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Rapid Identification of Staphylococcal Strains from Positive-Testing Blood Culture Bottles by Internal Transcribed Spacer PCR Followed by Microchip Gel Electrophoresis

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PCR analysis of the 16S-23S rRNA gene internal transcribed spacer (ITS) followed by microchip gel electrophoresis (MGE) was evaluated for its usefulness in identification of staphylococci. Forty ITS PCR patterns were demonstrated among 228 isolated colonies of *Staphylococcus aureus*: 26 patterns for methicillin-susceptible *S. aureus* (MSSA; 91 strains), 11 patterns for methicillin-resistant *S. aureus* (MRSA; 99 strains), and 3 patterns for both MSSA and MRSA (38 strains). Thirty-seven control strains of coagulase-negative staphylococci (CNS) representing 16 species showed unique ITS PCR patterns (24 patterns) at the species and subspecies levels: two patterns for *S. caprae*, *S. cohnii*, *S. haemolyticus*, and *S. saprophyticus*; three patterns for *S. lugdunensis*; four patterns for *S. capitis*; and one pattern for each of the other CNS species. The combined PCR-MGE method was prospectively adapted to the positive blood culture bottles, and this method correctly identified MSSA and MRSA in 102 (89%) of 114 blood cultures positive for *S. aureus* on the basis of the ITS PCR patterns. Eight ITS PCR patterns were demonstrated from 166 blood culture bottles positive for CNS. The most frequent CNS species isolated from blood cultures were *S. epidermidis* (76%), *S. capitis* (11%), and *S. hominis* (8%). Overall, all 280 blood culture bottles shown to contain a single *Staphylococcus* species by routine phenotypic methods were correctly identified by the PCR-MGE method at the species level, whereas the organism failed to be identified in 8 culture bottles (3%) with mixed flora. The PCR-MGE method is useful not only for rapid identification (~1.5 h) of staphylococci in positive blood culture bottles, but also for strain delineation of *S. aureus*.

The coagulase-positive species *Staphylococcus aureus* has been well documented as an opportunistic human pathogen. Infections produced by *S. aureus* are often acute and pyogenic and, if left untreated, can spread to surrounding tissue or other organs. Identification of *S. aureus* in blood cultures begins with presumptive identification of gram-positive cocci in clusters (GPCC) in gram-stained smears of blood culture bottles, signaling a positive result, whereas final identification must await subculture and overnight incubation. Direct identification of methicillin-resistant *S. aureus* (MRSA) from GPCC-positive blood culture bottles may provide important diagnostic information, which would allow the selection of an appropriate course of treatment in a timely manner. A variety of rapid identification systems for direct identification of *S. aureus* from positive blood culture bottles have been evaluated, and although the overall specificity is excellent, a wide range of sensitivities have been reported (2, 32). Molecular techniques, such as gene probe hybridization assay (16), DNA sequencing of rRNA genes (25), fluorescence in situ hybridization (23), and PCR (18, 19, 35), have also been reported for the identification of *S. aureus* directly from positive blood cultures. Although these methods provide same-day results, it takes 4 to 8 h to obtain the results. In addition, some tests cannot be used for differential identification of methicillin-susceptible *S. aureus* (MSSA) and MRSA (23, 25, 35).

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The coagulase-negative *Staphylococcus* (CNS) species, a major component of the normal human microflora, are predominantly blood culture contaminants, but they are also a significant cause of bacteremia. Moreover, an increase in the documentation of infections due to CNS has been reported (30). A review of CNS summarized results that are helpful in identifying the etiological agent (14). These results included (i) the isolation of a strain in pure culture from the infected site or body fluid and (ii) the repeated isolation of the same strain over the course of infection. Therefore, a method for the rapid and accurate identification of *Staphylococcus* strains from clinical specimens is needed, so that the clinical disease produced by this group of bacteria can be precisely delineated and the etiological agent can be determined.

In the clinical laboratory setting, *Staphylococcus* species can be identified using commercially available kits based on their phenotypic characteristics. However, DNA analysis has become the preferred method of identification, as it provides more stable and rapid identification of the isolate. The CNS isolates that were not identified at the species level or that were misidentified by conventional microbiological phenotypic tests were correctly identified by sequence analysis of the *sodA* gene (24), the DNA-DNA hybridization method (12), and PCR-amplified tRNA gene intergenic spacer length polymorphism analysis (17). Spacer regions separating the two prokaryotic rRNA genes are characterized by a high degree of sequence and length variation at both the genus and species levels (7, 8, 20). The PCR assay, which detects polymorphisms in the 16S-23S rRNA gene spacer region, has also been reported to be potentially useful for the identification of CNS isolates (20,

28). The aim of the present study was to establish a rapid and accurate method for the identification of *Staphylococcus* species from isolated colonies, as well as positive blood culture bottles, by PCR amplification of the 16S-23S rRNA gene spacer region.

MATERIALS AND METHODS

Bacterial strains. A total of 342 staphylococcal strains (given the prefix K) isolated from clinical samples between January 1997 and December 1999 at Kanazawa University Hospital were used. The strains consisted of 228 epidemiologically unrelated isolates of *S. aureus*, 16 of *S. capitis*, 12 of *S. caprae*, 45 of *S. epidermidis*, 18 of *S. haemolyticus*, 10 of *S. hominis*, 1 of *S. hyicus*, 2 of *S. lugdunensis*, 4 of *S. saprophyticus*, 2 of *S. simulans*, 3 of *S. warneri*, and 1 of *S. xylosus*. Thirty-four control strains representing 17 different species of staphylococci (*S. aureus* [$n = 3$], *S. auricularis* [$n = 1$], *S. capitis* [$n = 3$], *S. caprae* [$n = 1$], *S. cohnii* [$n = 3$], *S. epidermidis* [$n = 4$], *S. haemolyticus* [$n = 1$], *S. hominis* [$n = 1$], *S. hyicus* [$n = 1$], *S. intermedius* [$n = 1$], *S. lugdunensis* [$n = 1$], *S. saprophyticus* [$n = 3$], *S. schleiferi* [$n = 2$], *S. sciuri* [$n = 2$], *S. simulans* [$n = 2$], *S. warneri* [$n = 2$], and *S. xylosus* [$n = 3$]) were obtained from the American Type Culture Collection (ATCC) (Manassas, Va.), the Gifu Type Culture Collection (GTC) (Gifu University, Gifu, Japan), the Japan Collection of Microorganisms (JCM) (Wako, Japan), and the Czechoslovak Collection of Microorganisms (CCM) (Brno, Czechoslovakia). Nonstaphylococcal control strains used in the present study included *Bacillus cereus* JCM2152, *Corynebacterium jeikeium* JCM9384, *Enterococcus faecalis* JCM5803, *Leuconostoc lactis* JCM6123, *Micrococcus luteus* JCM1464, *Propionibacterium acnes* JCM6425, *Stomatococcus mucilaginosus* GTC311, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Identification of *Staphylococcus* strains. In the present study, all strains were identified by colony morphology, Gram stain characteristics, and detection of coagulase production by the Staphyslide test (Eiken Chemical Co., Ltd., Tokyo, Japan). Non-*aureus* staphylococcal strains were identified from the results of the N-ID test SP-18 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) performed according to the manufacturer's protocol. The tests included acid production from fructose, mannose, maltose, lactose, trehalose, mannitol, raffinose, sucrose, *N*-acetylglucosamine, turanose, ribose, and arabinose; decarboxylation of arginine; production of urease, β-glucuronidase, acetoin, and alkaline phosphatase; and reduction of nitrate. The susceptibility of staphylococci to antimicrobial agents was measured by the standard agar diffusion method (22), with the following disks: oxacillin, cefazolin, cefmetazole, impinem-cilastatin sodium, clarithromycin, minocycline, gentamicin, arbekacin, levofloxacin, fosfomycin, vancomycin, and clindamycin. If necessary, a novobiocin susceptibility test was performed. Methicillin resistance was confirmed by the presence of the *mecA* gene, determined by PCR as described previously (36). This PCR amplifies a segment of ~150 bp of the *mecA* gene; *mecA*-positive *S. aureus* strains were defined as MRSA, and *mecA*-negative strains were defined as MSSA in the present study.

