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Species-Specific and Ubiquitous DNA-Based Assays for Rapid Identification of *Staphylococcus epidermidis*

FRANCIS MARTINEAU,[1,2] FRANÇOIS J. PICARD,[1] PAUL H. ROY,[1,3] MARC OUELLETTE,[1,2] AND MICHEL G. BERGERON[1,2]*

Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, Ste-Foy, Québec, Canada G1V 4G2,[1] and Département de Biochimie,[3] and Département de Microbiologie,[2] Université Laval, Ste-Foy, Québec, Canada G1K 7P4

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***Staphylococcus epidermidis* is an aerobic gram-positive coccus that is now recognized among the coagulase-negative staphylococci as an etiological agent with an important range of pathogenicity in humans. Several diagnostic kits based on biochemical or immunological reactions can efficiently identify *Staphylococcus aureus*. However, these tests are often unreliable for the identification of coagulase-negative staphylococcal species including *S. epidermidis*. Since DNA-based assays for the species-specific identification of *S. epidermidis* remain unavailable, we have developed such tests in order to improve the accuracy and the rapidity of tests for the diagnosis of *S. epidermidis* infections. On the basis of the results of hybridization assays with clones randomly selected from an *S. epidermidis* genomic library, we identified a chromosomal DNA fragment which is specific and 100% ubiquitous for the identification of *S. epidermidis*. This 705-bp fragment was sequenced and used to design PCR amplification primers. PCR assays with the selected primers were also highly specific and ubiquitous for the identification from bacterial cultures of clinical isolates of *S. epidermidis* from a variety of anatomic sites. While three strains of *S. capitis* were misidentified as *S. epidermidis* with the API Staph-Ident system and 2.5% of the *S. epidermidis* identifications were inconclusive with the MicroScan Autoscan-4 system, the PCR assay was highly specific and allowed for the correct identification of all 79 *S. epidermidis* strains tested. The PCR assays developed are simple and can be performed in about 1 h. These DNA-based tests provide novel diagnostic tools for improving the diagnosis of *S. epidermidis* infections.**

Coagulase-negative staphylococci (CoNS) are a major component of the normal skin flora and mucous membranes, and they are among the most frequently isolated bacteria in the clinical microbiology laboratory (11, 13, 20, 26). *Staphylococcus epidermidis* is frequently associated with bacteremia (19, 28), urinary tract infections (17), and postcatheterization infections (20). The etiological importance of this opportunistic nosocomial pathogen in osteomyelitis and wound infections is well documented (12, 23). An association of *S. epidermidis* with peritonitis during continuous ambulatory dialysis and transient or permanent medical devices (intravascular catheters and prosthetic devices) in seriously ill and immunocompromised patients is also recognized (12, 23).

Several manual and automated methods for the identification of staphylococci are commercially available (6, 27). Since rapid systems or methods for the specific identification of *S. epidermidis* directly from clinical specimens are not available, this species and other CoNS can only be identified from bacterial cultures with systems based on biochemical tests which require from 4 to 48 h of incubation (2, 8–10). Furthermore, commercially available panels, which are based on functional differences in metabolic pathways, do not allow for the reliable distinction of *S. epidermidis* from other CoNS (21). Because these procedures are not optimal for the diagnosis of *S. epidermidis* infections, the development of a rapid and reliable method for the identification of this pathogen that can also be applied for detection directly from clinical specimens is needed.

* Corresponding author. Mailing address: Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, 2705 Boul. Laurier, Ste-Foy, Québec, Canada G1V 4G2. Phone: (418) 654-2705. Fax: (418) 654-2715.

This study deals with the development of species-specific DNA-based assays for the identification of *S. epidermidis*. For this purpose, an *S. epidermidis* genomic library was screened by hybridization to DNAs from an array of both gram-positive and gram-negative bacterial species in order to identify a clone carrying an insert suitable as a probe for *S. epidermidis* identification. A genomic DNA fragment probe which is species specific and ubiquitous for the identification of *S. epidermidis* was isolated. This genomic DNA fragment was sequenced and used to design PCR amplification primers for *S. epidermidis*-specific PCR assays. These PCR assays provide improvements for the diagnosis of *S. epidermidis* infections in terms of both rapidity and accuracy.

