Restriction Endonuclease Analysis of *Staphylococcus epidermidis* DNA May Be a Useful Epidemiological Marker

FRANÇOIS RENAUD,^{1*} JEAN FRENEY,¹ JÉRÔME ETIENNE,² MICHÈLE BES,³ YVONNE BRUN,¹
ODILE BARSOTTI,⁴ SYLVIANE ANDRE,¹ AND JEAN FLEURETTE¹

Laboratoire de Bactériologie-Virologie, Centre National de référence des Staphylocoques, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin,¹ Laboratoire de Microbiologie-Immunologie, Faculté d'Odontologie,⁴ and Centre d'Etudes et de Recherches Bactériologiques, Institut Pasteur de Lyon,³ 69372 Lyon Cédex 08, and Laboratoire de Bactériologie, Hôpital Cardiologique, 69393 Lyon Cédex 3,² France

Received 14 December 1987/Accepted 18 May 1988

We compared the epidemiological markers of 13 *Staphylococcus epidermidis* strains isolated from an adult inpatient during a febrile episode and 23 *S. epidermidis* strains isolated during a presumptive outbreak of nosocomial infection in a neonatal ward. The total DNA restriction endonuclease analysis (REA) was processed along with the following conventional markers: biotyping, serotyping, phage typing, antibiotic susceptibility profiles, and plasmid profiles. The REA method was reproducible, giving stable results both in vitro and in vivo. For the hospitalized adult patient, the conventional markers of the 13 strains were concordant and the restriction profiles were identical. Five restriction groups were demonstrated during the course of the outbreak. Within two of the groups, the identities of all of the markers were used to verify whether all of the isolates belonged to the same cell clone. In a third group, combined analysis of the conventional markers and REA had to be used to demonstrate isolate similarity. On the other hand, in another group, none of the markers were similar; interpretation was not easy. An epidemiological study of *S. epidermidis* infections in hospitals must take into account all of the epidemiological markers: biotypes, serotypes, phage types, antibiograms, plasmid profiles, and REA.

Staphylococcus epidermidis, generally considered a commensal organism in humans, may be responsible for pathogenic processes, usually of iatrogenic origin, particularly in immunodeficient patients (15).

An outbreak of *S. epidermidis* is not easy to investigate because of the poor discriminating property of the conventional epidemiological markers biotype, serotype, antibiotic susceptibility pattern, plasmid profile, and phage type (31). Cleavage of chromosomal DNA by a restriction enzyme and visualization of bands obtained after electrophoretic separation (restriction endonuclease analysis [REA]) appear to be useful taxonomic and epidemiological tools.

The technique was applied successfully to the following organisms: *Actinomyces* sp. (13), *Bacteroides* sp. (7), *Candida* sp. (36), *Campylobacter jejuni* (33), *Campylobacter pylori* (25), *Citrobacter diversus* (28), *Clostridium difficile* (24), *Corynebacterium* sp. group JK (20), *Legionella* sp. (41), *Leptospira* sp. (26, 40), *Mycobacterium* sp. (10, 11, 32), *Mycoplasma* sp. (9, 35), *Neisseria meningitidis* (6, 21-23), *Streptomyces* sp. (17), *Streptococcus zooepidemicus* (38), and *Vibrio cholerae* (19).

No study of REA of *S. epidermidis* has been published. The aim of this work was to compare the existing epidemiological markers with the REA patterns of total DNA on strains isolated either from a patient with a lethal infection or during a so-called nosocomial outbreak.

MATERIALS AND METHODS

Patient. An inpatient of the Hôpital Cardiologique in Lyon, France, who had cardiac insufficiency demonstrated a positive serology to *Chlamydia trachomatis*; he was receiving pefloxacin and vibramycin. The patient underwent prosthetic aortic-mitral valve surgery. Seven days later, he had a

febrile episode. Thirteen isolates were collected over 9 days from the following samples: a Swan-Ganz catheter, the left and right eyes, a scar, a pleural drain (two isolates), surgical wounds (two isolates), pericardial fluid, blood culture (two isolates), and left and right mediastinal drains. The patient died of septic shock 19 days after surgery although he had been receiving vancomycin-pefloxacin therapy.