A total of 620 blood culture bottles (501 patients) that became positive testing from January 2000 to December 2003 were used for molecular identification of bacteria.

Our laboratory uses the Bact/Alert blood culture system (Organon Teknika Corp., Durham, N.C.), which is an automated colorimetric CO_2 microbial growth detection system. For each blood culture, 5% sheep blood agar (Denka Seiken, Tokyo, Japan) and modified Drigalski agar (Eiken Chemical Co., Ltd., Tokyo, Japan) were inoculated with a drop of broth for isolation.

DNA preparation. Organisms were cultured onto blood agar plates. A colony grown overnight at 35°C was suspended in 0.2 ml of TE buffer (10 mM Tris-HCl–1 mM EDTA, pH 8.0) at a density of ~10^8 CFU/ml. Aliquots of 10 μl of a 10-mg/ml solution of achromopeptidase (Sigma Chemical Co., St. Louis, Mo.) were added to the bacterial solutions and incubated at 60°C for 5 min. Then, 5 μl of proteinase K solution (20 mg/ml) (Takara Shuzo Co., Ltd., Ohtsu, Japan) was added, followed by incubation at 60°C for 5 min to lyse the bacterial cells. After 7 min of incubation in a boiling water bath, the lysate was centrifuged at 9,000 × *g* for 5 min, and the supernatant was used as the template DNA.

Processing of positive blood culture bottles for PCR was performed as described previously (5) with slight modifications. Briefly, ~10 ml of 0.1% sodium dodecyl sulfate was added to 0.1 to 0.2 ml of blood culture fluids, and the mixture was centrifuged at 4,000 × *g* for 5 min. The pellet was suspended in 1.5 ml of distilled water and centrifuged again at 9,000 × *g* for 5 min. Then, 100 μl of TE buffer and 10 μl of achromopeptidase solution were added to the pellet, and the mixture was incubated at 60°C for 5 min. Purified DNA was obtained from the mixture using a MagExtract DNA extraction kit (Toyobo Co. Ltd., Osaka, Japan). In addition to DNA preparations from blood culture bottles, DNA was prepared from all culture isolates.

PCR and electrophoresis. Internal transcribed spacer (ITS) PCR was performed using the primers described by Saruta et al. (26): primer IX (5′-GGTG AAGTCGTAACAAG) and primer II (5′-TGCCAAGGCATCCACC). The PCR mixture consisted of primers (80 pmol each), 5 μl of 10× buffer (Takara), 4 μl of deoxynucleoside triphosphate mixture (2.5 mM each; Takara), 1.5 U of *Z-Taq* DNA polymerase (Takara), and 2 μl of template DNA. Amplification was performed on a Perkin-Elmer (Norwalk, Conn.) thermocycler in 50-μl reaction mixtures. The program consisted of an initial denaturation step at 94°C for 2 min, followed by 22 cycles of 1 s at 94°C, 2 s at 55°C, and 10 s at 72°C, with a final extension step at 72°C for 4 min.

Microchip gel electrophoresis (MGE) was performed as described previously (5), except that a 1,000-bp DNA size marker (GenSura Laboratories, Inc. San Diego, Calif.) was used instead of an 800-bp size marker. Briefly, a mixture of 9 μl of PCR product and 1 μl of loading buffer containing the internal standards was loaded into one of the sample wells of the microchip. Then, the mixture was run for 4 min with a microchip electrophoresis instrument (model SV1100; Hitachi Electronics Engineering Co. Ltd., Tokyo, Japan). The ITS PCR patterns of the clinical isolates were compared visually with those of the control strains. Identification was assigned to those isolates with ITS PCR patterns that matched any of the control strains. When additional characterization of isolates was needed to complete ITS PCR results, biochemical tests using a commercial identification system and partial 16S rRNA gene sequence analysis were performed. It took ~15 min to extract DNA from isolated colonies and 40 min to extract it from positive blood culture bottles. The PCR procedure required ~40 min, and MGE took ~4 min. Therefore, the overall turnaround time of the PCR-MGE assay was ~1 h with isolated colonies and 1.5 h with positive blood culture bottles.

Partial 16S rRNA gene sequence analysis. The ITS PCR patterns of non-*aureus Staphylococcus* isolates K12464 (*S. capitis*; N-ID test SP-18 code no. 130663), K332 (*S. capitis*; code no. 310662), K469 (*S. caprae*; code no. 712643), K15486 (*S. lugdunensis*; code no. 734445), K541 (*S. haemolyticus*; code no. 436766), and K2342 (*S. lugdunensis*; code no. 734564) differed from those of the 31 control strains obtained from the reference laboratories. To determine identity, an ~500-bp portion of the 16S rRNA gene was sequenced. Briefly, aliquots of 2 μl of template DNA were added to 48 μl of PCR mixture containing 5 μl of 10× buffer (Takara), 4 μl of deoxynucleoside triphosphate mixture (2.5 mM each; Takara), two primers (60 pmol each), and 1.5 U of Ex*Taq* DNA polymerase (Takara). After 3 min of incubation at 95°C, the template was amplified in a thermal cycler with the following program: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C for 35 cycles. The two primers used in the present study were 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-GTATTACCGCGGCTGCT GG-3′ (9). Amplified products were recovered from the agarose gel using a QIAquick extraction kit (QIAGEN Inc., Mississauga, Ontario, Canada), and sequencing was performed on an ABI PRISM 377 (Perkin-Elmer). Sequences were aligned using FASTA with the DNA Data Bank of Japan as published on the World Wide Web (http://www.nig.ac.jp/), and identification was assessed as >99% similarity with one of the database sequences. The six CNS strains K12464, K332, K469, K541, K15486, and K2342 were confirmed to be *S. capitis* (homology, 99.6%), *S. capitis* (homology, 100%), *S. caprae* (homology, 99.2%), *S. haemolyticus* (homology, 99.8%), *S. lugdunensis* (homology, 99.6%), and *S. lugdunensis* (homology, 99.8%), respectively, based on partial sequence analysis of the 16S rRNA gene. In addition to the control strains described above, these six strains were used as controls throughout the present study. When the results of identification differed between the phenotypic method and the PCR-MGE method, partial 16S rRNA gene sequence analysis was performed.

