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Ribotyping of Coagulase-Negative Staphylococci with Special Emphasis on Intraspecific Typing of *Staphylococcus epidermidis*

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Coagulase-negative staphylococci (CoNS), particularly *Staphylococcus epidermidis*, are increasingly being recognized as opportunistic pathogens. They are often multiply antibiotic resistant and can cause nosocomial outbreaks. For clinical and epidemiological reasons, accurate species identification and typing are imperative. Ribotyping, i.e., the generation of characteristic fragment patterns by hybridization of restriction endonuclease fragments of total DNA with labeled standard rRNA from *Escherichia coli*, has been applied to CoNS for species identification by various investigators. The present study, involving 115 randomly collected clinical isolates of CoNS, provides ambiguous evidence with respect to those findings. Eighty six *S. epidermidis* strains were ribotyped intraspecifically. Eleven different ribotypes were found after digestion with *Eco*RI, and 10 were found with *Hin*dIII. A combination of the two restriction endonucleases resulted in an increase in the discriminatory power (DP) from 14.3 to 31.6%. A combination of ribotyping with biotyping raised the DP to a maximum of 48.6%. The reproducibility of ribotyping was 100% after >400 generations of growth. No correlation between methicillin resistance and certain ribotypes among the *S. epidermidis* strains was observed. Ribotyping is considered a useful tool for the intraspecific typing of CoNS for epidemiological purposes. The DP can be increased by the use of additional restriction endonucleases.

Coagulase-negative staphylococci (CoNS) are abundant in the normal flora of human mucocutaneous sites. Therefore, these organisms are among the most frequently isolated bacteria in clinical specimens. Because their pathogenic potential is low, they are mostly considered contaminants. Over the past decade, however, some species of CoNS have become recognized as important opportunistic pathogens, also causing nosocomial outbreaks. Although host defects, such as impaired immune defense (31), are important in the pathogenesis of CoNS infections, perhaps the most important factor contributing to the increasing number of CoNS infections is the frequent application of indwelling devices. Staphylococci are able to adhere to plastic surfaces of foreign bodies and to form biofilms (4), which provide a protective microenvironment for the organisms. This factor may also be the reason why CoNS have become the major cause of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (24).

Efforts to establish the sources of nosocomial CoNS infections and the modes of transmission of the organisms have been impaired by the lack of an efficient typing system. Until recently, epidemiological analysis was based on the determination of classical phenotypic markers, such as serotype, biotype, bacteriophage type, and antibiotic susceptibility pattern (32), some of which became the basis of commercially available kits for species identification (39). Some modern methods, such as the production of protein patterns (33), gas-liquid chromatography (23), and pyrolysis mass spectrometry (11), turned out to be too expensive or labor-intensive to become widely applicable. Recent developments in DNA analysis techniques, such as plasmid analysis (27), comparison of genomic DNA fragment patterns (5, 36), and the use of nucleic acid probes, have reduced the dependence on phenotypic traits in the study of CoNS epidemics. However, plasmid analysis has the disadvantages that it is limited to strains containing plasmids and that plasmids are unstable genetic elements (6). The disadvantage of standard chromosomal restriction fragment analysis (REA) is that it produces a large number of bands for analysis, rendering reproducible identification of specific patterns difficult (5, 36). Three approaches have been suggested to reduce the number of interpretable DNA fragments: (i) large-fragment REA in combination with pulsed-field gel electrophoresis (13), (ii) small-fragment REA in combination with polyacrylamide gel electrophoresis (18), and (iii) hybridization of standard REA fragments with specific gene probes that represent only a small percentage of a bacterial genome. One such probe was introduced by Grimont and Grimont (15). It consists of *Escherichia coli* rDNA (DNA coding for rRNA), and provides a broad spectrum of hybridization with virtually all bacterial genomes. Because of the strong evolutionary conservation of rRNA genes, such a probe offers a broad spectrum of applications. This method is now called ribotyping, and it has shown considerable discriminatory power (DP) in identification to the species level as well as in typing of various bacteria (1, 15, 16, 19, 26, 29, 40, 42). It has also been applied, mainly for taxonomic purposes, to CoNS (5, 10, 41).

The present study evaluates the use of an *E. coli* rRNA gene probe in the typing of a random selection of CoNS strains belonging to various species. Special emphasis is placed on the intraspecific typing of *Staphylococcus epidermidis*, the pathogen most frequently isolated in disease caused by CoNS.

