JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2003, p. 1219–1224 Vol. 41, No. 3
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.3.1219–1224.2003

Five-Test Simple Scheme for Species-Level Identification of Clinically Significant Coagulase-Negative Staphylococci

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Received 5 August 2002/Returned for modification 23 October 2002/Accepted 23 November 2002

A working scheme developed in our laboratory for identification (by species group and species) of coagulase-negative staphylococci (CNS) was evaluated with 201 consecutive isolates and then validated by using the reference method of Kloos and Schleifer (W. E. Kloos and K. H. Schleifer, J. Clin. Microbiol. 1:82–88, 1975). This five-test simple scheme (referred to here as the simple scheme) combines the novobiocin susceptibility test with tests for urease, pyrrolidonyl arylamidase, ornithine decarboxylase, and aerobic acid from mannose. The addition of one or two tests within a particular species group could then positively identify the isolate. Two commercial systems, Staph-Zym (Rosco) and API-Staph (bioMérieux), along with results obtained by using Rosco diagnostic tablets (nongrowth tests), were also compared with the reference method. One isolate could not be identified even by the reference method. Of the remaining 200 strains, 191 (95.5%) strains were correctly identified with Staph-Zym and 171 strains (85.5%) were correctly identified with API-Staph. The most frequent clinical CNS species isolated were *Staphylococcus epidermidis* (50.5%), *S. haemolyticus* (18.5%), *S. saprophyticus* subsp. *saprophyticus* (16.0%), *S. lugdunensis* (6.0%), and *S. warneri* (2.5%). The simple scheme validated with the reference method has demonstrated an excellent correlation in the identification of the three most frequent species isolated: *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus* subsp. *saprophyticus*. With the simple scheme, identification of CNS was possible within 24 h after the enzymatic tests were used, whereas up to 72 h is necessary for the growth tests. This methodology would be very useful in any clinical microbiology laboratory for the presumptive identification of CNS species groups and species.

Many studies have been initiated since 1958 as a result of the growing recognition that coagulase-negative staphylococci (CNS) are clinically important (28) in an attempt to classify these organisms (1, 21). In the 1970s, W. E. Kloos and K. H. Schleifer determined the natural relationships of CNS based on systematic studies that allowed these researchers to resolve and characterize different CNS species (14). Species were identified based on an ensemble of morphological, physiological, and biochemical characteristics, antibiotic susceptibility patterns, and cell wall composition.

In the last decade, molecular studies contributed to a notable progress in the classification of staphylococci and in the development of methods for identifying them at the genus, species, subspecies, and strain levels (5). Although *Staphylococcus epidermidis* accounts for most CNS infections, many other species have been identified in association with human infections (3, 27, 29). The importance of CNS as major nosocomial pathogens is mainly associated with prosthetic and indwelling devices such as prosthetic joints, heart valves, pacemaker implants, ventricular-peritoneal shunts, and peritoneal dialysis catheters and with specific clinical disease settings such as postoperative sternotomy, wound infections, urinary tract infections, chronic prostatitis, native valve endocarditis, and septicemia in immunocompromised patients (10, 22, 24). CNS are nosocomial pathogens associated with multiple antimicrobial resistance mechanisms including, in particular, methicillin resistance (25). CNS are reservoirs of antimicrobial resistance determinants; hence, rapid and reliable identification is necessary in order to predict early in the course of infection the potential pathogenicity or antibiotic susceptibility of each clinical isolate (5).

CNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures, or determine the cause of specific infections. The reference method of Kloos and Schleifer is too cumbersome and time-consuming to use routinely, and most commercial systems, unfortunately, were designed for the identification of all known CNS species (i.e., clinical, veterinary, and alimentary isolates) and thus are not very specific. Other systems do not have enough strain information in the associated databases and so are not accurate to the species level. In fact, some systems have good agreement within themselves but show highly variable species results when compared to one another (17).