RESULTS

ITS PCR patterns of *S. aureus*. Electrophoretic analysis of the amplified products consistently showed two to six intense, sharp fragments (major fragments) for each sample, ranging in size from 222 to 615 bp. The fragments with intensities of <10% of the peak intensity were not taken into account in the present study. On the basis of a single band difference of major bands, 342 *S. aureus* strains were divided into 40 types. Table 1 shows the distribution of the *S. aureus* strains analyzed, and Fig. 1 shows the six representative ITS PCR patterns of MSSA and MRSA. The 165 MSSA strains were divided into 29 types

TABLE 1. ITS PCR patterns of *S. aureus* obtained from clinical materials

ITS-PCR type	No. of DNA samples[a]		Mean sizes (bp) of fragments[b]
	mecA negative	*mecA* positive	
aur1	1		222, 413, 440, 501, 611
aur2		1	320, 424, 438
aur3		3	322, 428, 441, 458, 532
aur4		3 (1)	322, 427, 440, 528
aur5	5 (2)		372, 415, 487, 501, 541
aur6	12 (7)		408, 440, 457, 539, 604
aur7	3		412, 437, 530, 608
aur8	4 (1)		413, 427, 438, 501, 608
aur9		3 (2)	413, 441, 499, 541, 600
aur10	8	6 (2)	415, 441, 458, 536
aur11	7 (4)	8 (2)	415, 441, 458, 499, 532, 605
aur12	7 (3)		419, 448, 465, 545, 611
aur13	7 (3)		421, 450, 543
aur14	5 (1)	16 (3)	422, 438, 511, 542, 603
aur15	4 (3)		424, 457, 530, 600
aur16	4 (1)		425, 520, 608
aur17	1		427, 473, 499, 534
aur18	21 (8)		427, 441, 541, 612
aur19		24 (7)	439, 529
aur20	13 (8)		440, 457, 470, 503
aur21		9 (3)	440, 460, 506, 536
aur22		90 (30)	440, 460, 533
aur23		7 (4)	440, 458, 526, 543
aur24	21 (5)		444, 469, 527, 605
aur25	4		450, 476, 505, 513, 534
aur26	9 (6)		450, 461, 469, 515, 608
aur27	1		451, 463, 506
aur28	3 (1)		451, 474, 611
aur29	2		451, 473, 513, 608
aur30	12 (3)		452, 477, 499, 516, 545, 608
aur31		2	461, 561, 637
aur32	2		461, 470, 499, 532
aur33	1		463, 474, 503, 513, 532
aur34	4 (2)		463, 536
aur35		3	462, 498, 532
aur36	1		461, 498, 511, 530
aur37	1		465, 479, 516, 615
aur3	1		470, 520, 541, 566
aur39		2 (1)	472, 530
aur40	2 (1)		481, 504, 516, 545, 615
Total	165 (59)	177 (55)	

[a] DNA samples were obtained from isolated colonies and positive blood culture fluid. When two or more samples showing similar ITS PCR patterns were present from the same patient, only one sample was included. Parentheses indicate the number of samples from positive blood culture fluid.

[b] Intense, sharp, and constantly observed fragments.

by ITS PCR pattern, and the 177 MRSA strains were divided into 14 types. Of the 40 ITS PCR types, three (aur10, aur11, and aur14) were distributed in both MSSA and MRSA strains. The remaining 37 types were homogenous with regard to the presence of the *mecA* gene, i.e., they included only MSSA or MRSA strains. The ITS PCR type aur22 was more frequent than the others, typing ~51% of all MRSA strains. Seven (4%) of 174 oxacillin-sensitive *S. aureus* isolates, determined by the agar diffusion method, had the *mecA* gene, and these strains belonged to type aur14 (five strains), aur19 (one strain), and aur31 (one strain). Three MSSA control strains tested, i.e., ATCC 29213, JCM2179, and ATCC 25923, belonged to types aur10, aur34, and aur38, respectively, and these strains were not included in Table 1.

ITS PCR patterns of non-*aureus* staphylococci. Electrophoretic analysis of the amplification products showed one or two major fragments for each sample, ranging in size from 289 to 644 bp (Table 2). Although two or more patterns were observed with *S. capitis* (four patterns), *S. lugdunensis* (three patterns), *S. caprae* (two patterns), *S. cohnii* (two patterns), *S. haemolyticus* (two patterns), and *S. saprophyticus* (two patterns), other species showed one ITS PCR pattern. All 15 *S. caprae* isolates showed identical ITS PCR patterns, but the pattern differed from that of the control *S. caprae* strain. Among the 35 *S. capitis* isolates from clinical materials, 17 (49%) belonged to type cap3, 13 (37%) belonged to type cap4, 4 (11%) belonged to type cap1, and 1 (3%) belonged to type cap2. Figure 2 shows the six representative ITS PCR patterns of clinically relevant non-*aureus* staphylococcal species. All of the species studied had unique ITS PCR patterns, although those of *S. epidermidis* and *S. sciuri* were very similar. However, these two species showed differences in the sizes of the first and second bands: 72 to 78 bp for *S. sciuri* and 82 to 89 bp for *S. epidermidis*. The ITS PCR patterns obtained for the control strains of the two *S. cohnii* subspecies were indistinguishable, and the same was also verified for the two *S. schleiferi* subspecies. On the other hand, the type strains of the two *S. capitis* subspecies showed different patterns.

Identification of staphylococcal strains from positive blood culture bottles by PCR-MGE. Of the 620 blood culture specimens tested, 288 were positive for staphylococci as determined by the conventional culture method. Of these 288 blood culture bottles, 280 were identified by PCR-MGE and included seven species: 114 isolates of *S. aureus*, 126 isolates of *S. epidermidis*, 14 isolates of *S. hominis*, 19 isolates of *S. capitis*, 3 isolates of *S. caprae*, 2 isolates of *S. haemolyticus*, and 2 isolates of *S. lugdunensis* (Tables 1 and 2). The ITS PCR patterns were the same as those observed for the culture isolates. The remaining eight bottles (3%) were not identified by PCR-MGE because they contained more than one species; *S. epidermidis* was detected mixed with *Enterococcus* spp. (two specimens), with *S. capitis* (one specimen), with *S. haemolyticus* (one specimen), and with a gram-negative rod (one specimen), and MSSA was detected mixed with gram-negative rods (two specimens) and with *Enterococcus faecium* (one specimen). No false-positive results were observed with the PCR-MGE method. With regard to species level identification of staphylococcus strains in blood culture bottles, the method showed a sensitivity of 97% and a specificity of 100%.