MATERIALS AND METHODS

Bacterial strains. One hundred fifteen clinical isolates of CoNS were randomly collected from patients at the University Hospital of Zürich and nearby hospitals between March and July 1988. *E. coli* MM290 was kindly provided by M.

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Altwegg of the Institute of Medical Microbiology, Zürich, Switzerland. This strain harbors plasmid pKK3535, which contains rDNA from *E. coli* on a 7.5-kb *Bam*HI fragment (7).

Identification. Species identification was performed with the Staph-Ident system (API, Bio-Merieux, Montalieu-Vercieu, France) (hereafter referred to as API) (22).

Antimicrobial susceptibility testing. Agar diffusion tests were performed in accordance with the guidelines of the National Committee for Clinical Laboratory Standards of the United States (30).

Isolation of whole-cell DNA. Strains were grown overnight in 120 ml of Luria-Bertani broth (38) at 37°C. Cells were pelleted by centrifugation at 7,000 × *g* for 10 min and resuspended in 4 ml of lysis mixture (0.1 M Tris-HCl [pH 7.5], 0.15 M NaCl, 0.1 M EDTA, 0.05 mg of lysostaphin [Sigma, St. Louis, Mo.] per ml, 0.025 mg of lysozyme [Sigma] per ml). The suspension was frozen at −70°C for 30 min, thawed at 50°C for 5 min, and incubated at 37°C for 3 h with gentle shaking. Lysis was completed by the addition of 0.5 ml of 5% sodium dodecyl sulfate (SDS) in 45% ethanol and incubation of the mixture at room temperature for 30 min. The lysate was extracted with 1 ml of Tris-saturated phenol-chloroform (38), 2 volumes of cold ethanol were added to the supernatant, and the DNA was spooled out on a glass rod. The DNA was dissolved in 2 ml of TE buffer (pH 8) (38).

Isolation of probe DNA. Plasmid pKK3535 is a pBR322 derivative containing the *rrnB* rDNA operon of *E. coli* encoding 5S, 16S, and 23S rRNAs as well as $tRNA^{Glu}$. The plasmid was prepared essentially as described previously (20) and digested with *Bam*HI. The fragments were separated by preparative electrophoresis in a 0.6% agarose gel, and the 7.5-kb fragment was recovered from the agarose by standard protocols (38).

Restriction and electrophoresis. Restriction endonucleases were purchased from Boehringer (Mannheim, Germany) and used in accordance with the manufacturer's instructions. Agarose gel electrophoresis was performed with 0.5 to 0.8% agarose gels at 1 to 4 V/cm. DNA was visualized under UV light after the gels were stained with ethidium bromide. Phage λ DNA (Boehringer) digested with *Bst*EII was used as a molecular size standard.

Radioactive labeling of rRNA and rDNA. rRNA from *E. coli* (Boehringer) was end labeled with [γ-^{32}P]ATP (Amersham International, Amersham, United Kingdom) by use of a 5′ DNA-RNA terminus labeling kit with T4 polynucleotide kinase (Boehringer) in accordance with the instructions of the manufacturer.

The 7.5-kb *Bam*HI fragment from pKK3535 was labeled with [α-^{32}P]dCTP by use of the Random-Primed DNA Labeling kit (Boehringer) in accordance with the instructions of the manufacturer.

Southern transfer and hybridization. Alkaline Southern blotting of DNA from agarose gels to GeneScreen Plus nylon membranes (New England Nuclear, Boston, Mass.) was done as previously described (35). Prehybridization and hybridization were performed at 66°C for 4 and 20 h, respectively. The membranes were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (38) at room temperature for 5 min, twice in 2× SSC with 1% SDS at 66°C for 30 min, and twice in 0.1× SSC at 22°C for 30 min. The wet membranes were sealed in plastic bags, and the labeled bands were visualized on X-ray films (Fuji RX) with intensifying screens at −80°C for 1 day to 2 weeks, depending on the kind of probe used.

Analysis and interpretation of data. Original X rays from different gels were standardized in size and then drawn in scale on a McIntosh SE/30 with the graphics software Super Paint 2.0. Three classes of signal strengths were created by visual estimation. Each different banding pattern thus derived was considered a ribotype and given a designation. The DP (percentage) was calculated with the formula (number of types/total number of strains) × 100 (25).