MATERIALS AND METHODS

Bacterial strains. The bacterial isolates used in this study were selected from the culture collection of the Microbiology Laboratory of the Centre Hospitalier de l'Université Laval (CHUL). *S. epidermidis* ATCC 12228 and ATCC 14990 were also used. Duplicate isolates from the same patients, even if the site of infection was different, were excluded from this study. Strains were cultured onto sheep blood agar or in brain heart infusion (BHI) broth. Bacterial cultures were stored frozen (−80°C) in BHI broth containing 10% glycerol.

The specificity of the DNA-based tests was verified by using a battery of clinical isolates consisting of 41 gram-negative and 20 gram-positive bacterial species (Table 1). This group of bacterial strains includes isolates obtained from both the American Type Culture Collection (ATCC) and the Microbiology Laboratory of CHUL. The ubiquity of the DNA-based tests was verified with a set of 80 clinical isolates of *S. epidermidis* selected from the culture collection of the Microbiology Laboratory of CHUL. *S. epidermidis* isolates (80 strains) were from blood (46 strains), catheter tips (16 strains), osteomyelitis lesions (5 strains), urine (4 strains), wound infections (4 strains), synovial fluid (3 strains), and cerebrospinal fluid (2 strains). The identification of all strains was reconfirmed as *S. epidermidis* by using both the API Staph-Ident system (bioMérieux, Saint-Laurent, Québec, Canada) and the MicroScan Autoscan-4 system equipped with the Positive BP Combo Panel Type 6 (Dade Diagnostics, Mississauga, Ontario, Canada).

Genomic DNA library construction. Genomic DNA from *S. epidermidis* ATCC

TABLE 1. Bacterial species used to test the specificity of selected probes and PCR primers

Bacterial species (no. of species)	No. of strains tested
Gram-negative species (41)	
Proteus mirabilis	2
Klebsiella pneumoniae	2
Pseudomonas aeruginosa	2
Escherichia coli	2
Moraxella catarrhalis	1
Proteus vulgaris	1
Morganella morganii	1
Enterobacter cloacae	1
Providencia stuartii	1
Enterobacter agglomerans	1
Providencia rettgeri	1
Neisseria mucosa	1
Providencia rustigianii	1
Burkholderia cepacia	1
Enterobacter aerogenes	1
Stenotrophomonas maltophilia	1
Pseudomonas fluorescens	1
Comamonas acidovorans	1
Pseudomonas putida	1
Haemophilus influenzae	1
Haemophilus parainfluenzae	1
Bordetella pertussis	1
Haemophilus parahaemolyticus	1
Haemophilus haemolyticus	1
Haemophilus aegyptius	1
Kingella indologenes	1
Moraxella atlantae	1
Neisseria caviae	1
Neisseria subflava	1
Moraxella urethralis	1
Shigella sonnei	1
Shigella flexneri	1
Klebsiella oxytoca	1
Serratia marcescens	1
Salmonella typhimurium	2
Yersinia enterocolitica	1
Acinetobacter calcoaceticus	1
Acinetobacter lwoffi	1
Hafnia alvei	1
Citrobacter diversus	1
Citrobacter freundii	1
Gram-positive species (20)	
Streptococcus pneumoniae	4
Streptococcus salivarius	2
Streptococcus viridans	2
Streptococcus pyogenes	2
Staphylococcus aureus	2
Staphylococcus epidermidis	5
Staphylococcus saprophyticus	8
Micrococcus luteus	2
Corynebacterium diphtheriae	2
Streptococcus agalactiae	2
Staphylococcus simulans	1
Staphylococcus lugdunensis	1
Staphylococcus capitis	2
Staphylococcus haemolyticus	2
Staphylococcus hominis	2
Enterococcus faecalis	4
Enterococcus faecium	2
Staphylococcus warneri	1
Enterococcus durans	1
Streptococcus bovis	1