One hundred-fourteen blood culture bottles that were growth positive for only *S. aureus* showed 24 ITS PCR patterns. Twenty-one of these patterns were homogenous with regard to the presence of the *mecA* gene, i.e., they included only MSSA or MRSA strains. Therefore, the PCR-MGE method differentiated between MSSA and MRSA in 102 (89%) of the 114 culture bottles positive for *S. aureus*. Based on the ITS PCR patterns defined for culture isolates, this method had a sensitivity of 87% and a specificity of 89% for the detection of MRSA in blood culture bottles.

PCR-MGE analysis showed nine patterns among 166 blood cultures positive for only coagulase-negative staphylococci by culture. The most common species detected were *S. epidermidis* (126 strains), *S. capitis* (19 strains), and *S. hominis* (14 strains), which accounted for nearly 94% of the CNS strains

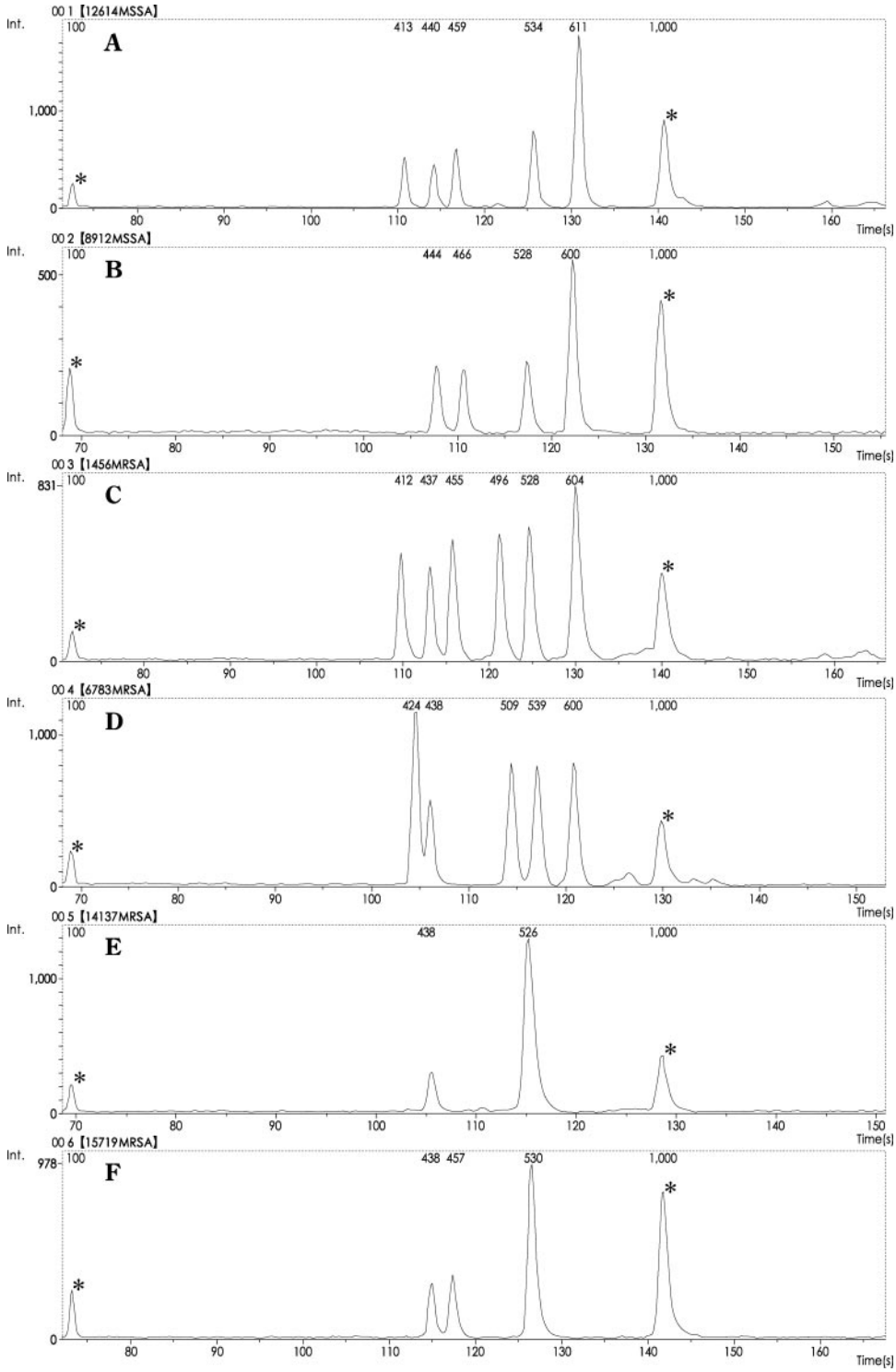

FIG. 1. Representative internal transcribed spacer PCR patterns of *S. aureus* strains. (A) MSSA K12614 (ITS PCR type, aur6); (B) MSSA K8912 (aur24); (C) MRSA K1456 (aur11); (D) MRSA K6783 (aur14); (E) MRSA K14137 (aur19); (F) MRSA K15719 (aur22). Asterisks indicate DNA size markers of 100 (left) and 1,000 (right) bp. Int., intensity.

isolated from blood culture. Two or more blood cultures positive for the same species (i.e., with the same ITS PCR pattern) in bacteremic episodes were obtained with *S. epidermidis* (33 episodes), *S. haemolyticus* (2 episodes), *S. capitis* (2 episodes), and *S. hominis* (1 episode).

Comparison of identification results by the genetic and phenotypic methods. The results of genotypic versus phenotypic identification for the 313 CNS strains isolated from clinical materials are shown in Table 3. Forty-one strains (13%) that showed discrepancies between identifications were identified

