1-10.
15. Ludlam HA, Noble WC, Marples RR, Bayston R, Phillips I. The epidemiology of peritonitis caused by coagulase-negative staphylococci in continuous ambulatory peritoneal dialysis. *J Med Microbiol* 1989; **30**: 167-174.
 16. Mickelsen PA, Plorde JJ, Gordon KP *et al*. Instability of antibiotic resistance in a strain of *Staphylococcus epidermidis* isolated from an outbreak of prosthetic valve endocarditis. *J Infect Dis* 1985; **152**: 50-58.
 17. Van Den Broek PJ, Lampe AS, Berbée GAM, Thompson J, Mouton RP. Epidemic of prosthetic valve endocarditis caused by *Staphylococcus epidermidis*. *Br Med J* 1985; **291**: 949-950.
 18. Christensen GD, Baddour LM, Madison BM *et al*. Colonial morphology of staphylococci on Memphis agar: phase variation of slime production, resistance to β -lactam antibiotics, and virulence. *J Infect Dis* 1990; **161**: 1153-1169.
 19. Etienne J, Renaud F, Bes M *et al*. Instability of characteristics amongst coagulase-negative staphylococci causing endocarditis. *J Med Microbiol* 1990; **32**: 115-122.
 20. Richardson JF, Marples RR. Changing resistance to antimicrobial drugs, and resistance typing in clinically significant strains of *Staphylococcus epidermidis*. *J Med Microbiol* 1982; **15**: 475-484.
 21. Marples RR. Laboratory assessment in the epidemiology of infections caused by coagulase-negative staphylococci. *J Med Microbiol* 1986; **22**: 285-286.
 22. Parisi JT, Lampson BC, Hoover DL, Khan JA. Comparison of epidemiologic markers for *Staphylococcus epidermidis*. *J Clin Microbiol* 1986; **24**: 56-60.
 23. Pfaller MA, Herwaldt LA. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clin Microbiol Rev* 1988; **1**: 281-299.
 24. Thore M, Kühn I, Löfdahl S, Burman LG. Drug-resistant coagulase-negative skin staphylococci. *Epidemiol Infect* 1990; **105**: 95-105.
 25. Hartstein AI, Valvano MA, Morthland VH, Fuchs PC, Potter SA, Crosa JH. Antimicrobial susceptibility and plasmid profile analysis as identity tests for multiple blood isolates for coagulase-negative staphylococci. *J Clin Microbiol* 1987; **25**: 589-593.
 26. Hébert GA, Cooksey RC, Clark NC, Hill BC, Jarvis WR, Thornsberry C. Biotyping coagulase-negative staphylococci. *J Clin Microbiol* 1988; **26**: 1950-1956.
 27. Ludlam HA, Noble WC, Marples RR, Phillips I. The evaluation of a typing scheme for coagulase-negative staphylococci suitable for epidemiological studies. *J Med Microbiol* 1989; **30**: 161-165.
 28. Parisi JT, Hamory BH. Simplified method for the isolation, identification, and characterisation of *Staphylococcus epidermidis* in epidemiologic studies. *Diagn Microbiol Infect Dis* 1986; **4**: 29-35.
 29. Schumacher-Perdreau F, Jansen B, Peters G, Pulverer G. Typing of coagulase-negative staphylococci isolated from foreign body infections. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 270-273.
 30. Froggatt JW, Johnston JL, Galetto DW, Archer GL. Antimicrobial resistance in nosocomial isolates of *Staphylococcus haemolyticus*. *Antimicrob Agents Chemother* 1989; **33**: 460-466.
 31. Witte W, Bräulke C, Halle E, Spencker FB, Handrick W. Typing of multiple isolates of coagulase-negative staphylococci from blood cultures and cerebrospinal fluid in neonates and children: an approach to discriminate contaminants. *Zentralbl Bakteriol* 1990; **274**: 382-389.
 32. Bes M, Brun Y, Fleurette J. Nouveaux bactériophages de *Staphylococcus epidermidis*: évaluation de leur intérêt épidémiologique. *Ann Microbiol (Inst Pasteur)* 1984; **135B**: 165-176.