12228 was isolated from an overnight culture in BHI broth as described by Sambrook et al. (25), except that lysostaphin (Sigma, St. Louis, Mo.) at 200 μg/ml was added to the lysis solution. Staphylococcal genomic DNA was then digested with the restriction enzyme *Sau*3AI (New England Biolabs Ltd., Mississauga, Ontario, Canada) and cloned into the *Bam*HI site of the plasmid vector pGEM-7Zf (Promega Corp., Madison, Wis.) by using T4 DNA ligase (New England BioLabs). Recombinant plasmids were transformed into *Escherichia coli* DH5α competent cells by standard procedures (1, 25).

Plasmid DNA isolation was done either by the method of Birnboim and Doly (4) for small-scale preparations or by using the Wizard Maxiprep kit (Promega Corp.) for large-scale preparations.

Probe preparation and labeling. Genomic DNA inserts from randomly selected clones were cut out from the recombinant plasmids by double digestion with *Sac*I and *Cla*I restriction endonucleases (New England BioLabs). Fragments were separated by electrophoresis in 1% agarose gels. The band corresponding to a fragment of inserted genomic DNA was excised from the gel, and the DNA was purified by using the Sephaglass BandPrep kit (Pharmacia Biotech Inc., Baie d'Urfé, Québec, Canada). In some instances, the restriction digest yielded more than one fragment of *S. epidermidis* genomic DNA due to the presence of *Sac*I and/or *Cla*I sites within the genomic DNA insert. In these cases, each fragment was gel purified and individually tested. Purified DNA fragments (approximately 100 ng) were labeled with [α-^{32}P]dATP (DuPont NEN Research Products, Mississauga, Ontario, Canada) by random priming with Klenow fragment of *E. coli* DNA polymerase (New England Biolabs) and were used as probes in hybridization assays (25). All reactions were performed according to the manufacturer's instructions.

Dot blot hybridization. Genomic DNA was extracted from *S. epidermidis* strains as described by Ubukata et al. (30). Denatured genomic DNA was spotted onto a nylon membrane by using a dot blot apparatus (Minifold 2 Slot-Blot System; Schleicher & Schuell, Keene, N.H.) and was irreversibly fixed. The genomic DNA extraction methodology used for bacterial species other than staphylococcal species was identical except that lysozyme at a concentration of 100 μg/ml instead of lysostaphin was incorporated into the lysis solution.

Prehybridization was performed at 65°C for 15 min in prehybridization solution containing 1 M NaCl, 10% dextran sulfate, 1% sodium dodecyl sulfate (SDS), and 100 μg of salmon sperm DNA per ml. The hybridization step was done with fresh prehybridization solution containing the ^{32}P-labeled probe at 65°C overnight. Two posthybridization washes were done with 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate) containing 1% SDS at 65°C; this was followed by two washes in 1× SSC containing 1% SDS and one wash in 0.1× SSC containing 0.1% SDS. All washes were performed at 65°C for 15 min. The detection of selectively hybridized probes was done by autoradiography.

DNA sequencing. Both strands of genomic DNA fragments were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers by using the Applied Biosystems automated DNA sequencer (model 373A) with the PRISM Sequenase Terminator Double-Stranded DNA Sequencing Kit (Perkin-Elmer Corp., Applied Biosystems Division, Foster City, Calif.).

PCR amplification. Oligonucleotide primers were synthesized with a model 391 DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division). For all bacterial species, amplification was performed either directly from a bacterial colony or from a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 bacteria per ml. A portion of an isolated colony or 2 μl of the standardized bacterial suspension was transferred directly to a 50-μl PCR mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM (each) the two PCR primers, 200 μM (each) the four deoxynucleoside triphosphates, and 1.25 U of *Taq* DNA polymerase (Promega Corp.). In order to reduce the formation of nonspecific extension products, the hot-start protocol (5) was performed by adding the PCR buffer and the polymerase last, once the other components of the reaction mixture reached a temperature of 85°C. The PCR mixtures were subjected to thermal cycling (3 min at 96°C and then 30 cycles of 1 s at 95°C for the denaturation step and 1 s at 55°C for the annealing-extension step) with a Perkin-Elmer 480 thermal cycler (Perkin-Elmer Canada Ltd., Mississauga, Ontario, Canada). The quick lysis and the rapid cycling for PCR amplification required slightly less than 1 h.