TABLE 2. ITS PCR patterns of non-*aureus Staphylococcus* strains

Species	Control strain	ITS PCR type	No. of DNA samples from clinical materials[a]	Mean sizes (bp) of fragments[b]
S. auricularis	GTC326 (ATCC 33753^T)	auc1		322 (±2.2), 415 (±2.1), 466 (±5.1), 487 (±4.2)
S. capitis subsp. *urealyticus*	GTC727 (ATCC 49326^T)	cap1	4 (1)	356 (±1.9), 408 (±3.1), 438 (±1.6), 538 (±4.6)
S. capitis subsp. *capitis*	GTC287 (ATCC 27840^T) ATCC 35661	cap2	1	355 (±4.5), 407 (±3.6), 436 (±2.2), 499 (±3.4)
S. capitis	K12464	cap3	13 (9)	352 (±6.3), 408 (±5.4), 438 (±4.0), 464 (±4.5), 611 (±6.5)
S. capitis	K332	cap4	17 (9)	413 (±4.8) 468 (±3.8), 475 (±4.2), 485 (±5.5), 640 (±6.5)
S. caprae	GTC378 (CCM3573^T)	car1		371 (±1.7), 420 (±1.8), 459 (±3.9), 485 (±2.4)
S. caprae	K469	car2	15 (3)	371 (±3.0), 421 (±2.7), 486 (±3.3)
S. cohnii subsp. *cohnii*	JCM2417 (ATCC 29974^T)	coh1		388 (±4.3), 454 (±3.6), 505 (±7.1), 526 (±3.0)
S. cohnii subsp. *urealyticum*	GTC728 (ATCC 49330^T)	coh1		391 (±5.3), 458 (±4.6), 501 (±4.3), 525 (±2.2)
S. cohnii	ATCC 35662	coh2		320 (±2.2), 416 (±3.0), 487 (±5.0)
S. epidermidis	ATCC 14990^T, ATCC 12228 ATCC 49134, ATCC 49461	epi1	171 (126)	374 (±5.4), 458 (±5.8), 521 (±7.7)
S. haemolyticus	GTC290 (ATCC 29970^T)	hae1	19 (2)	455 (±5.2), 519 (±4.4), 558 (±4.4)
S. haemolyticus	K541	hae2	1	434 (±4.1), 444 (±5.2), 455 (±5.1), 520 (±4.4)
S. hominis subsp. *hominis*	GTC485 (ATCC 27844^T)	hom1	24 (14)	411 (±4.9), 429 (±4.1), 497 (±5.6)
S. hyicus subsp. *hyicus*	JCM2423 (ATCC 11249^T)	hyi1	1	474 (±3.1), 522 (±4.6), 534 (±4.1)
S. intermedius	GTC266 (ATCC 29663^T)	int1		421 (±1.3), 471 (±5.9), 533 (±9.4)
S. lugdunensis	GTC458 (ATCC 43809^T)	lug1	2 (1)	548 (±4.6), 575 (±5.7), 615 (±6.2), 657 (±6.8)
S. lugdunensis	K15486	lug2	2 (1)	551 (±5.2), 633 (±5.1), 677 (6.0)
S. lugdunensis	K2342	lug3	1	644 (±5.5), 690 (±6.0)
S. saprophyticus	GTC265 (ATCC 15305^T) ATCC 49907	sap1	4	390 (±3.4), 455 (±2.9), 532 (±4.8)
S. saprophyticus	ATCC 49453	sap2		389 (±3.8), 405 (±2.4), 456 (±3.2), 521 (±4.6)
S. schleiferi subsp. *coagulans*	JCM7470 (ATCC 49545^T)	sch1		356 (±3.3), 436 (±2.9)
S. schleiferi subsp. *schleiferi*	GTC483 (ATCC 43808^T)	sch1		352 (±3.0), 434 (±3.9)
S. sciuri subsp. *sciuri*	ATCC 29060 GTC291 (ATCC 29062^T)	sci1		370 (±1.9), 445 (±3.4), 516 (±4.6)
S. simulans	GTC292 (ATCC 27848^T) ATCC 27851	sim1	2	344 (±4.7), 429 (±3.8), 487 (±3.9)
S. warneri	GTC293 (ATCC 27836^T) ATCC 49454	war1	3	440 (±2.5), 469 (±2.4), 496 (±3.4)
S. xylosus	GTC294 (ATCC 29971^T) ATCC 49148, ATCC 35663	xyl1	1	394 (±6.4), 462 (±5.4), 527 (±2.2)
Total			281 (166)	

[a] When two or more strains showing similar ITS PCR patterns were present from the same patient, only one strain was included. The parentheses indicate the number of DNA samples from positive blood culture fluid.

[b] Mean amplicon sizes were obtained from the results of five experiments for each control strain. The parentheses indicate standard deviation, and underlines indicate the most intense fragment.

correctly by the PCR-MGE method but not by the N-ID test SP-18. There was 100% agreement for phenotypic and genotypic identification methods for *S. auricularis*, *S. haemolyticus*, *S. hyicus*, *S. intermedius*, *S. lugdunensis*, *S. sciuri*, and *S. simulans* and 80% or more agreement for *S. capitis*, *S. caprae*, *S. epidermidis*, *S. saprophyticus*, and *S. warneri*. In contrast, only 12 of 25 *S. hominis* strains (48%) were identified correctly by the phenotypic method. The control *S. caprae* strain GTC378 (ITS PCR type car1) was positive for acid production from mannitol but negative for acid production from maltose. On the other hand, all 15 *S. caprae* isolates from clinical materials were positive for acid production from mannitol (77%) and maltose (100%).

Reproducibility testing. Two staphylococcal strains (*S. epidermidis* K13168 and *S. aureus* K9287) were tested 10 times by the PCR-MGE method to evaluate the variation caused by differences in DNA preparation, PCR, and electrophoresis runs. The sizes (means ± standard deviations) of PCR products from *S. epidermidis* were 375 ± 2.4, 456 ± 2.7, and 522 ± 3.0 bp, and those of MSSA K9287 (type aur23) were 449 ± 2.8, 471 ± 4.0, 540 ± 2.7, and 611 ± 5.1 bp. Thus, the standard deviation did not exceed 10 bp, indicating good reproducibility. Pattern reproducibility was ensured by comparing the ITS PCR patterns of five randomly selected strains 10 times, and these controls gave satisfactory results for the patterns.

DISCUSSION

Rapid identification of MRSA from blood culture bottles is important for the establishment of effective antibiotic therapy. In the present study, 147 (83%) of 177 MRSA strains were identified correctly as MRSA on the basis of ITS PCR patterns, and the remaining 30 MRSA strains were identified as *S. aureus* only. Similar results were reported by Dolzani et al. (4), who showed that two of eight ITS PCR types were heterogeneous with regard to methicillin susceptibility. Therefore, in addition to PCR-MGE analysis, detection of the *mecA* gene is necessary for identification of MRSA, as well as borderline resistant *S. aureus* (oxacillin MICs, 4 μg/ml). Although attempts to use PCR for the detection of *mecA* have been reported, it takes ~2 to 8 h to obtain results using the methods reported previously (10, 18, 19, 27, 34). For rapid detection of the *mecA* gene, we used *Z-Taq* polymerase to shorten the time for PCR, and we used the MGE procedure to detect PCR products instead of the laborious agarose gel electrophoresis procedure. Consequently, the overall turnaround times of the