RESULTS

Biochemical identification. Of the 115 strains examined, 86 (75%) were *S. epidermidis*, 4 were *S. capitis*, 6 were *S. haemolyticus*, 8 were *S. hominis*, 3 were *S. warneri*, 1 was *S. intermedius*, 1 was *S. saprophyticus*, 1 was *S. cohnii*, 1 was *S. simulans*, 1 was *S. sciuri*, and 3 were unclassified by the API.

REA versus ribotyping. The following restriction endonucleases were used for the digestion of staphylococcal whole-cell DNA to achieve clean, well-resolved fragment banding patterns over a wide size range: *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Pst*I, *Pvu*II, *Sal*I, *Xba*I, and *Xho*II. *Eco*RI and *Hin*dIII yielded the most satisfying results (Fig. 1).

The method used for the preparation of staphylococcal DNA preserved plasmid DNA apart from the chromosome. Plasmid bands were readily seen after electrophoresis of undigested (data not shown) and also digested DNA preparations because of the increased brightness of the bands (Fig. 1, top panel, e.g., lane Eep-4). Seventy nine percent of all strains, 83% of the *S. epidermidis* strains, and 69% of the non-*S. epidermidis* strains harbored one or more plasmids.

REA of chromosomal DNA revealed variability between strains. However, elucidation of strain-specific differences was difficult, time-consuming, and often restricted to fragments of >5 kb. It was essential to run small numbers of samples side by side on one gel. Slight differences in DNA concentrations per lane as well as bright plasmid bands interfered with precise interpretation (Fig. 1). Furthermore, the number of bands to deal with was high in REA (several hundreds) but low in ribotyping (approximately 10 to 25) (Fig. 1). This reduction in the number of bands rendered the patterns more easily interpretable, as was obvious with a particular pair of strains. Their REA patterns were indistinguishable, but they belonged to the two strikingly different ribotypes E-ep2 and E-ep4 (see Fig. 2A).

Ribotyping. One hundred two isolates were ribotyped after digestion with *Eco*RI, and 79 were ribotyped after digestion with *Hin*dIII. The profiles obtained were recorded as drawings in Fig. 2. The number of strains contained in each ribotype varied between 1 and 35 (Fig. 2).

Identification of ribotypes in a number of cases depended on the high resolution of closely migrating hybridizing bands. Ambiguities could always be eliminated by changing the agarose concentration of the gel. Two concentrations (0.5 and 0.8%) yielded excellent resolution over a fragment size range of 500 to 20,000 bp (data not shown).

Except for some slight differences in the intensity of the signals, the number and position of hybridizing bands were always identical whether radioactive rRNA or rDNA (7.5-kb *Bam*HI fragment of pKK3535) was used as a probe (data not shown). Consequently, the DNA probe was preferred because it reduced the exposure time for the X-ray film from 1 to 3 weeks to 0.5 to 3 days.

The stability of the ribotypes was tested by examination of two randomly chosen strains after 5, 10, 15, and 20 overnight subcultures in broth. No difference in the patterns was observed (data not shown).