We sought here to validate the accuracy, by using the reference method, of a simple, rapid, and easily performed scheme developed in our laboratory to identify CNS species or species groups (i.e., a group approach) that can be utilized by most clinical laboratories. This five-test simple scheme (referred to here as the simple scheme) of presumptive identification is concentrated on the species or species groups encountered among our clinical isolates: the *S. epidermidis* group (i.e., *S. epidermidis*, *S. capitis* subsp. *ureolyticus*, and *S. caprae*), the *S. haemolyticus* group (*S. haemolyticus*, *S. auricularis*, and *S. casseolyticus*), the *S. saprophyticus* group (*S. saprophyticus* subsp. *saprophyticus*, and *S. hominis* subsp. *novobiosepticus*),

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the *S. warneri* group (*S. warneri*, and *S. hominis* subsp. *hominis*), the *S. cohnii* group (*S. xylosus* and *S. cohnii* subsp. *urealyticum*), *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi*, *S. capitis* subsp. *capitis*, *S. simulans*, and *S. cohnii* subsp. *cohnii*. The species groups were defined according to their biochemical reactions rather than their phylogenetic relationships. The addition of one or two tests within each particular species group could complete the strain identification, even in the case of the most infrequently isolated species. We also compared two commercial identification methods, Staph-Zym (Rosco, Taastrup, Denmark) and API-Staph (bioMérieux, Marcy l'Étoile, France), along with the results obtained by using Rosco diagnostic tablets (nongrowth tests), with the reference method.

MATERIALS AND METHODS

Strains. A total of 201 consecutive and clinically significant strains of CNS were isolated between July 1999 and July 2001 in the Department of Microbiology, Instituto de Investigaciones Médicas A. Lanari, Universidad de Buenos Aires, Buenos Aires, Argentina. The strains were collected from blood cultures in cases of significant bacteremia with ≥2 blood samples with the same strain ($n = 26$), central venous catheters ($n = 22$), deep wounds ($n = 30$), peritoneal dialysis fluids ($n = 19$), cerebrospinal fluids ($n = 2$), external otitis ($n = 2$), urine samples (present in pure culture at $\geq 10^5$ CFU/ml; $n = 72$), and other sources ($n = 28$). CNS isolates were frozen at −70°C in 15% glycerol broth until the study. The organisms were tested by Gram staining, catalase and benzidine tests, and the determination of anaerobic acid production from glucose. Bacitracin (0.04 U), furazolidone (100 μg), and erythromycin (0.4 μg/ml) susceptibilities were determined to exclude *Micrococcus*, *Planococcus*, and *Stomatococcus* spp. (15). A coagulase test (rabbit plasma tube test, read after 4 and 24 h) and a test for anaerobic acid from mannitol (6) were performed to exclude *Staphylococcus aureus* and other coagulase-positive species.

Reference strains. The reference strains used in the present study were *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, *S. saprophyticus* ATCC 15305, *S. warneri* ATCC 49454, *S. lugdunensis* ATCC 700328, *S. capitis* ATCC 35661, *S. cohnii* ATTC 35662, *S. sciuri* ATCC 29060, *S. xylosus* 29971, and *S. simulans* ATCC 27851.

Identification methods. (i) Reference method. The 201 CNS isolates and 10 reference strains were identified to the species level by the conventional method of Kloos and Schleifer (14) based on colony size and pigment; anaerobic and aerobic growth; the presence of clumping factor, hemolysins, oxidase, ornithine decarboxylase, urease, and pyrrolidonyl arylamidase (PYR); novobiocin and polymyxin B resistance; arginine utilization; acetoin production; nitrate reduction; esculin hydrolysis; and the generation of acid (aerobically) from D-trehalose, D-mannitol, D-mannose, D-turanose, D-xylose, D-cellobiose, L-arabinose, maltose, α-lactose, sucrose, *N*-acetylglucosamine, and raffinose. Alkaline phosphatase activity was determined by a previously published modification of the Pennock and Huddy technique (7). β-Galactosidase activity was determined by using 2-naphthol-β-D-galactopyranoside and Fast Blue BB (15), and β-glucuronidase and β-glucosidase activities were determined by commercial rapid identification tests.