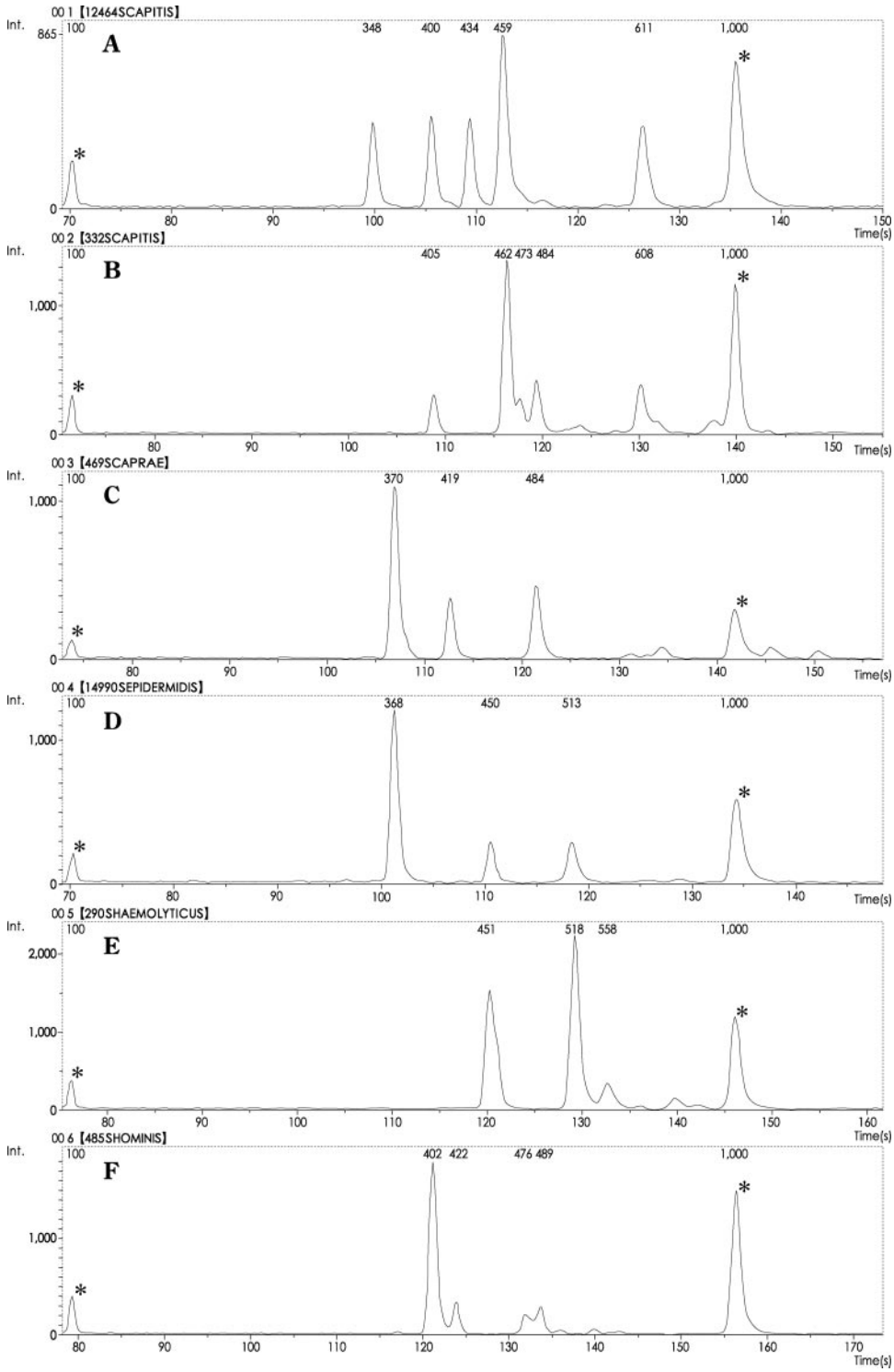

FIG. 2. Representative internal transcribed spacer PCR patterns of non-*aureus* staphylococcal strains. (A) *S. capitis* K12464 (ITS PCR type, cap3); (B) *S. capitis* K332 (cap2); (C) *S. caprae* K469 (car2); (D) *S. epidermidis* ATCC14990^T (epi1); (E) *S. haemolyticus* GTC290 (hae1); (F) *S. hominis* GTC485 (hom1). Asterisks indicate DNA size markers of 100 (left) and 1,000 (right) bp. Int., intensity.

PCR-MGE assay for the *mecA* gene were ~1 h with isolated colonies and 1.5 h with positive blood culture bottles.

The results of the *mecA* gene detection corresponded closely with those obtained by susceptibility testing. However, 7 (4%) of 172 oxacillin-susceptible strains had the *mecA* gene. Similar false-positive results were reported by other investigators (19, 27). Interestingly, five of seven *mecA*-positive and oxacillin-susceptible *S. aureus* strains belonged to ITS PCR type aur14. Therefore, oxacillin-susceptible *S. aureus* strains belonging to type aur14 should be tested for *mecA* and/or penicillin-binding

TABLE 3. Comparison of results of identification by ITS PCR analysis and using the commercially available identification kit (N-ID test SP-18) for 313 non-*aureus Staphylococcus* strains

Species (total no. of isolates examined[a])	ITS PCR pattern(s) (no. of isolates)	No. (%) of isolates obtained by the kit			
		Correctly identified	Identified with low discrimination[b]	Incorrectly identified	Not identified
S. auricularis (1)	auc1 (1)	1 (100)			
S. capitis (43)	cap1 (5) cap2 (3) cap3 (14) cap4 (21)	37 (88)	2 (5)	3 (5); *S. lugdunensis*, *S. warneri*, *S. cohnii*	1 (2)
S. caprae (17)	car1 (1) car2 (16)	16 (94)		1 (6); *S. capitis*	
S. cohnii (4)	coh1 (3) coh2 (1)	3 (75)		1 (25); *S. caprae*	
S. epidermidis (170)	epi1 (170)	154 (90)	10 (6)	5 (3); *S. caprae*, *S. capitis*, *S. capitis*, *S. hominis*, *S. warneri*	1 (1)
S. haemolyticus (21)	hae1 (20) hae2 (1)	21 (100)			
S. hominis (25)	hom1 (25)	12 (48)	8 (32)	3 (12); *S. lugdunensis*, *S. lugdunensis*, *S. warneri*	2 (8)
S. hyicus (2)	hyi1 (2)	2 (100)			
S. intermedius (1)	int1 (1)	1 (100)			
S. lugdunensis (5)	lug1 (2) lug2 (2) lug3 (1)	5 (100)			
S. saprophyticus (7)	sap1 (6) sap2 (1)	6 (86)			1 (24)
S. schleiferi (2)	sch1 (1) sch2 (1)	1 (50)			1 (50)
S. sciuri (2)	sci1 (2)	2 (100)			
S. simulans (4)	sim1 (4)	4 (100)			
S. warneri (5)	war1 (5)	4 (80)		1 (20); *S. hominis*	
S. xylosus (4)	xyl1 (4)	3 (75)		1 (25); *S. delphini*	

[a] Number of isolates identified using a combination of the N-ID test SP-18 and PCR-MGE. When discrepancies occurred between identifications, partial 16S rRNA gene sequence analysis was performed, as described in Materials and Methods.
[b] Two or more possible species-level identities for the strain.

protein 2′. In our laboratory, simultaneous detection of the *mecA* gene and rRNA gene spacer length polymorphism from GPCC-positive blood culture bottles by multiplex PCR are under way, as the product of the *mecA* gene has no effect on the ITS PCR patterns of staphylococcal strains.