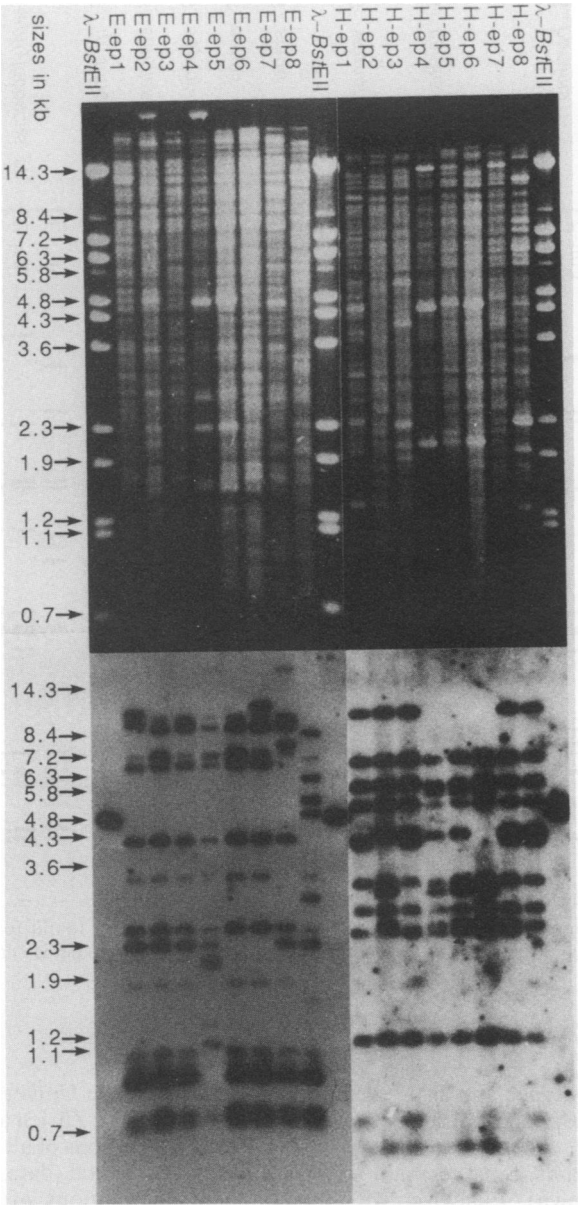

FIG. 1. Ethidium bromide-stained gel showing selected restriction endonuclease fragment patterns of *S. epidermidis* strains (top) and an X ray showing the same patterns after hybridization with the rDNA probe from pKK3535 (bottom).

Species identification by ribotyping. To be useful as a system for species identification of CoNS, ribotyping would have to yield both closely related banding patterns within a single species as well as sufficient variation from species to species. Our results were ambiguous. On the one hand, a close similarity of ribotypes with >10 comigrating bands could be found within a species, e.g., E-ep1, E-ep2, E-ep3, E-ep5, and E-ep6 or E-ho1, E-ho2, and E-ho3, and less or no similarity of ribotypes was generally observed with different species, e.g., E-in1, E-si1, or E-sa1 (Fig. 2A). On the other hand, obvious intraspecific dissimilarities (e.g., E-ep4 and E-ep8 or E-wa1, E-wa2, and E-wa3) as well as striking interspecific similarities (e.g., E-ca1 and E-ep8) were also detected (Fig. 2A).

Intraspecific ribotyping. The descriptions in this section are based exclusively on our *S. epidermidis* strains. The total of 86 strains was subdivided into 17 different API biotypes, giving rise to a DP of 19.8%. Because DP is the percentage of different types among the total number of strains tested (25), it is a measure of the ability of a method to detect small differences between distinct strains of a species, i.e., of its power to classify strains into defined types.

Ribotyping of 73 strains by use of *Eco*RI yielded 11 types (DP, 15.1%) (Fig. 2A). Ribotyping of 70 strains by use of *Hin*dIII yielded 10 types (DP, 14.3%) (Fig. 2B).

Our hypothesis was that two strains belonging to a single *Eco*RI ribotype would be sufficiently related, with respect to their rRNA genes, that they would tend to be classified within the same *Hin*dIII ribotype as well, meaning that each *Eco*RI type should correspond to a particular *Hin*dIII type and indicating that the distribution of strains among different *Eco*RI types would characteristically reflect their distribution among different *Hin*dIII types and vice versa.

To test this hypothesis, we set up a crossed table (Table 1). Both sets of types were listed in descending order of the number of strains in the types (see totals in Table 1). This arrangement allowed qualitative judgment of the relatedness of the two sets, in that strong relatedness should lead to alignment of data along the diagonal (boldfacing in Table 1). However, this expectation seemed to be supported only by a few strains which belonged to E-ep4 and H-ep4 or to E-ep1 and H-ep7 and were barely or not represented by other types (Table 1). In contrast, the majority of combined *Eco*RI-*Hin*dIII types were scattered about a large area of the diagram. As an example, the eight strains in E-ep3 were split into four different *Hin*dIII types (Table 1).

This finding implied that ribotyping with two or more restriction endonucleases rather than only one should enhance the sensitivity of the method in detecting small differences between strains. Indeed, the DP increased from 12.2% (*Eco*RI typing alone) or 15.8% (*Hin*dIII typing alone) to 31.6% (combined *Eco*RI-*Hin*dIII typing). Calculation of DPs was based on the data in Table 1. Among the total of 57 strains, 7 *Eco*RI types, 9 *Hin*dIII types, and 18 *Eco*RI-*Hin*dIII types were counted. (Small discrepancies in the DPs, e.g., 14.3% versus 15.8% for *Hin*dIII typing, are random variations because the calculations were based on slightly different numbers of strains involved).