Primer sequences derived from highly conserved regions of the bacterial 16S rRNA gene (5′-GGAGGAAGGTGGGGATGACG and 5′-ATGGTGTGACG GGCGGTGTG) were used to provide an internal control for all PCRs. These primers are useful for providing an internal control because they can amplify a 241-bp product from any bacterial species (universal bacterial amplification). The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. The internal control and the *S. epidermidis*-specific amplifications were performed simultaneously in multiplex PCR assays with 0.4 μM (each) species-specific primer and 0.04 μM (each) 16S rRNA universal primer. The universal primers were used in a limiting concentration to avoid detrimental competition with the species-specific amplification.

Twenty microliters of the PCR-amplified mixture was resolved by electrophoresis in a 2% agarose gel containing 0.5 μg of ethidium bromide per ml at 170 V for 15 min. The size of the amplification products was estimated by comparison with a 50-bp ladder molecular mass marker. The total time for the PCR assays

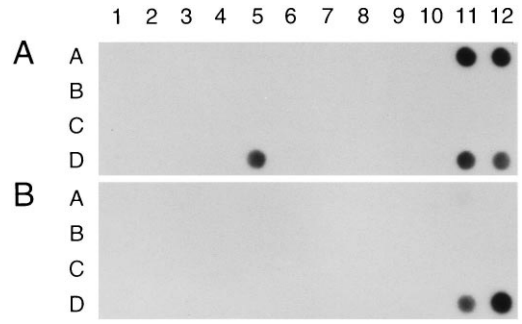

FIG. 1. Specificity test by dot blot hybridization by using the ^{32}P-labeled *S. epidermidis*-specific 705-bp DNA fragment as a probe and DNAs from a variety of gram-positive (A; locations 1A to 12D) and gram-negative (B; locations 1A to 12D) bacterial species (Table 1) as targets. DNAs from *S. epidermidis* isolates are at locations 11A (ATCC 12228), 12A (ATCC 14990), 5D, 11D, and 12D for panel A and at locations 11D (ATCC 12228) and 12D (ATCC 14990) for panel B.

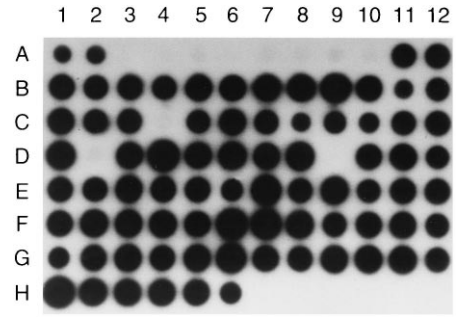

FIG. 2. Ubiquity test by dot blot hybridization by using the ^{32}P-labeled *S. epidermidis*-specific 705-bp DNA fragment as a probe and DNAs from 82 clinical isolates of *S. epidermidis* as targets. DNAs from the *S. epidermidis* strains are at locations 1A (ATCC 12228), 2A (ATCC 14990), and 11A to 6H (80 clinical strains from CHUL). A battery of eight different staphylococcal species including *S. aureus*, *S. saprophyticus*, *S. simulans*, *S. lugdunensis*, *S. capitis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* were used as negative controls (locations 3A to 10A, respectively). Isolates incorrectly identified as *S. epidermidis* by the API Staph-Ident system and which did not hybridize with the probe are at locations 4C, 2D, and 9D. These three strains were identified as *S. capitis* with the Autoscan-4 system.

from a bacterial colony or from a standardized bacterial suspension was approximately 1 h.