(ii) Staph-Zym. The Staph-Zym method consists of 10 tests for β-glucosidase, β-galactosidase, β-glucuronidase, alkaline phosphatase, urease, pyrrolidonyl amonipeptidase, arginine dihydrolase, nitrate reduction, and acid from trehalose and maltose. Susceptibilities to furazolidone, polymyxin, novobiocin, and deferoxamine were also determined. The manufacturer's instructions were followed to interpret the results. Fosfomycin susceptibility and other additional tests were sometimes also required. All drugs were supplied as Neo-Sensitabs from Rosco.

(iii) API-Staph. API-Staph consists of a strip containing dehydrated test substrates in individual microtubes. The tests are reconstituted by adding an aliquot of the API-Staph medium to each tube inoculated with the strain to be studied. The tests included acid production from D-glucose, D-trehalose, D-mannitol, D-mannose, xylose, maltose, lactose, sucrose, *N*-acetylglucosamine, raffinose, D-fructose, D-melibiose, xylitol, and α-methyl-glucosamine; nitrate reduction; and alkaline phosphatase, arginine dihydrolase, urease, and acetoin production. The manufacturer's instructions were followed to interpret the results. All drugs were supplied from bioMérieux, Buenos Aires, Argentina.

(iv) Simple scheme. The simple scheme is a rapid and inexpensive working scheme for the identification of the CNS species most commonly isolated in clinical samples. The scheme combines one susceptibility test, the novobiocin resistance test, with tests for urease, PYR, ornithine decarboxylase, and aerobic acid from mannose. These tests were performed according to the reference method (15), and the urease, ornithine decarboxylase, and mannose tests were also performed by nongrowth methods with incubation for 4 h up to 18 to 24 h. Only one or two additional tests were used to resolve each species group: trehalose and mannitol for the *S. epidermidis* group, acetoin production and lactose for the *S. haemolyticus* group, trehalose for the *S. saprophyticus* group, β-glucosidase and/or anaerobic thioglycolate growth for the *S. warneri* group, and xylose for the *S. cohnii* group (Table 1). These analyses were performed by using growth and nongrowth tests to confirm some species and to verify others that are much less frequently isolated in clinical samples.

TABLE 1. Presumptive identification of CNS by simple scheme[a]

Species	Novobiocin (5 μg)	Urease	Acid from D-mannose	PYR	Ornithine decarboxylase
More frequently isolated					
S. epidermidis group[b]	S	+	+	−	d
S. haemolyticus group[c]	S	−	−	+	−
S. saprophyticus group[d]	R	+	−	−	−
S. lugdunensis	S	d	+	+	+
S. warneri group[e]	S	+	−	−	−
S. schleiferi subsp. *schleiferi*	S	−	+	+	−
Less frequently isolated					
S. simulans	S	+	d	+	−
S. capitis subsp. *capitis*	S	−	+	−	−
S. cohnii subsp. *cohnii*	R	−	d	−	−
S. cohnii group[f]	R	+	+	d	−

[a] +, Positive results; −, negative results; d, positive or negative results; R, resistant, S, susceptible.
[b] When ornithine decarboxylase is negative: trehalose negative and mannitol negative, *S. epidermidis* is confirmed; trehalose positive, mannitol negative, *S. caprae* is suspected; trehalose negative, mannitol positive, *S. capitis* subsp. *ureolyticus* is suspected.
[c] Acetoin production positive, *S. haemolyticus* is confirmed; acetoin production negative, lactose negative, *S. auricularis* is suspected; acetoin production negative, lactose positive, *S. casseolyticus* is suspected.
[d] Trehalose positive, *S. saprophyticus* subsp. *saprophyticus* is confirmed; trehalose negative, *S. hominis* subsp. *novobiosepticus* is suspected.
[e] β-Glucosidase positive, anaerobic growth positive, *S. warneri* is confirmed; β-glucosidase negative, anaerobic growth negative, *S. hominis* subsp. *hominis* is suspected.
[f] Xylose positive, *S. xylosus* is suspected; xylose negative, *S. cohnii* subsp. *urealyticum* is suspected.