 33. de Saxe MJ, Crees-Morris JA, Marples RR, Richardson JF. Evaluation of current phage-typing systems for coagulase-negative staphylococci. *Zentralbl Bakteriol* 1981; Suppl **10**: 197-204.
 34. Marples RR, Richardson JF. Characters of coagulase-negative staphylococci collected for a collaborative phage-typing study. *Zentralbl Bakteriol* 1981; Suppl **10**: 175-180.
 35. Martin-de-Nicolas MM, Vindel A, Sáez-Nieto JA. Development of a new set of phages as an epidemiological marker in *Staphylococcus epidermidis* causing nosocomial infections. *Epidemiol Infect* 1990; **104**: 111-118.
 36. Richardson JF, Marples RR. Phage typability of coagulase-negative staphylococci in relation to antibiotic resistance. *Zentralbl Bakteriol* 1987; Suppl **16**: 235-246.
 37. Rosdahl VT, Gahrn-Hansen B, Møller JK, Kjaeldgaard P. Phage-typing of coagulase-negative staphylococci. *APMIS* 1990; **98**: 299-304.
 38. Verhoef J, Van Boven CPA, Winkler KC. Phage-typing of coagulase-negative staphylococci. *J Med Microbiol* 1972; **5**: 9-19.
 39. Pillet J, Orta B. Analyse sérologique des staphylocoques coagulase négatifs: étude des agglutinogènes caractéristiques des souches types des neuf espèces individualisées par Schleifer et Kloos. *Ann Microbiol (Inst Pasteur)* 1977; **128B**: 475-486.
 40. Archer GL. Molecular epidemiology of multiresistant *Staphylococcus epidermidis*. *J Antimicrob Chemother* 1988; **21** Suppl C: 133-138.
 41. Archer GL, Karchmer AW, Vishniavsky N, Johnston JL. Plasmid-pattern analysis for the differentiation of infecting from noninfecting *Staphylococcus epidermidis*. *J Infect Dis* 1984; **149**: 913-920.
 42. Brown E, Wenzel RP, Hendley JO. Exploration of the microbial anatomy of normal human skin by using plasmid profiles of coagulase-negative staphylococci: search for the reservoir of resident skin flora. *J Infect Dis* 1989; **160**: 644-650.
 43. Etienne J, Poitevin-Later F, Renaud F, Fleurette J. Plasmid profiles and genomic DNA restriction endonuclease patterns of 30 independent *Staphylococcus lugdunensis* strains. *FEMS Microbiol Lett* 1990; **67**: 93-98.
 44. Parisi JT, Hecht DW. Plasmid profiles in epidemiologic studies of infections by *Staphylococcus epidermidis*. *J Infect Dis* 1980; **141**: 637-643.
 45. Bialkowska-Hobrzanska H, Jaskot D, Hammerberg O. A method for DNA restriction endonuclease fingerprinting of coagulase-negative staphylococci. *J Microbiol Meth* 1990; **12**: 41-49.
 46. Burnie JP, Lee W. A comparison of DNA and immunoblot fingerprinting of the SII biotype of coagulase negative staphylococci. *Epidemiol Infect* 1989; **101**: 203-212.
 47. Bialkowska-Hobrzanska H, Harry V, Jaskot D, Hammerberg O. Typing of coagulase-negative staphylococci by Southern hybridization of chromosomal DNA fingerprints using a ribosomal RNA probe. *Eur J Clin Microbiol Infect Dis* 1990; **9**: 588-594.
 48. De Buyser M-L, Morvan A, Grimont F, El Solh N. Characterization of *Staphylococcus* species by ribosomal RNA gene restriction patterns. *J Gen Microbiol* 1989; **135**: 989-999.
 49. El Solh N, De Buyser ML, Morvan A *et al*. Use of *Bacillus subtilis* 16S rRNA as a probe to identify species, subspecies, and types in the genus staphylococcus. In: Novick RP, Skurray RA (eds) *Molecular biology of staphylococci*. 1990: 585-593.
 50. Pennington TH, Harker C, Thompson-Carter F. Identification of coagulase-negative staphylococci by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and rRNA restriction patterns. *J Clin Microbiol* 1991; **29**: 390-392.