As in Table 1, strains contained within individual ribotypes were compared with strains contained within individual API biotypes. Again, no correlation was found (data not shown), meaning that knowing the ribotype of a particular strain by no means allowed the API code to be predicted and vice versa. As a consequence, the DPs increased when data from biotyping and ribotyping were analyzed in combination. Among 70 strains, 10 *Hin*dIII types (DP, 14.3%), 15 API types (DP, 21.4%), and 34 *Hin*dIII-API types (DP, 48.3%) were found. Among 73 strains, 11 *Eco*RI types (DP, 15.1%), 15 API types (DP, 20.5%), and 31 *Eco*RI-API types (DP, 42.5%) were found.

Ribotyping and Mec^r. Methicillin resistance (Mec^r) and multiple antibiotic resistance in *S. epidermidis* are clinically significant. Of our 86 *S. epidermidis* strains, 36 were Mec^r (41.9%). Of the 29 non-*S. epidermidis* CoNS, only 1 (*S. haemolyticus*) was Mec^r. Mec^r in *S. epidermidis* was strongly associated with multiple antibiotic resistance (data not shown).

We determined the percentage of Mec^r *S. epidermidis* strains among all different *Eco*RI and *Hin*dIII ribotypes and found that it varied in a wide range. In addition, Mec^r strains