For determination of the sensitivity for the PCR assays, a culture of *S. epidermidis* in the logarithmic phase of growth (optical density at 600 nm, ≈0.7 to 0.8) (24) was diluted in phosphate-buffered saline. Each dilution (2 μl) was tested in PCR assays to determine the minimal number of CFU which can be detected. The number of CFU was estimated by standard plating procedures. A similar approach was applied to determine the minimal number of recombinant plasmid molecules which can be detected.

RESULTS

Identification of *S. epidermidis*-specific genomic DNA fragments. The *S. epidermidis* genomic library was selected for clones carrying species-specific DNA inserts by hybridization to DNAs from an array of bacterial species (Table 1). A total of 36 randomly selected clones had to be tested in order to obtain a species-specific probe. For each clone, the genomic DNA insert was excised from the vector by digestion with the restriction endonucleases *Sac*I and *Cla*I. The resulting 62 genomic DNA fragments of sizes ranging from 100 bp to 1.2 kbp were each gel purified, labeled, and individually tested in hybridization assays. Using this strategy, we isolated a fragment of genomic DNA of 705 bp which was shown to be *S. epidermidis* specific (Fig. 1). This probe hybridized only to DNAs from the *S. epidermidis* isolates, and no hybridization signal was observed with DNAs from the other bacterial species listed in Table 1. Ubiquity tests performed with this *S. epidermidis*-specific probe with an array of 82 clinical strains of *S. epidermidis* showed that DNAs from 79 of the 82 strains hybridized specifically with the probe. The three isolates from the CHUL collection (locations 4C, 2D, and 9D in Fig. 2) whose DNA did not hybridize with the *S. epidermidis*-specific probe were found to be incorrectly identified by the API Staph-Ident system. In fact, all three isolates were identified by this system as *S. epidermidis* with a qualification of "low discrimination" between *S. epidermidis*, *S. capitis*, *S. warneri*, *S. simulans*, and *S. aureus*. A reconfirmation of the identification with the MicroScan Autoscan-4 system identified all three isolates as *S. capitis* at a probability of 99.5%. Consequently, in ubiquity tests the 705-bp DNA probe detected 100% of the 79 clinical *S. epidermidis* isolates tested. The Autoscan-4 system identified these 79 strains as *S. epidermidis* with a probability of 99.5% (77 strains) or with a probability of 62.0% (2 strains).

Subcloning and sequencing of the *S. epidermidis*-specific probe. The *S. epidermidis*-specific 705-bp genomic DNA fragment probe was subcloned into pGEM-7Zf. Subsequently, the sequences of both strands were determined. Searches in data banks did not reveal any significant homologies with known sequences. Two sets of PCR primers derived from this sequence were designed with the help of Oligo, version 4.0, Primer Analysis software (National Biosciences, Plymouth, Minn.) (Table 2).

PCR assays. Specificity tests performed with the panel of gram-positive and gram-negative bacterial species listed in Table 1 showed that both selected PCR primer pairs amplified only DNA from clinical isolates of *S. epidermidis*. In order to ensure that the negative PCR results obtained with the bacterial species other than the target species were not attributable to PCR inhibitors or to the inadequacy of the PCR assays, all cell lysates were simultaneously amplified in a multiplex PCR assay with both the *S. epidermidis*-specific primers and the universal primers specific for the highly conserved bacterial

TABLE 2. PCR primer pairs selected from the 705-bp *S. epidermidis*-specific genomic DNA probe

PCR primer	Nucleotide sequence	Annealing positions	Product length (bp)
Pair 1			
Se705-1	5′-ATC AAA AAG TTG GCG AAC CTT TTC A-3′	21–45	124
Se705-2	5′-CAA AAG AGC GTG GAG AAA AGT ATC A-3′	145–121	
Pair 2			
Se705-3	5′-TCT CTT TTA ATT TCA TCT TCA ATT CCA TAG-3′	448–477	174
Se705-4	5′-AAA CAC AAT TAC AGT CTG GTT ATC CAT ATC-3′	622–593	