Nosocomial outbreak. A significant rise in the number of *S. epidermidis* isolates was observed over 1 month in the neonatal ward of the Hôpital Edouard-Herriot in Lyon, France. The epidemiological investigation that was carried out demonstrated no overload of activity during that period (the number of samples remained stable) and no increase in antibiotic therapy. However, 80% of the infants in the ward were premature (less-than-36-week pregnancy). Twenty-three *S. epidermidis* strains were isolated from eight neonates (A, F, H, J, M, S, V, and Z) sampled simultaneously in the same unit (see Table 1). Of the 23 isolates, 12 were recovered from the same infant (H), a twin. He weighed 700 g at birth, which necessitated total parenteral feeding. Strains 4, 5, 10, 14, 12, 13, and 7 were isolated over 10 days from patient H (see Table 1). On day 11, the baby died of septic shock. *S. epidermidis* (no. 6, 8, 9, 11, and 34) was isolated from the samples recovered during the shock period and postmortem. The other isolates originated from various specimens collected from the other seven infants (see Table 1). The bacterial analysis of the environment revealed the presence of *S. epidermidis* in the mother's milk to patient Z (no. 104).

Biotyping. The species diagnosis established according to the criteria of Schleifer (37) was performed by using a micromethod described previously (8). The biotype was represented by the numeric profile of the API Staph Gallery (API System, Montalieu-Vercieu, France).

Serotyping. Serotyping was performed by agglutination

* Corresponding author.

with sera 260 and 180 (Pasteur Institute, Paris, France) as described by Pillet and Orta (34).

Phage typing. The eight phages isolated in our laboratory were tested on all of the strains by a method described previously (5).

Antibiotic susceptibility pattern. The antibiograms were realized by using disks and Mueller-Hinton medium (Bio-Mérieux, Marcy L'Etoile, France). The antibiotics tested were penicillin G, oxacillin, gentamicin, tetracycline, chloramphenicol, erythromycin, trimethoprim-sulfamethoxazole, fosfomycin, streptomycin, lincomycin, pristinamycin, and rifampin. The results were expressed as susceptible, intermediate, or resistant by the criteria of the Comité français de l'Antibiogramme (1). Oxacillin susceptibility was determined at 30°C.

Plasmid profile analysis. The plasmid DNAs of isolates were obtained by a rapid method (16, 18), modified as described previously (14).

Restriction endonuclease analysis. (i) Total DNA extraction. The strains were cultivated overnight at 35°C in 10% glycerol broth. After centrifugation, total DNA extraction was performed by the simultaneous action of lysozyme (Sigma Chemical Co., La Verpillière, France) and TBL₁ achromopeptidase (AMI-Labo, Lyon, France) by a previously described method (4).

(ii) Cleavage with restriction endonuclease. DNA (1.5 µg) was dissolved in a final volume of 20 µl buffer, 7.5 U of *EcoRI* enzyme (Boehringer Mannheim, Meylan, France) was added, and the reaction mixture was allowed to stand for 1.5 h at 37°C. A further 7.5 U of the enzyme was added, and the mixture was kept for another 1.5 h. The sticky ends were removed by heat treatment for 10 min at 64°C. After addition of 3 µl of staining solution (bromophenol blue, Ficoll 400), the digests were loaded into the electrophoresis well.

(iii) Electrophoresis. Electrophoresis of the DNA digest was performed on a 0.8% agarose gel. The restriction fragments were run at 50 V for 18 h. A 0.089 M Tris-borate buffer, pH 8.0, was used. The gel was stained with a 0.5-g/liter solution of ethidium bromide and photographed under UV light with a Wratten red filter.

(iv) Intrasystem reproducibility of the REA. Six total DNA extractions were performed from the overnight broth culture of the same *S. epidermidis* (type strain, CCM 2124). The six profiles obtained after enzyme digestion and electrophoresis were compared.

(v) Intersystem reproducibility of the REA. Restriction pattern stability was tested by reculturing five nutrient broths inoculated with the same *S. epidermidis* type strain 25 times by using a method described previously (36). Initial and terminal profiles were compared.

RESULTS

Reproducibility of the REA method. After digestion with *EcoRI*, the six REA patterns of the same strain were identical, which indicates good reproducibility of the method. After 25 successive cultures, the DNA digest patterns were identical and similar to those of the strain kept frozen during the experiment, which confirmed stability during in vitro culture.

Adult patient. For 8 of the 13 isolates, the biotype number was 6606113 (mannose negative), and for the other 5 it was 6706113 (mannose positive) (Table 1). The two biotypes were considered to be identical, since the mannose characteristic is often slightly positive.