(v) Acid production. Acid production from mannitol, mannose, arabinose, lactose, sucrose, raffinose, and ornithine decarboxylase was also tested by using Rosco diagnostic tablets, with incubation for 4 h up to 18 to 24 h, and the results were compared to those obtained by the reference method.

Interpretation of results. Identifications obtained by the two commercial systems and by the simple scheme were compared to those obtained by the reference method. When discrepant results were obtained, the isolate was then retested by the four systems to exclude technical errors. A different result (compared to the one obtained by the reference method) obtained repeatedly in a given system was considered a misidentification.

RESULTS

All reference strains were correctly identified by the four methods except for *S. sciuri* subsp. *sciuri* (ATCC 29060), which was misidentified as *S. cohnii* subsp. *cohnii* by both the Staph-Zym method and the simple scheme. This species is not part of the Staph-Zym human index, and the simple scheme was designed only for the most clinical significant *Staphylococcus* species. Of 201 strains, 200 (99.5%) were identified by the reference method; only one isolate, obtained from a central venous catheter, could not be identified. This strain, which was novobiocin resistant, could be considered *S. cohnii* subsp. *cohnii* or

TABLE 2. Frequency of CNS isolation from clinical specimens

Species	No. (%)	No. of positive clinical specimens from[a]:							
		Blood	Catheters	Wounds	PDF	CSF	Otitis	Urine	Other sources
S. epidermidis	101 (50.5)	16	19	11	12	1	2	26	14
S. haemolyticus	37 (18.5)	3	1	7	4	1		14	7
S. saprophyticus subsp. *saprophyticus*	32 (16.0)	1						30	1
S. lugdunensis	12 (6.0)	2		6	1			1	2
S. warneri	5 (2.5)	1		1	1				2
S. capitis subsp. *ureolyticus*	3 (1.5)	1	1	1					
S. simulans	3 (1.5)			1	1			1	
S. schleiferi subsp. *schleiferi*	2 (1.0)			2					
S. sciuri subsp. *sciuri*	2 (1.0)		1	1					
S. hominis subsp. *hominis*	1 (0.5)	1							
S. capitis subsp. *capitis*	1 (0.5)								1
S. cohnii subsp. *urealyticum*	1 (0.5)	1							
Total	200 (100.0)	26	22	30	19	2	2	72	27

[a] PDF, peritoneal dialysis fluid; CSF, cerebrospinal fluid.

S. kloosii, but it had a variety of aberrant characters for each option. None of the methods could identify it, and it was excluded from the study. Therefore, 200 strains were analyzed.

Table 2 shows the frequencies of isolation from the clinical specimens, and Table 3 presents the CNS species identifications obtained by the two commercial systems compared to those obtained by the reference method.

Of 200 strains, 191 (95.5%) strains were correctly identified with Staph-Zym and 171 strains (85.5%) were correctly identified with API-Staph. *S. epidermidis* isolates were correctly identified by the two methods; only 1 of 101 of these isolates was misidentified by Staph-Zym, and only 3 of these isolates were misidentified by API-Staph. Of 37 *S. haemolyticus* isolates, 1 was misidentified by Staph-Zym, whereas the API-Staph method failed to identify 6 isolates and misidentified 4 isolates. The Staph-Zym method correctly identified 32 *S. saprophyticus* subsp. *saprophyticus* isolates, whereas the API-Staph method failed to identify 2 isolates and misidentified 7 isolates. Both methods correctly identified 12 *S. lugdunensis* isolates, except for 1 isolate that was misidentified by Staph-Zym and 1 isolate that was not identified by API-Staph. Of five *S. warneri* strains, one was misidentified by Staph-Zym and one was not identified by API-Staph. Three *S. capitis* subsp. *ureolyticus* isolates were correctly identified by Staph-Zym and were not