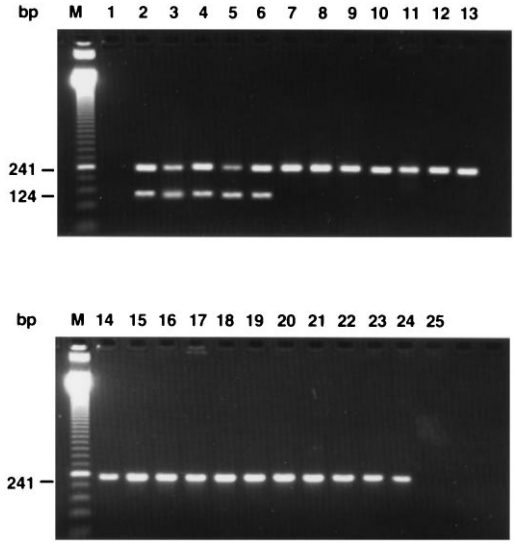

FIG. 3. Multiplex PCR amplification with the *S. epidermidis*-specific PCR primer pair 1 and the universal primers, which were used to provide an internal control. PCR assays were performed from 2 μl of a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard prepared from ATCC strains or from clinical isolates from CHUL. The content of each lane is as follows: 2, *S. epidermidis* ATCC 12228; 3, *S. epidermidis* ATCC 14990; 4 to 6, *S. epidermidis*; 7 to 9, *Staphylococcus aureus*; 10, *Staphylococcus saprophyticus*; 11, *Staphylococcus simulans*; 12, *Staphylococcus lugdunensis*; 13, *Staphylococcus capitis*; 14, *Staphylococcus haemolyticus*; 15, *Staphylococcus hominis*; 16, *Staphylococcus warneri*; 17, *Micrococcus luteus*; 18, *Enterococcus faecalis*; 19, *Enterococcus faecium*; 20, *Streptococcus pneumoniae*; 21, *Streptococcus pyogenes*; 22, *Streptococcus salivarius*; 23, viridans group streptococcal strain; 24, *Streptococcus agalactiae*. Lanes 1 and 25, controls to which no DNA was added; lanes M, 50-bp ladder molecular size standard.

16S rRNA gene. The results showed that all bacterial species were efficiently amplified by the universal primers, thereby showing the absence of PCR inhibitors and the suitability of the PCR assays for amplifying DNA from the wide variety of bacterial species tested (Fig. 3). It is important to note that the PCR assays with both species-specific primer pairs did not yield any amplification product with eight staphylococcal species other than *S. epidermidis* (Fig. 3).

The ubiquity of each primer pair was tested by performing PCR assays with the 79 clinical isolates of *S. epidermidis* previously used for the hybridization assays. The ubiquity test showed that DNAs from all isolates were specifically amplified with each pair of PCR primers. As expected, the three isolates identified as *S. epidermidis* by the API Staph-Ident system but as *S. capitis* by the Autoscan-4 system were not amplified with both *S. epidermidis*-specific PCR primer pairs.

Sensitivity assays performed with primer pair 1 indicated a detection limit of approximately 1.2×10^3 copies of the recombinant plasmid carrying the 705-bp DNA fragment probe. In terms of numbers of CFU, the detection limit with logarithmically growing *S. epidermidis* cultures was approximately 1.5×10^3 CFU. For primer pair 2, the detection limit was approximately 2×10^5 CFU. These results show that primer pair 1 amplifies the targeted *S. epidermidis* sequence much more efficiently than primer pair 2, even though both primer pairs appeared optimal for PCR on the basis of computer analysis with the Oligo program. Although suitable for the identification of *S. epidermidis* from bacterial cultures, the PCR assay with primer pair 2, which is approximately 100-fold less sensitive than PCR assays with primer pair 1, has not been retained for further evaluation in more exhaustive clinical studies with both bacterial cultures and clinical specimens, which are in progress.