All of the isolates were agglutinated by serum 186; none of them were lysed by the phages in our collection. The antibiotic resistance profiles of the 13 isolates were identical; the plasmid profiles (Fig. 1) and restriction profiles (Fig. 2) were similar. These data demonstrate that all of the isolates derived from a single strain that had infected the prosthetic valve.

Nosocomial outbreak. Of the 23 isolates, 16 revealed a 6(6/7)06113 biochemical profile (Table 1). Five of the remaining isolates were alkaline phosphatase negative (Pal⁻). One of these failed to hydrolyze arginine (Adh⁻), and another did not reduce nitrates to nitrite (Nit⁻). Of the 23 isolates, 2 were Voges-Proskauer reaction negative (Vp⁻), and one of them was Nit⁻. Of the 23 isolates, 15 were agglutinated by serum 260, 2 others were agglutinated by serum 186, 5 revealed no agglutination (nontypable), and 1 agglutinated spontaneously (autoagglutinable). Of the 23 isolates, 5 were lysed by phage 392, 1 was weakly lysed by several phages, and the other 17 were not lysed by any of the phages.

All of the isolates were penicillin G resistant and pristinamycin susceptible. The 23 isolates were divided into seven plasmid profiles (A to G); 8 isolates had no plasmid. Five restriction profile types (I to V) were revealed.

Patient H. Six isolates (no. 4, 5, 10, 14, 12, and 13) were collected from patient H during the various septic episodes. They all had the same serotype, and they were non-phage typable. The biochemical profiles were diverse. The isolates were all oxacillin and gentamicin resistant but tetracycline susceptible. Four of them (no. 5, 10, 14, and 13) had no plasmid and were susceptible to chloramphenicol, erythromycin, and lincomycin, contrary to those that presented the E plasmid profile (no. 4 and 12). Two of the four plasmid-free isolates (no. 10 and 14) were streptomycin resistant. All of the strains possessed the same restriction profile. During the septic shock responsible for the infant's death, isolates 6, 8, 9, 11, and 34 were recovered from pre- and postmortem samples. All of the strains showed the same biotype, were not serotypable, and were lysed by phage 392. Their antibiograms were identical (resistant to penicillin G and tetracycline). They presented the same plasmid profile and restriction profile. The 2.7-megadalton plasmid may be responsible for the resistance to tetracycline (12).

REA groups. The three group I isolates had identical epidemiological markers (one of the isolates was spontaneously agglutinated); they were samples collected from two different patients (Table 1; Fig. 3). The three group II isolates all had different markers. The 10 group III isolates originated from five patients; these isolates had five biotypes and one serotype (260) and were not phage typable. Of the 10 isolates, 3 revealed the same plasmid profile and antibiogram, whereas the seven others were plasmid free and had comparable antibiograms, apart from four isolates that proved to be streptomycin resistant. The five group IV isolates were investigated at the same time as patient H. The two group V isolates had only one similar biotype and serotype in common.

DISCUSSION

It is often difficult to ascertain the evidence of an outbreak of infections caused by *S. epidermidis*. This bacterium is widely present on human skin and mucous membranes and in environmental reservoirs. In addition, the infections caused are usually torpid and the outbreaks rarely occur as "exploding" epidemics, differing from nosocomial infections caused by *S. aureus*. Consequently, and also because