TABLE 3. Comparison of CNS species identification by two comercial systems with identification by the reference method

Species	No of isolates identified by the reference method (%)	No. of isolates CI, UI, or MI[a] by:					
		Staph-Zym			API-Staph		
		CI	UI	MI	CI	UI	MI
S. epidermidis	101 (50.5)	100	0	1 (*S. hominis*)	98	0	2 (*S. xylosus*), 1 (*S. capitis*)
S. haemolyticus	37 (18.5)	36	0	1 (*S. epidermidis*)	27	6	1 (*S. epidermidis*), 1 (*S. hominis*), 1 (*S. sciuri*), 1 (*S. saprophyticus*)
S. saprophyticus subsp. *saprophyticus*	32 (16.0)	32	0	0	23	2	1 (*S. hominis*), 4 (*S. xylosus*), 1 (*S. aureus*), 1 (*S. warneri*)
S. lugdunensis	12 (6.0)	11	0	1 (*S. warneri*)	11	1	0
S. warneri	5 (2.5)	4	0	1 (*S. hominis*)	4	1	0
S. capitis subsp. *ureolyticus*	3 (1.5)	3	0	0	0	3	0
S. simulans	3 (1.5)	2	0	1 (*S. lugdunensis*)	1	1	1 (*S. epidermidis*)
S. schleiferi subsp. *schleiferi*	2 (1.0)	1	1	0	2	0	0
S. sciuri subsp. *sciuri*	2 (1.0)	0	0	2 (*S. cohnii* subsp. *cohnii*)	2	0	0
S. hominis subsp. *hominis*	1 (0.5)	1	0	0	1	0	0
S. capitis subsp. *capitis*	1 (0.5)	0	1	0	1	0	0
S. cohnii subsp. *urealyticum*	1 (0.5)	1	0	0	1	0	0
Total no. (% identified)	200 (100.0)	191 (95.5)	2 (1.0)	7 (3.5)	171 (85.5)	14 (7.0)	15 (7.5)

[a] CI, correctly identified; UI, unidentified; MI, misidentified.

TABLE 4. Results obtained by the simple scheme that were validated by the reference method

Group, species, or subspecies identified by simple scheme	No. of isolates identified	Species or subspecies identified by reference method	No. of isolates identified	Simple scheme plus additional tests (no. of isolates identified)
S. epidermidis group	104	*S. epidermidis*	101	101
		S. capitis subsp. *ureolyticus*	3	3
		S. caprae	0	0
S. haemolyticus group	37	*S. haemolyticus*	37	37
		S. auricularis	0	0
		S. casseolyticus	0	0
S. saprophyticus group	32	*S. saprophyticus* subsp. *saprophyticus*	32	32
		S. hominis subsp. *novobiosepticus*	0	0
S. warneri group	3[a]	*S. warneri*	5	2
		S. hominis subsp. *hominis*	1	1
S. cohnii group	1	*S. cohnii* subsp. *urealyticum*	1	1
		S. xylosus	0	0
S. lugdunensis	12	*S. lugdunensis*	12	12
S. simulans	6[a]	*S. simulans*	3	6
S. schleiferi subsp. *schleiferi*	2	*S. schleiferi* subsp. *schleiferi*	2	2
S. capitis subsp. *capitis*	1	*S. capitis* subsp. *capitis*	1	1
S. cohnii subsp. *cohnii*	2[b]	*S. cohnii* subsp. *cohnii*	0	2
S. sciuri subsp. *sciuri*	0	*S. sciuri* subsp. *sciuri*	2	0
Total	200		200	200

[a] Three strains were incorrectly identified as *S. simulans* because of PYR+ reactions, whereas they were identified as *S. warneri* according to the method of Kloos and Schleifer (14).