 51. Thompson-Carter FM, Carter PE, Pennington TH. Differentiation of staphylococcal species and strains by ribosomal RNA gene restriction patterns. *J Gen Microbiol* 1989; **135**: 2093-2097.
 52. Burnie JP, Lee W, Matthews RC, Bayston R. Immunoblot fingerprinting of coagulase-negative staphylococci. *J Clin Pathol* 1988; **41**: 103-110.
 53. Clink J, Pennington TH. Staphylococcal whole-cell polypeptide analysis: evaluation as a taxonomic and typing tool. *J Med Microbiol* 1986; **22**: 41-44.
 54. Maggs AF, Pennington TH. Temporal study of staphylococcal species on the skin of human subjects in isolation and clonal analysis of *Staphylococcus capitis* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J Clin Microbiol* 1989; **27**: 2627-2632.
 55. Stephenson JR, Tabaqchali S. New method for typing coagulase-negative staphylococci. *J Clin Pathol* 1986; **39**: 1271-1275.
 56. Thompson-Carter FM, Pennington TH. Characterization of coagulase-negative staphylococci by sodium dodecyl sul-

- fate-polyacrylamide gel electrophoresis and immunoblot analyses. *J Clin Microbiol* 1989; **27**: 2199–2203.
57. Kotilainen P, Huovinen P, Eerola E. Application of gas-liquid chromatographic analysis of cellular fatty acids for species identification and typing of coagulase-negative staphylococci. *J Clin Microbiol* 1991; **29**: 315–322.
 58. Monzon-Moreno C, Aubert S, Morvan A, El Solh N. Usefulness of three probes in typing methicillin resistant *Staphylococcus aureus* (MRSA) strains. *J Med Microbiol* 1991; **34**: 80–88.
 59. Iglesias A, Ceglowski P, Trautner TA. Plasmid transformation in *Bacillus subtilis*. Effects of the insertion of *Bacillus subtilis* rRNA genes into plasmids. *Mol Gen Genet* 1983; **192**: 149–155.
 60. Ferretti JJ, Gilmore KS, Courvalin P. Nucleotide sequence analysis of the gene specifying the bifunctional 6'-aminoglycoside acetyltransferase 2'-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. *J Bacteriol* 1986; **167**: 631–638.
 61. El Solh N, Moreau N, Ehrlich SD. Molecular cloning and analysis of *Staphylococcus aureus* chromosomal aminoglycoside resistance genes. *Plasmid* 1986; **15**: 104–118.
 62. Brun Y, Bes M, Boeufgras JM *et al.* International collaborative evaluation of the ATB 32 Staph gallery for identification of the *Staphylococcus* species. *Zentralbl Bakteriol* 1990; **273**: 319–326.
 63. El Solh N, Allignet J, Bismuth R, Buret B, Fouace J-M. Conjugative transfer of staphylococcal antibiotic resistance markers in the absence of detectable plasmid DNA. *Antimicrob Agents Chemother* 1986; **30**: 161–169.
 64. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–685.
 65. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press. 1989.
 66. Kado CI, Liu S-T. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981; **145**: 1365–1373.
 67. Smith GE, Summers MD. The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. *Anal Biochem* 1980; **109**: 123–129.
 68. Dice LR. Measures of the amount of ecologic association between species. *Ecology* 1945; **26**: 297–302.
 69. Byrne ME, Rouch DA, Skurray RA. Nucleotide sequence analysis of IS256 from the *Staphylococcus aureus* gentamicin-tobramycin-kanamycin-resistance transposon Tn4001. *Gene* 1989; **81**: 361–367.
 70. Rouch DA, Byrne ME, Kong YC, Skurray RA. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn4001 from *Staphylococcus aureus*; expression and nucleotide sequence analysis. *J Gen Microbiol* 1987; **133**: 3039–3052.
 71. Thomas WD, Archer GL. Mobility of gentamicin resistance genes from staphylococci isolated in the United States: identification of Tn4031, a gentamicin resistance transposon from *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 1989; **33**: 1335–1341.