TABLE 1. Comparison of *S. epidermidis* epidemiological markers^a

Reference no.	Patient	Sample origin	Biotype no.	Serotype	Phage type	Antibiotic resistance										Plasmid profile	REA group	
						PG	OX	GT	TE	CL	ER	SXT	FF	ST	LC			PR
	Adult		6(6/7)06113	186	NT	R	R	R	R	R	R	R	R	R	R	R	A	I
20	F	Catheter	6(6/7)06113	AA	NT	R	R	R	R	R	R	R	R	R	R	R	A	I
30	A	Catheter	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	A	I
31	A	Catheter	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	A	I
23	M	Tracheal aspirate	6(6/7)06113	186	NT	R	R	R	R	R	R	R	R	R	R	R	B	II
63	F	Eye	Pal ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	C	II
50	V	Catheter	Pal ⁻ Adh ⁻	186	NT	R	R	R	R	R	R	R	R	R	R	R	D	II
4 ^b	H	Tracheal aspirate	Nit ⁻ Pal ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
5 ^b	H	Tracheal aspirate	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
10 ^b	H	Tracheal aspirate	Vp ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
14 ^b	H	Tracheal aspirate	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
12 ^b	H	Thoracic drain	Nit ⁻ Vp ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
13 ^b	H	Blood culture	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
18	M	Catheter	Pal ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
19	J	Catheter	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
24	Z	Gastric fluid	Pal ⁻	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
60	S	Tracheal aspirate	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	E	III
6 ^c	H	Peripheral catheter	6(6/7)06113	NT	392	R	R	R	R	R	R	R	R	R	R	R	F	IV
8 ^c	H	Gastric fluid	6(6/7)06113	NT	392 (41) ^d	R	R	R	R	R	R	R	R	R	R	R	F	IV
9 ^c	H	Blood culture	6(6/7)06113	NT	392 (41) ^d	R	R	R	R	R	R	R	R	R	R	R	F	IV
11 ^c	H	Pleural fluid	6(6/7)06113	NT	392	R	R	R	R	R	R	R	R	R	R	R	F	IV
34 ^c	H	Pulmonary biopsy	6(6/7)06113	NT	392	R	R	R	R	R	R	R	R	R	R	R	F	IV
7	H	Thoracic drain	6(6/7)06113	260	— ^e	R	R	R	R	R	R	R	R	R	R	R	G	V
104	Z	Mother's milk	6(6/7)06113	260	NT	R	R	R	R	R	R	R	R	R	R	R	G	V

^a The most common biotype was 6(6/7)/06113. Only reactions which differed from the prototype profile are indicated. Abbreviations: NT, not typable; PG, penicillin G; OX, oxacillin; GT, gentamicin; TE, tetracycline; CL, chloramphenicol; ER, erythromycin; SXT, trimethoprim-sulfamethoxazole; FF, fosfomycin; ST, streptomycin; LC, lincomycin; PR, pristinamycin; RF, rifampin.

^b This was isolated during the various septic episodes of patient H.

^c This isolate was recovered from pre- and postmortem samples of patient H.

^d Parentheses, Weak reaction.

^e —, Weak reactions (41, 63, 118^d, 245, and 392).

FIG. 1. Schematic representation of plasmid profiles of the seven groups (A to G) from the outbreak and one group (V) from the adult patient. Lane 1 contains molecular size standards from *Escherichia coli*. Molecular size markers in megadaltons (MD) are shown on the left; base pair (BP) markers (Appligene, France) are shown on the right.

of the lack of reference methods, the evaluation of the discriminating capacity of new epidemiological markers is not easy.

The use of the API-Staph gallery for species identification is not recommended for epidemiological studies; too many strains belong to the same [6(6/7)06113] biotype, and the other types are not always in agreement with the other markers (group III REA). Although serotyping is a very stable marker, it is not sufficiently discriminating and other serotypes need to be described. Phage typing is a useful method, but unfortunately many strains remain nontypable. The antibiogram is established by using routine techniques; it has often proved most useful in epidemiological studies. Unfortunately, it is closely related to and as unstable as the plasmid profile (2, 3, 27, 30).

Among the criteria used to select an epidemiological marker, two are important: stability and discriminating ca-

capacity. Our in vitro experiments demonstrated satisfactory intra- and extrasystem reproducibility of the REA method. The origin and identity of the markers of the isolates from the adult patient demonstrate that the isolates belong to the same cell clones. This hypothesis was confirmed by the similarity of their REA profiles, which revealed moreover that the REA patterns remained stable for several days in various biotopes of the human body (in vivo reproducibility). This has been observed previously with *Corynebacterium* sp. group JK (20) and *Campylobacter pylori* (25). Equivalent results were obtained when isolates 6, 8, 9, 11, and 34 from infant H were examined. All of the markers confirmed that

FIG. 2. *S. epidermidis* total DNA REA of adult patient. Lanes: 1, phage λ DNA digested with *Hind*III used as size markers (bp, base pairs); 2 to 14, 13 DNA strains isolated during a febrile episode.