DISCUSSION

We were able to isolate a genomic DNA fragment probe of 705 bp from an *S. epidermidis* genomic library which is species specific and ubiquitous for the identification of *S. epidermidis* in hybridization assays. This assay is therefore adequate for *S. epidermidis* culture confirmation. In order to simplify the assay as well as to improve its rapidity and sensitivity, the sequence of the 705-bp DNA probe, which is of unknown coding potential, was used to develop PCR assays suitable for the rapid and accurate diagnosis of *S. epidermidis* infections. The PCR assays, which were performed directly from bacterial colonies or from a standardized bacterial suspension, were designed and optimized to be simple and performed in approximately 1 h. Our data indicate that these PCR assays are also highly specific and ubiquitous for *S. epidermidis.*

These diagnostic tests provide improvements for the diagnosis of *S. epidermidis* infections in terms of both rapidity and accuracy. In fact, none of the existing diagnostic tools can reliably distinguish *S. epidermidis* from other species of CoNS. For example, the widely used API Staph-Ident system requires 24 h and sometimes 48 h to provide species identification and may incorrectly or poorly identify *S. epidermidis* (15). When we used this system to reconfirm the *S. epidermidis* identifications of the 82 strains selected for this study, only 24.3% (20 strains) were identified as *S. epidermidis*, with excellent discrimination. The other strains were identified as *S. epidermidis* as follows: 17.0% (14 strains) with a very good discrimination, 48.8% (40 strains) with a good discrimination, 6.1% (5 strains) with an acceptable discrimination, and 3.7% (3 strains) with a low discrimination. The three strains identified with a low discrimination turned out to be species of CoNS other than *S. epidermidis* on the basis of testing by our hybridization and PCR assays as well as by the MicroScan Autoscan-4 system. In fact, the Autoscan-4 system identified all three strains as *S. capitis* with a probability of 99.5%. The other 79 strains were also identified as *S. epidermidis* by the hybridization assay, both PCR assays, and the Autoscan-4 system. Preliminary studies with *S. epidermidis* strains from various countries indicate that our most sensitive PCR assay (with primer pair 1) remains 100% ubiquitous for *S. epidermidis* identification.

With regard to automated systems, the MicroScan Autoscan-4 system equipped with Positive BP Combo Panel Type 6 is efficient at only 36% for the identification of species of CoNS after overnight incubation and at 83.4% after 48 h of incubation (6, 27). More specifically, the *S. epidermidis* identification with this system was correct for 95% (7) or 97% (27) of the strains tested. Our results with the Autoscan-4 system for the identification of the 79 *S. epidermidis* strains from this study show that all strains were identified as *S. epidermidis* with excellent discrimination (probability of at least 99.5%) except for two strains which were identified as *S. epidermidis* with a probability of only 62.0%. For these two strains, the other identification possibilities were *Staphylococcus capitis* at a probability of 25.9%, *Staphylococcus warneri* at a probability of 7.9%, and *Staphylococcus hominis* at a probability of 4.0%. On the other hand, our hybridization and both PCR assays identified clearly all of these 79 strains as *S. epidermidis*, thereby showing a 100% ubiquity. The fastest identification system, the autoScan-Walk-Away system, identifies a wide panel of gram-positive organisms (including staphylococcal species) from isolated bacterial colonies in only 2 h. However, this system may have a relatively high margin of error, especially with bacterial

species other than members of the family *Enterobacteriaceae* (31). For staphylococcal species there was about 91.6% correct identifications (31).