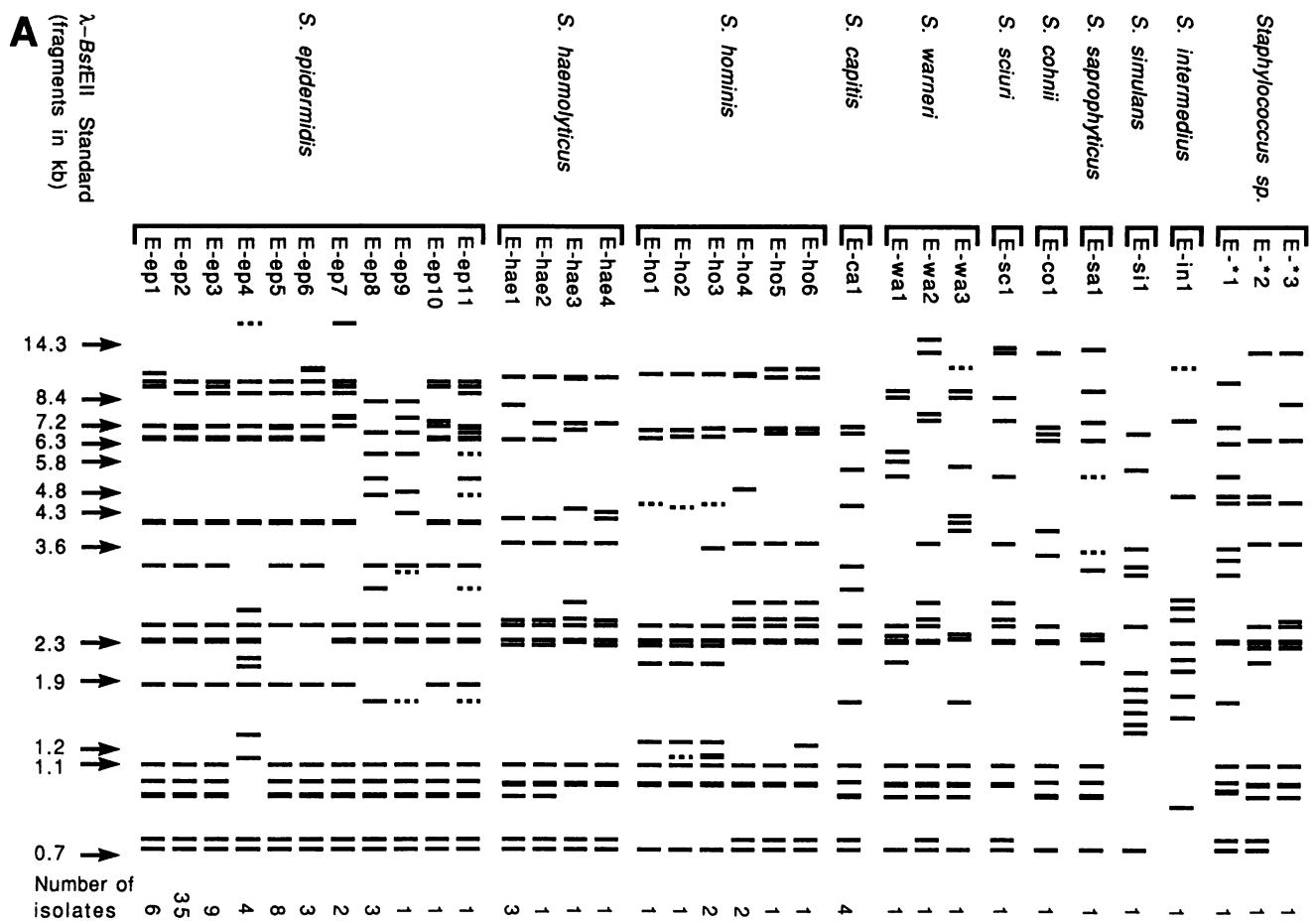

FIG. 2. Ribotypes drawn from original X rays. (A) *Eco*RI ribotypes found for 102 isolates tested. (B) *Hin*dIII ribotypes found for 79 isolates tested. The number of isolates found per pattern is given. An asterisk indicates *Staphylococcus* isolates for which the species could not be determined with the API.

were distributed in 5 of 11 *Eco*RI and in 8 of 10 *Hin*dIII ribotypes. No correlation between certain ribotypes and Mec^r was found.

Epidemiological aspects. Three aspects were analyzed: (i) comparison of multiple isolates from single patients, (ii) distribution of ribotypes among different hospitals and hospital wards, and (iii) distribution of ribotypes among different samples.

The majority of the strains came from different patients. We received four, three, and two samples from only one, two, and seven patients, respectively. In five of these patients, the multiple isolates belonged to identical ribotypes, whereas in the other five patients, the multiple isolates belonged to different ribotypes. From the former five patients, samples had been taken within an average of 1.8 days (range, 0 to 5 days); from the latter five patients, samples had been taken within an average of 7 days (range, 0 to 17 days). Although the numbers were small and no information about the exact site of sampling in the patients was available, these numbers seemed to suggest that particular CoNS strains were present in individual patients only for a few days and tended to be replaced by other strains after more than about 5 days. However, more data on this issue are needed.

The distributions of the nine most abundant *S. epidermidis* ribotypes among 15 well-separated wards of the University Hospital of Zürich and five other hospitals in the Zürich area were all found to be very similar to the distributions of all 115 strains. Small deviations were purely coincidental (data not shown). Likewise, we found random distributions of the same abundant *S. epidermidis* ribotypes among the different samples from patients. These samples included wound swabs (24.1%), catheters (22.3%), urine (19.6%), blood cultures (16.1%), pus (11.6%), and various others (6.3%), such as cerebrospinal fluid, tracheal-bronchial secretions, urethral swabs, dialysates, and amniotic fluid.

On the basis of these data, we concluded that (i) no outbreak of any particular ribotype had occurred anywhere during the time of collection of the isolates (4.5 months) and that (ii) no ribotype was observed unexpectedly frequently in certain samples. However, these conclusions remain hypothetical because samples were taken only from real or suspected sites of infection in patients and no samples were taken from their environment, equipment, or health care personnel as controls.

DISCUSSION

The present study was designed to evaluate ribotyping as a tool for the differentiation of CoNS, with special emphasis