Pulsed-field gel electrophoresis (PFGE) is the "gold standard" technique for MRSA typing due to its high discriminatory power and excellent reproducibility (33). However, PFGE is a time-consuming procedure that requires specifically trained personnel and sophisticated equipment. Faster molecular typing approaches based on DNA amplification by PCR have been published over the last decade (33). The possibility of using polymorphisms in the spacer regions between 16S and 23S rRNA genes for typing *S. aureus* has been evaluated (4, 8). Gurtler and Barrie (7) characterized the 16S-23S rRNA gene spacer sequences of *S. aureus* and reported that the variations in 16S-23S spacer length were due, in part, to the number and type of $tRNA^{Ala}$ or $tRNA^{Ile}$ that the strain may contain. They divided the 48 MSSA strains into 26 ITS PCR patterns and the 274 MRSA strains into 9 patterns. Of these MRSA strains, 266 (97%) belonged to only two patterns, suggesting the limited usefulness of this method for epidemiological study of MRSA infections. In the present study, 55% of MRSA strains tested belonged to the ITS PCR type aur22. The ability to type MRSA isolates by the ITS PCR method has been shown to be inferior to PFGE (15). In the present study, however, the PCR-MGE method seemed a practical tool to evaluate nosocomial MRSA infections, as the ITS PCR assay has good discriminatory power, particularly for MSSA, and the method is also capable of discriminating to some extent among MRSA strains. In addition, as our method provides rapid information (within 1 h) from isolated colonies, it may provide very useful information as the first approach in investigating an epidemic outbreak.

With regard to identification of CNS, it has been reported that phenotypic identification of some CNS species, particularly those isolated infrequently, was difficult because no phenotypic criteria were available to unequivocally distinguish them (6, 38). Kawamura et al. (12) reported 80 isolates recognized as *S. caprae* by DNA-DNA hybridization that were initially misidentified as *S. haemolyticus*, *S. warneri*, *S. hominis*, and *S. epidermidis* by conventional methods or commercial kits. Even with automated systems, there is clearly room for improvement in identifying some CNS strains, particularly *S. hominis* (31).

Furthermore, phenotypic identification systems require 24 to 48 h to provide results after pure cultures are obtained. On the other hand, identification of staphylococci by the ITS PCR method has been studied with considerable success (11, 20, 28), with the exception of the report by Couto et al. (3). They studied 617 isolates of *Staphylococcus* strains belonging to 29 species by ITS PCR followed by agarose gel electrophoresis and reported that some staphylococcal strains showed very similar ITS PCR patterns, i.e., *S. saprophyticus*, *S. cohnii*, *S. gallinarum*, *S. xylosus*, *S. lentus*, *S. equorum*, and *S. chromogenes*. In the present study, however, species-specific patterns of *S. saprophyticus*, *S. cohnii*, and *S. xylosus* were recognized. On the other hand, the PCR-MGE assay did not allow discrimination of subspecies within a species, except for *S. capitis*.

In addition to *S. aureus*, which was the most heterogeneous species among the *Staphylococcus* strains examined, CNS strains,

such as *S. capitis*, *S. caprae*, *S. lugdunensis*, and *S. saprophyticus*, were also shown to be heterogeneous in the present study. Of these strains, type car1 from *S. caprae* and type sap2 from *S. saprophyticus* were represented by only one control strain. Two different groups of *S. caprae* have been proposed based on the results of biochemical tests (37), ribotyping (37), and DNA-DNA hybridization (12). The results of a PCR-MGE assays with *S. caprae* strains showed that all 15 *S. caprae* strains obtained from human clinical specimens had identical ITS PCR patterns, but this pattern differed from that of the control strain isolated from goat milk, supporting previous reports that the *S. caprae* species contains two different evolutionary groups (12, 37). Random amplification of polymorphic DNA analysis (1) and PFGE (1, 29) have both been shown to be able to determine the species of *Staphylococcus* and type of *S. epidermidis* and *S. haemolyticus*. In the present study, only one ITS PCR pattern was demonstrated in *S. epidermidis* strains. These discrepancies may have been due to the low discriminatory power of the PCR-MGE method.

Our results regarding the distribution of *Staphylococcus* species from blood cultures were similar to those reported previously; the CNS species most often isolated from blood cultures were *S. epidermidis*, *S. haemolyticus*, *S. capitis*, and *S. hominis* (13, 38). Although *S. caprae* was reported as the third most common CNS species isolated from clinical materials (12), the species was isolated from blood cultures at a frequency of only 1.9% in the present study, whereas *S. epidermidis*, *S. capitis*, and *S. hominis* were the species isolated most frequently from blood cultures. However, two or more positive blood cultures for the same species were found in only 1 (7%) of 14 *S. hominis* bacteremic episodes and 2 (11%) of 19 *S. capitis* bacteremic episodes. In contrast, *S. epidermidis* strains were isolated from successive blood cultures in 33 (26%) of 126 *S. epidermidis* bacteremic episodes. Our results support the finding that *S. epidermidis* is the most important species among CNS strains, although other CNS species are responsible for bloodstream infections (14).

In a recent study, Munson et al. (21) found that the most important information provided by the clinical microbiology laboratory with respect to antimicrobial treatment of bloodstream infections was the initial phone call reporting the results of gram staining. Providing a PCR-MGE result at the initial contact with the physician could result in a rapid and accurate diagnostic strategy for bloodstream staphylococcal infections. However, due to the inability of our procedure to identify microorganisms when several are present in the same specimen, further studies are needed with regard to polymicrobial infections. In conclusion, accurate identification of staphylococcal strains from positive blood culture bottles by the PCR-MGE method can be performed easily within 1.5 h in a routine clinical laboratory. We are generating a database for use in the PCR-MGE method that can be applied comprehensively to pathogenic bacteria.