FIG. 3. *S. epidermidis* total DNA REA of nosocomial outbreak. Lanes: 1, phage λ DNA digested with *Hind*III used as size markers (bp, base pairs); the other DNAs were digested with *Eco*RI; 2 to 4, DNAs 20, 30, and 31 (REA group I); 5 and 6, DNAs 23 and 63 (REA group II); 7 and 8, DNAs 4 and 12 (REA group III); 9 and 10, DNAs 6 and 11 (REA group IV); 11 and 12, DNAs 7 and 104 (REA group V). The other DNA patterns are not represented.

the fatal septic shock, with associated bacteremia and pleuropulmonary infection, was caused by a single strain that was also isolated from the gastric fluid and peripheral catheter. The origin of the strain was not investigated.

Strains 4, 5, 10, 14, 12, and 13 isolated from patient H may derive from a single strain, since the antibiotic resistance characteristics and plasmid profiles were closely related. The 2.5-megadalton plasmid demonstrated in the E plasmid profile may explain the chloramphenicol resistance; the 1.7-megadalton plasmid is probably responsible for the resistance to erythromycin and lincosamine (39). All of these isolates revealed the same REA profile, which thus became a useful epidemiological marker. This led us to believe that the same strain was responsible for the infant-to-infant cross-infection.

Restriction profiles IV and V were very similar, with minor differences, such as band intensity (Fig. 3, arrow). This is important, since the phenomenon is reproducible (isolates 9 and 10 and 11 and 12 in Fig. 3). These differences are apparently not due to the presence of plasmids, since these isolates contain no plasmid of a size equal to that of the bands involved in the REA modification (~2,260 base pairs). As regards strains 23, 63, and 50, the REA pattern did not seem to be discriminating, since this was the only characteristic they have in common.

In conclusion, the REA method may be an additional and useful marker for epidemiological studies of infections caused by *S. epidermidis*. It confirms or specifies the identities of isolates when they demonstrate variations in some of their bacteriological characteristics. This method appears to be reproducible and discriminating, but our preliminary results must be validated by a large-scale study. In particular, we must examine the problem of similar profiles and, in that case, perhaps use a different restriction enzyme so as to reveal minor differences undetected by the first endonuclease. A combined study of all of the epidemiological markers is nonetheless essential to establish the identity of the *S. epidermidis* strains implicated in an epidemiological process (29).

ACKNOWLEDGMENTS

We are grateful to G. Chambon and V. Delorme for competent technical assistance and to M. Courcier for translation of the paper.