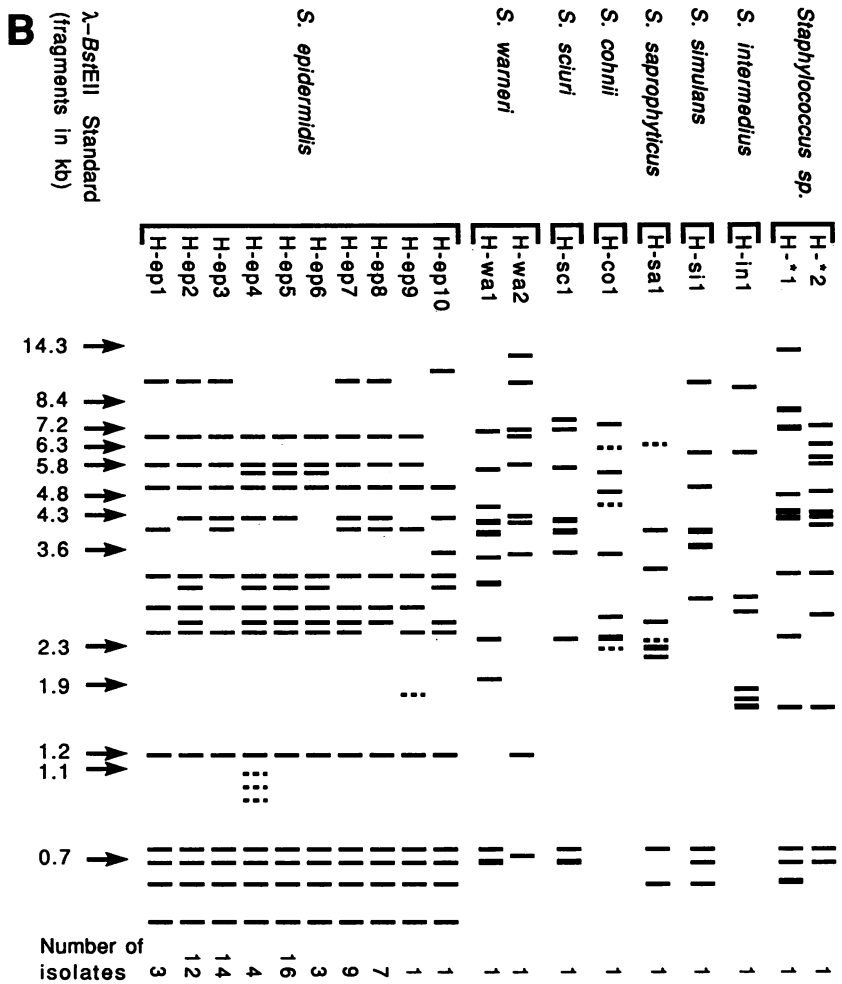

FIG. 2—*Continued.*

on the intraspecific typing of *S. epidermidis*. Classical methods based on antimicrobial or phage susceptibility (25), physiological properties (21), and pathogenic properties (e.g., slime production) (24) are important for species identification and typing. Many of them are inexpensive, fast, and easy to perform and may have considerable DP, as has recently been shown for antimicrobial susceptibility testing (25). Nevertheless, classical biological methods have the major disadvantage of limited reproducibility and/or specificity.

TABLE 1. Classification of 57 *S. epidermidis* strains in combined *Eco*RI-*Hin*dIII ribotypes[a]

*Hin*dIII ribotype	No. of strains with the following *Eco*RI ribotype:							
	E-ep2	E-ep3	E-ep5	E-ep1	E-ep4	E-ep6	E-ep7	Total
H-ep5	**14**	2	–	–	–	–	–	16
H-ep3	7	**2**	–	–	–	–	1	10
H-ep7	2	–	–	5	–	–	–	7
H-ep2	3	2	2	–	–	–	–	7
H-ep8	3	2	–	1	–	–	–	6
H-ep4	–	–	–	–	4	–	–	4
H-ep1	1	–	2	–	–	–	–	3
H-ep6	–	–	3	–	–	–	–	3
H-ep9	–	–	–	–	–	1	–	1
Total	30	8	7	6	4	1	1	57

[a] Ribotypes are listed in descending order of the number of strains per type from top to bottom (*Hin*dIII) and from left to right (*Eco*RI) (see totals). In this order strong relatedness between *Eco*RI and *Hin*dIII ribotypes would be shown by an accumulation of strains on and close to the diagonal of the table. The diagonal is indicated by boldfacing.

Molecular methods for the analysis of proteins or nucleic acids usually overcome this limitation. Evidence for the high reproducibility of restriction fragment patterns was obtained previously (6) and in this study as well. Ribotypes were stably reproducible after 20 passages of strains on agar, accounting for at least 400 cycles.

Although they are highly accurate, molecular methods also have shortcomings. Plasmid typing may be unreliable because of the spontaneous loss or acquisition of plasmids (6, 28, 32). Furthermore, plasmid-deficient strains remain untypeable, as would have been the case with 21% of all the CoNS in this study. This percentage is within the range found by other workers (32). REA was shown to be useful for the epidemiological analysis of CoNS (6). However, that study (6) as well as the present study showed that the interference of bright plasmid bands as well as large numbers of chromosomal bands rendered interpretation difficult. Only a limited number of strains might be typed efficiently. Our results indicated that ribotyping overcame the shortcomings of plasmid typing and REA.