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REFERENCES

1. **Burnie, J. P., M. Naderi-Nasab, K. W. Loudon, and R. C. Matthews.** 1997. An epidemiological study of blood culture isolates of coagulase-negative staphylococci demonstrating hospital-acquired infection. J. Clin. Microbiol. **35:**1746–1750.
2. **Chapin, K., and M. Musgnug.** 2003. Evaluation of three rapid methods for direct identification of *Staphylococcus aureus* from positive blood cultures. J. Clin. Microbiol. **41:**4324–4327.
3. **Couto, I., S. Pereira, M. Miragaia, I. S. Sanches, and H. de Lencastre.** 2001. Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. J. Clin. Microbiol. **39:**3099–3103.
4. **Dolzani, L., E. Tonin, C. Lagatolla, and C. Monti-Bragadin.** 1994. Typing of *Staphylococcus aureus* by amplification of the 16S-23S rRNA intergenic spacer sequences. FEMS Microbiol. Lett. **119:**167–174.
5. **Fujita, S., Y. Senda, S. Nakaguchi, and T. Hashimoto.** 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J. Clin. Microbiol. **39:**3617–3622.
6. **Grant, C. E., D. L. Sewell, M. Pfaller, R. V. Bumgardner, and J. A. Williams.** 1994. Evaluation of two commercial systems for identification of coagulase-negative staphylococci to species level. Diagn. Microbiol. Infect. Dis. **18:**1–5.
7. **Gurtler, V., and H. D. Barrie.** 1995. Typing of *Staphylococcus aureus* by PCR-amplification of variable-length 16S–23S rDNA spacer regions: characterization of spacer sequences. Microbiology **141:**1255–1265.
8. **Gurtler, V., and V. A. Stanisich.** 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology **142:**3–16.
9. **Hiraishi, A.** 1992. Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. Lett. Appl. Microbiol. **15:**210–213.
10. **Jaffe, R. I., J. D. Lane, S. V. Albury, and D. M. Niemeyer.** 2000. Rapid extraction from and direct identification in clinical samples of methicillin-resistant staphylococci using the PCR. J. Clin. Microbiol. **38:**3407–3412.
11. **Jensen, M. A., J. A. Webster, and N. Straus.** 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl. Environ. Microbiol. **59:**945–952.
12. **Kawamura, Y., X.-G. Hou, F. Sultana, K. Hirose, M. Miyake, S. Shu, and T. Ezaki.** 1998. Distribution of *Staphylococcus* species among human clinical species and emended description of *Staphylococcus caprae*. J. Clin. Microbiol. **36:**2038–2042.
13. **Kleeman, K. T., T. L. Bannerman, and W. E. Kloos.** 1993. Species distribution of coagulase-negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. J. Clin. Microbiol. **31:**1318–1321.
14. **Kloos, W. E., and T. L. Bannerman.** 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. **7:**117–140.
15. **Kumari, D. N. P., V. Keer, P. M. Hawkey, P. Parnell, N. Joseph, J. F. Richardson, and B. Cookson.** 1997. Comparison and application of ribosomal spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. **35:**881–885.
16. **Levi, K., and K. J. Towner.** 2003. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in blood with the EVIGENE MRSA detection kit. J. Clin. Microbiol. **41:**3890–3892.
17. **Maes, N., Y. De Gheldre, R. De Ryck, M. Vaneechoutte, H. Meugnier, J. Etienne, and M. J. Struelens.** 1997. Rapid and accurate identification of *Staphylococcus* species by rRNA intergenic spacer length polymorphism analysis. J. Clin. Microbiol. **35:**2477–2481.
18. **Maes, N., J. Magdalena, S. Rottiers, Y. De Gheldre, and M. J. Struelens.** 2002. Evaluation of a triplex PCR assay to discriminate *Staphylococcus aureus* from coagulase-negative staphylococci and determine methicillin resistance from blood cultures. J. Clin. Microbiol. **40:**1514–1517.
19. **Mason, W. J., J. S. Blevins, K. Beenken, N. Wibowo, N. Ojha, and M. Smeltzer.** 2001. Multiplex PCR protocol for the diagnosis of staphylococcal infection. J. Clin. Microbiol. **39:**3332–3338.
20. **Mendoza, M., H. Meugnier, M. Bes, J. Etienne, and J. Freney.** 1998. Identification of *Staphylococcus* species by 16S-23S rDNA intergenic spacer PCR analysis. Int. J. Syst. Bacteriol. **48:**1049–1055.
21. **Munson, E. L., D. J. Diekema, S. E. Beekmann, K. C. Chapin, and G. V. Doern.** 2003. Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. J. Clin. Microbiol. **41:**495–497.
22. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests, 6th ed. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
23. **Oliveira, K., G. W. Procop, D. Wilson, J. Coull, and H. Stender.** 2002. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence *in situ* hybridization using PNA probes. J. Clin. Microbiol. **40:**247–251.
24. **Poyart, C., G. Quesne, C. Boumaila, and P. Trieu-Cuot.** 2001. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. J. Clin. Microbiol. **39:**4296–4301.

25. **Qian, Q., Y. Tang, C. P. Kolbert, C. A. Torgerson, J. G. Hughes, E. A. Vetter, W. S. Harmsen, S. O. Montogomery, F. R. Cockerill III, and D. H. Persing.** 2001. Direct identification of bacteria from positive blood cultures by amplification and sequencing of the 16S rRNA gene: evaluation of BACTEC 9240 instrument true-positive and false-positive results. J. Clin. Microbiol. **39:**3578–3582.
26. **Saruta, K., T. Matsunaga, M. Kono, S. Hoshina, S. Ikawa, O. Sakai, and K. Machida.** 1997. Rapid identification and typing of *Staphylococcus aureus* by nested PCR amplified ribosomal DNA spacer region. FEMS Lett. **146:** 271–278.
27. **Shimaoka, M., M. Yoh, A. Segawa, Y. Takarada, K. Yamamoto, and T. Honda.** 1994. Development of enzyme-labeled oligonucleotide probe for detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **32:**1866–1869.
28. **Shittu, A., J. Lin, D. Morrison, and D. Kolawole.** 2004. Isolation and molecular characterization of multiresistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. J. Med. Microbiol. **53:**51–55.
29. **Snopkova, S., F. Gotz, J. Doskar, and S. Rosypal.** 1994. Pulsed-field gel electrophoresis of the genomic restriction fragments of coagulase-negative staphylococci. FEMS Microbiol. Lett. **124:**131–140.
30. **Souvenir, D., D. E. Anderson, Jr., S. Palpant, H. Mroch, S. Askin, J. Anderson, J. Claridge, J. Eiland, C. Malone, M. W. Garrison, P. Watson, and D. M. Campbell.** 1998. Blood culture for coagulase-negative staphylococci: antisepsis, pseudobacteremia, and therapy of patients. J. Clin. Microbiol. **36:** 1923–1926.
31. **Spanu, T., M. Sanguinetti, D. Ciccagione, T. D'Inzeo, L. Romano, F. Leone, and G. Fadda.** 2003. Use of the VITEK 2 system for rapid identification of clinical isolates of staphylococci from bloodstream infections. J. Clin. Microbiol. **41:**4259–4263.
32. **Speers, D. J., T. R. Olma, and G. L. Gilbert.** 1998. Evaluation of four methods for rapid identification of *Staphylococcus aureus* from blood cultures. J. Clin. Microbiol. **36:**1032–1034.
33. **Stranden, A., R. Frei, and A. F. Widmer.** 2003. Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? J. Clin. Microbiol. **41:**3181–3186.
34. **Tan, T. Y., S. Corden, R. Barnes, and B. Cookson.** 2001. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. **39:**4529–4531.
35. **Turenne, C. Y., E. Witwicki, D. J. Hoban, J. A. Karlowsky, and A. M. Kabani.** 2000. Rapid identification of bacteria from positive blood cultures by fluorescence-based PCR-single-strand conformation polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol. **38:**513–520.
36. **Ubukata, K., S. Nakaguchi, A. Nitta, A. Yamane, S. Kawakami, M. Sugiura, and M. Konno.** 1992. Rapid detection of the *mecA* gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain reaction products. J. Clin. Microbiol. **30:**1728–1733.
37. **Vandenesch, F., S. Eykyn, M. Bes, H. Meugnier, J. Fleurette, and J. Etienne.** 1995. Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. J. Clin. Microbiol. **33:**888–892.
38. **Weinstein, M. P., S. Mirrett, L. V. Pelt, M. McKinnon, B. L. Zimmer, W. Kloos, and L. B. Reller.** 1998. Clinical importance of identifying coagulase-negative staphylococci isolated from blood cultures: evaluation of MicroScan rapid and dried overnight gram-positive panels versus a conventional reference method. J. Clin. Microbiol. **36:**2089–2092.