LITERATURE CITED

1. Acar, J., E. Bergogne-Bérézin, Y. Chabbert, R. Cluzel, A. Courtieu, P. Courvalin, H. Dabernat, H. Drugeon, J. Duval, J. Fleurette, C. Morel, A. Philippon, J. Sirot, C. J. Soussy, A. Thabaut, and M. Véron. 1986. Communiqué du Comité de l'Antibiogramme de la Société française de Microbiologie. *Pathol. Biol.* 34:313-314.
2. Archer, G. L., A. W. Karchmer, N. Vishniavsky, and J. L. Johnston. 1984. Plasmid-pattern analysis for the differentiation of infecting from noninfecting *Staphylococcus epidermidis*. *J. Infect. Dis.* 149:913-920.
3. Archer, G. L., N. Vishniavsky, and H. Grant Stiver. 1982. Plasmid pattern analysis of *Staphylococcus epidermidis* isolates from patients with prosthetic valve endocarditis. *Infect. Immun.* 35:627-632.
4. Barsotti, O., F. Renaud, J. Freney, G. Benay, D. Decoret, and J. Dumont. 1987. Rapid isolation of DNA from *Actinomyces*. *Ann. Microbiol. (Inst. Pasteur)* 138:529-536.
5. Bes, M., Y. Brun, and J. Fleurette. 1984. Nouveaux bactériophages de *Staphylococcus epidermidis*: évaluation de leur intérêt épidémiologique. *Ann. Microbiol. (Inst. Pasteur)* 135B:165-176.
6. Bjorvatn, B., V. Lund, B.-E. Kristiansen, L. Korsnes, O. Spanne, and B. Lindqvist. 1984. Applications of restriction endonuclease fingerprinting of chromosomal DNA of *Neisseria meningitidis*. *J. Clin. Microbiol.* 19:763-765.
7. Bradbury, W. C., R. G. E. Murray, C. Mancini, and V. L. Morris. 1985. Bacterial chromosomal restriction endonuclease analysis of the homology of *Bacteroides* species. *J. Clin. Microbiol.* 21:24-28.
8. Brun, Y., J. Fleurette, and F. Forey. 1978. Micromethod for biochemical identification of coagulase-negative staphylococci. *J. Clin. Microbiol.* 8:503-508.
9. Chan, H. W., and R. F. Ross. 1984. Restriction endonuclease analyses of two porcine mycoplasma deoxyribonucleic acids: sequence-specific methylation in the *Mycoplasma hyopneumoniae* genome. *Int. J. Syst. Bacteriol.* 34:16-20.
10. Collins, D. M., and G. W. De Lisle. 1985. DNA restriction endonuclease analysis of *Mycobacterium bovis* and other members of the tuberculosis complex. *J. Clin. Microbiol.* 21:562-564.
11. Collins, D. M., G. W. De Lisle, and D. M. Gabric. 1986. Geographic distribution of restriction types of *Mycobacterium bovis* isolates from brush-tailed possums (*Trichosurus vulpecula*) in New Zealand. *J. Hyg.* 96:431-438.
12. Cooksey, R. C., and J. N. Baldwin. 1985. Relatedness of tetracycline resistance plasmids among species of coagulase-negative staphylococci. *Antimicrob. Agents Chemother.* 27:234-238.
13. Crameri, R., G. Hintermann, and R. Hütter. 1983. Deoxyribonucleic acid restriction endonuclease fingerprint characterization of actinomycete strains. *Int. J. Syst. Bacteriol.* 33:652-655.
14. Etienne, J., Y. Brun, N. El Solh, V. Delorme, C. Mouren, M. Bes, and J. Fleurette. 1988. Characterization of clinically significant isolates of *Staphylococcus epidermidis* from patients with endocarditis. *J. Clin. Microbiol.* 26:613-617.
15. Gemmel, C. G. 1986. Coagulase-negative staphylococci. *J. Med. Microbiol.* 22:285-295.
16. Goering, R. V., and E. A. Ruff. 1983. Comparative analysis of conjugative plasmids mediating gentamicin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 24:450-452.
17. Hintermann, G., R. Crameri, T. Kieser, and R. Hütter. 1981. Restriction analysis of the *Streptomyces glaucescens* genome by agarose gel electrophoresis. *Arch. Microbiol.* 130:218-222.
18. Holmes, D. S., and M. Quigley. 1981. A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* 114:193-197.
19. Kaper, J. B., H. B. Bradford, N. C. Roberts, and S. Falkow. 1982. Molecular epidemiology of *Vibrio cholerae* in the U.S. Gulf Coast. *J. Clin. Microbiol.* 16:129-134.
20. Khabbaz, R. F., J. B. Kaper, M. R. Moody, S. C. Schimpff, and J. H. Tenney. 1986. Molecular epidemiology of group JK *Corynebacterium* in a cancer ward: lack of evidence for patient-to-patient transmission. *J. Infect. Dis.* 154:95-99.
21. Kristiansen, B. E., B. Sørensen, B. Bjorvatn, E. S. Falk, E. Fosse, K. Bryn, L. O. Frøholm, P. Gaustad, and K. Bøvre. 1985. An outbreak of group B meningococcal disease: tracing the causative strain of *Neisseria meningitidis* by DNA fingerprinting. *J. Clin. Microbiol.* 23:764-767.
22. Kristiansen, B. E., B. Sørensen, T. Simonsen, O. Spanne, V. Lund, and B. Bjorvatn. 1984. Isolates of *Neisseria meningitidis* from different sites in the same patient: phenotypic and genomic studies, with special reference to adherence, piliation, and DNA restriction endonuclease pattern. *J. Infect. Dis.* 150:389-396.
23. Kristiansen, B. E., B. Sørensen, O. Spanne, and B. Bjorvatn. 1985. Restriction fingerprinting and serology in a small outbreak of B15 meningococcal disease among Norwegian soldiers. *Scand. J. Infect. Dis.* 17:19-24.
24. Kuijper, E. J., J. H. Oudbier, W. N. H. M. Stuifbergen, A. Janz, and H. C. Zanen. 1987. Application of whole-cell DNA restriction endonuclease profiles to the epidemiology of *Clostridium difficile*-induced diarrhea. *J. Clin. Microbiol.* 25:751-753.
25. Langenberg, W., E. A. J. Rauws, A. Widjojokusumo, G. N. J. Tytgat, and H. C. Zanen. 1986. Identification of *Campylobacter pyloridis* isolates by restriction endonuclease DNA analysis. *J. Clin. Microbiol.* 24:414-417.
26. Marshall, R. B., B. E. Wilton, and A. J. Robinson. 1981.