Therefore, ribotyping can be considered a valuable tool for accurate and reproducible intraspecific typing. For our *S. epidermidis* strains, the DP varied from 14.3 to 15.1% when a single restriction enzyme was used. This DP was lower than the DP obtained for 29 *S. epidermidis* strains with REA (27.6%; 6) or with API biotyping (19.8%; this study). However, the use of an additional restriction enzyme for ribotyping increased the DP to 31.6%. This surprising increase was in contrast to the results reported by Bialkowska-Hobrzanska et al. (6), who found that their *Cla*I REA types were confirmed rather than that further subdivisions were detected when they replaced *Cla*I with *Pst*I or *Bgl*II. Moreover, when our results gained by the two unrelated procedures of ribotyping and biotyping were analyzed in combination, an increase in the DP to 42.5 to 48.6% was observed. This increase was in accordance with earlier findings obtained from a comparison of typing by plasmid profiling and REA (6). Despite these high DPs, we do not recommend the combination of ribotyping with biotyping or other classical methods for epidemiological purposes because the reliability of the former will be overshadowed by the latter.

The present study yielded evidence that many ribotypes of CoNS might be widespread geographically. Interestingly, certain *Eco*RI ribotypes of CoNS collected in the United Kingdom and tested by Thomson-Carter et al. (41) were indistinguishable from some in our collection; e.g., *S. epidermidis* 14, 8, and 7 (41) corresponded to our ribotypes Eep-2, Eep-1, and Eep-7, respectively (this study).

Our comparative study also revealed that intraspecific typing by different methods yielded different sets of types that showed surprisingly little relatedness to each other. This finding implied that the introduction of new typing methods and the combination of new and old typing methods will ever increase the DP to, finally, close to 100%. It is obvious that these methods will enhance clinicians' and microbiologists' chances to differentiate between epidemically relevant strains and harmless contaminants. Furthermore, ribotyping is an excellent additional tool for studying the in vivo transmission of resistance plasmids among chromosomally distinct backgrounds of CoNS because it allows accurate analysis of the chromosome whether plasmids are present or not. Hence, it should become easier to trace the sources and transmission of epidemic pathogens and to take preventive measures. However, even the most refined typing methods will not allow the prediction of the clinical significance of a particular strain of CoNS found in an individual patient. This conclusion is also supported by a study which failed to show an association between certain CoNS and clinical significance (37).

The taxonomic potential of ribotyping for the species identification of CoNS was pointed out earlier (5, 10, 41). The data for our 26 non-*S. epidermidis* strains seemed to support that finding. However, a careful comparison of these 26 ribotype banding patterns with those of the 86 *S. epidermidis* strains revealed obvious similarities between ribotypes of different species. This finding indicated that as soon as collections with large numbers of strains per species were ribotyped, intraspecific fragment pattern diversity interfered with interspecific discrimination, thus rendering species identification difficult. It also indicated that our collection of non-*S. epidermidis* strains (26 strains in nine species) was by far too small to allow judgment of the degree of intraspecific diversity of fragment patterns within those nine species. Hence, despite encouraging progress concerning the classification of CoNS by ribotyping, as reported earlier (5, 10, 41), caution should be exercised, since those investigators' strain collections contained relatively small numbers of isolates per species. We therefore recommend that species identification be done by classical methods and that it only be confirmed by molecular methods, such as ribotyping.

In recent years, nosocomial infection and colonization of patients and staff with multiply antibiotic resistant and/or Mec^r *S. aureus* (MRSA) strains have become a problem in many parts of the world (for a review see reference 8). Results of investigations of outbreaks caused in hospitals by MRSA became more reliable with the introduction of molecular methods (9, 12, 17, 34). These studies revealed that the outbreaks of MRSA infections were often due to the spread of a single epidemic strain within a hospital. Mec^r is known to occur even more frequently in *S. epidermidis* than in *S. aureus* (3, 32; this study). Analysis of the ribotypes of our Mec^r *S. epidermidis* strains revealed that there was no correlation between Mec^r and certain ribotypes. This result indicated that the Mec^r *S. epidermidis* strains in our hospitals had no common clonal origin. It also suggested that no outbreak caused by a particular Mec^r *S. epidermidis* strain was ongoing during the period of collection, confirming the conclusions drawn from the epidemiological interpretation of our data.

In conclusion, ribotyping is considered an excellent tool for the epidemiological analysis of CoNS, because it offers nearly 100% reproducibility and good intraspecific DP. If necessary, the DP of the method can easily be increased by the use of one or more additional restriction enzymes or by combination with equally highly reproducible methods for typing, such as large-fragment REA (13) or small-fragment REA (18). For routine testing, the rDNA probe rather than the rRNA probe should be used and radioactivity should be replaced by visualizing the hybridized probe with alternative labels, such as biotin (2) or acetylaminofluorene (14).

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