Other systems for the identification of *S. epidermidis* are commercially available, such as the STAPH-IDENT system (bioMérieux Vitek, Inc., Hazelwood, Mo.), which is marketed as a 5-h identification system for staphylococci. However, Rhoden and Miller (22) needed 26.6% more time in order to obtain a complete identification. Furthermore, 6% of the *S. epidermidis* strains tested were misidentified by this system. The conventional reference identification method of Kloos and Schleifer (14) is not applicable in the routine laboratory because of its complexity and its requirement for up to 5 days of incubation before obtaining the results. Methods based on gas-liquid chromatography of staphylococcal cellular fatty acids such as the microbial identification system manufactured by Microbial ID Inc. (Newark, Del.) are labor intensive and show an unacceptable performance for the identification of *S. epidermidis* (29). In fact, 16 of 36 strains of *S. hominis* were misidentified as *S. epidermidis* (16). The RapiDEC Staph system (RD-Staph; bioMérieux-Vitek) failed to detect alkaline phosphatase activity in many *S. epidermidis* isolates (only 70.3% correct identification) (10). The susceptibility test based on desferrioxamine cannot discriminate promptly between *S. epidermidis* and *S. hominis* and needs at least 18 h of incubation for the interpretation of the MICs of desferrioxamine (18). Finally, no commercially available immunological assays, such as latex agglutination assays, are available for the species-specific identification of any species of CoNS.

In this study, we have focused on PCR assays performed directly from bacterial colonies or from a standardized *S. epidermidis* bacterial suspension. We have developed a simple lysis protocol and a rapid thermal cycling procedure which allow the assay to be performed in about 1 h. Even though this PCR protocol includes only 30 cycles, our results show that the sensitivity levels achieved (i.e., about 1,500 CFU with primer pair 1) are sufficient for culture confirmation assays. Preliminary data indicate that this assay is also suitable for *S. epidermidis* identification from blood cultures. Furthermore, this assay is also suitable for the amplification of genomic DNA from a wide variety of bacterial species, as shown by the universal amplification assay. However, increased sensitivity levels will be required for PCR assays performed directly from clinical specimens, in which the number of target bacterial cells is much lower than that in a bacterial colony or in blood cultures. We have demonstrated that the sensitivities of PCR assays can be enhanced by increasing the number of cycles to 35 to 40 instead of 30, as described in this report. For example, when we used PCR assays with 40 cycles, as frequently used for direct detection from clinical specimens, the sensitivity level of the assay was increased by about 1,000-fold (data not shown). Although our lysis protocol is very rapid, it appears to lyse *S. epidermidis* bacterial cells efficiently, since the sensitivity levels measured by titrations of CFU counts and recombinant plasmids were both similar. We are adapting the protocol for PCR assays to be performed directly from a variety of clinical specimens. Multiplex PCR assays performed directly from a bacterial colony or from clinical specimens which allow for the identification of *S. epidermidis* as well as the simultaneous detection of clinically relevant antibiotic resistance genes are also under development.

In our research laboratory, we are developing rapid PCR assays for other important bacterial pathogens and associated antibiotic resistance genes. We have assays that can detect 13 species of the most frequently encountered bacterial pathogens, which account for approximately 80% of the bacteria routinely isolated in the microbiology laboratory. The *S. epidermidis* PCR assay reported here will be combined with these PCR assays as well as with others which are under development. All assays will be adapted for fluorescence-based amplicon detection, which is much faster and simpler than agarose gel electrophoresis. Furthermore, the assays should be performed in sealed wells from 96-well plates to prevent the carryover of target DNA. The simplicity, the rapidity, the high accuracy, and the potential for full automation of those assays should facilitate their integration in the microbiology laboratory. A direct impact of such diagnostic tests is that they should allow for the faster establishment of effective antibiotic therapy and a reduction of empirical treatments with broad-spectrum antibiotics, which are associated with high costs and toxicity (3). The consequent reduction in antibiotic use should reduce the emergence of resistance. Simple and rapid DNA-based diagnostic tests, such as the PCR assay described in this report, should improve the diagnosis of bacterial infections in the routine microbiology laboratory because they are highly specific and sensitive and are amenable to full automation.

In conclusion, we have developed DNA-based diagnostic assays which are much faster and more reliable for the identification of *S. epidermidis* than the commercially available biochemical identification systems API Staph-Ident and MicroScan Autoscan-4, which are both examples of classical identification systems commonly used in routine microbiology laboratories.

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