- Identification of leptospira serovars by restriction-endonuclease analysis. *J. Med. Microbiol.* **14**:163-166.
27. Mickelsen, P. A., J. J. Plorde, K. P. Gordon, C. Hargiss, J. McClure, F. D. Schoenknecht, F. Condie, F. C. Tenover, and L. S. Tompkins. 1985. Instability of antibiotic resistance in a strain of *Staphylococcus epidermidis* isolated from an outbreak of prosthetic valve endocarditis. *J. Infect. Dis.* **152**:50-58.
 28. Morris, J. G., Jr., F. Y. C. Lin, C. B. Morrison, R. J. Gross, R. Khabbaz, K. O. Maher, B. Rowe, E. Israel, and J. P. Libonati. 1986. Molecular epidemiology of neonatal meningitis due to *Citrobacter diversus*: a study of isolates from hospitals in Maryland. *J. Infect. Dis.* **154**:409-414.
 29. Parisi, J. T. 1986. Epidemiologic markers in *Staphylococcus epidermidis* infections, p. 139-144. In L. Leive (ed.), *Microbiology—1986*. American Society for Microbiology, Washington, D.C.
 30. Parisi, J. T., and D. W. Hecht. 1980. Plasmid profiles in epidemiologic studies of infections by *Staphylococcus epidermidis*. *J. Infect. Dis.* **141**:637-643.
 31. Parisi, J. T., B. C. Lampson, D. L. Hoover, and J. A. Khan. Comparison of epidemiological markers for *Staphylococcus epidermidis*. *J. Clin. Microbiol.* **24**:56-60.
 32. Patel, R., J. T. Kvach, and P. Mounts. 1986. Isolation and restriction endonuclease analysis of mycobacterial DNA. *J. Gen. Microbiol.* **132**:541-551.
 33. Penner, J. L., J. N. Hennessy, S. D. Mills, and W. C. Bradbury. 1983. Application of serotyping and chromosomal restriction endonuclease digest analysis in investigating a laboratory-acquired case of *Campylobacter jejuni* enteritis. *J. Clin. Microbiol.* **18**:1427-1428.
 34. Pillet, J., and B. Orta. 1970. Recherche sur l'analyse sérologique des staphylocoques coagulase négatifs: étude de deux sérotypes. *Ann. Microbiol. (Inst. Pasteur)* **119**:193-205.
 35. Razin, S., R. Harasawa, and M. F. Barile. 1983. Cleavage patterns of the mycoplasma chromosome, obtained by using restriction endonucleases, as indicators of genetic unrelatedness among strains. *Int. J. Syst. Bacteriol.* **33**:201-206.
 36. Scherer, S., and D. A. Stevens. 1987. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. *J. Clin. Microbiol.* **25**:675-679.
 37. Schleifer, K. H. 1986. Taxonomy of coagulase-negative staphylococci, p. 11-26. In P. A. March and K. H. Schleifer (ed.), *Coagulase-negative staphylococci*. Almquist and Wiksell International, Stockholm.
 38. Skjold, S. A., P. G. Quie, L. A. Fries, M. Barnham, and P. P. Cleary. 1987. DNA fingerprinting of *Streptococcus zooepidemicus* (Lancefield group C) as an aid to epidemiological study. *J. Infect. Dis.* **155**:1145-1150.
 39. Thakker-Varia, S., W. D. Jansen, L. Moon-McDermott, M. P. Weinstein, and D. T. Dubin. 1987. Molecular epidemiology of macrolides-lincosamides-streptogramin B resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *Antimicrob. Agents Chemother.* **31**:735-743.
 40. Thiermann, A. B., A. L. Handsaker, S. L. Moseley, and B. Kingscote. 1985. New method for classification of leptospiral isolates belonging to serogroup Pomona by restriction endonuclease analysis: serovar *kennewicki*. *J. Clin. Microbiol.* **21**:585-587.
 41. Van Ketel, R. J., J. ter Schegget, and H. C. Zanen. 1984. Molecular epidemiology of *Legionella pneumophila* serogroup 1. *J. Clin. Microbiol.* **20**:362-364.