[b] Two strains were incorrectly identified as *S. cohnii* subsp. *cohnii*, whereas they were identified as *S. sciuri* subsp. *sciuri* according to the method of Kloos and Schleifer (14).

identified by API-Staph. One of three *S. simulans* isolates was misidentified by Staph-Zym; API-Staph misidentified one *S. simulans* strain and failed to identify another. API-Staph correctly identified two *S. schleiferi* subsp. *schleiferi* isolates, but the Staph-Zym did not identify one of these. The only isolate of *S. hominis* subsp. *hominis* was correctly identified by both systems. API-Staph identified one isolate of *S. capitis* subsp. *capitis* that was not identified by Staph-Zym. The one strain of *S. cohnii* subsp. *urealyticum* was identified by both methods. Two *S. sciuri* subsp. *sciuri* strains were correctly identified by API-Staph; this species is not included in the Staph-Zym human index, and therefore it was not identified as *S. cohnii* subsp. *cohnii*.

Table 4 shows the results obtained by the simple scheme. All species groups were resolved to the species level by using additional tests in order to be validated by the reference method.

A total of 104 *S. epidermidis* group isolates corresponded to 101 *S. epidermidis* (trehalose and mannitol negative) and to 3 *S. capitis* subsp. *ureolyticus* (trehalose negative, mannitol positive) isolates; 37 *S. haemolyticus* group isolates corresponded to 37 *S. haemolyticus* (acetoin production positive) isolates, and 32 *S. saprophyticus* group corresponded to 32 *S. saprophyticus* subsp. *saprophyticus* (trehalose positive). Only three *S. warneri* group isolates were identified by the simple scheme; two strains corresponded to two *S. warneri* isolates (β-glucosidase and anaerobic thioglycolate growth positive), although five *S. warneri* strains were identified by the reference method. The remaining three strains were misidentified by the simple scheme as *S. simulans* because they were PYR positive, an aberrant characteristic for *S. warneri*; this test is crucial in our working scheme, and the rest of the characteristics were coincident with this species by the reference method. The other isolate from the *S. warneri* group was *S. hominis* subsp. *hominis* (β-glucosidase negative, anaerobic thioglycolate growth positive); this strain grew in anaerobic thioglycolate medium, a very uncommon characteristic for this species. One strain of the *S. cohnii* group corresponded to *S. cohnii* subsp. *urealyticum* (xylose negative). Twelve *S. lugdunensis*, two *S. schleiferi* subsp. *schleiferi*, one *S. capitis* subsp. *capitis*, and three *S. simulans* isolates corresponded to the same species identified by the reference method. Two isolates misidentified as *S. cohnii* subsp. *cohnii* by the simple scheme were identified as *S. sciuri* subsp. *sciuri* according to the reference method. Our working scheme plus one or two additional tests to resolve each species group identified 195 isolates from a total of 200 CNS (97.5%) to the species level.

There was an excellent correlation between the reference method and the Rosco diagnostic tablets in the following tests: ornithine decarboxylase, urease, and acid production from mannose, mannitol, arabinose, lactose, sucrose, raffinose, trehalose, and maltose. We had some difficulties (such as not being able to clearly determine the ending color) when the mannose tablets were evaluated. Of 123 mannose-positive strains, 4 were interpreted as negative, whereas they showed delayed positive reactions in the purple agar at 72 h. The same identifications were obtained in the simple scheme in both growth and nongrowth tests.

Different results were also obtained when β-galactosidase and alkaline phosphatase activities (commercial rapid identification tests) were compared to those proposed by Kloos and Schleifer. When *o*-nitrophenyl-β-D-galactopyranoside was used, 9.5% more positive reactions were obtained compared to the test with 2-naphthol-β-D-galactopyranoside as a substrate. Also, when *p*-nitrophenylphosphate was used 12.0% more pos-

itive reactions were obtained compared to the modification of the Pennock and Huddy technique.

DISCUSSION

The rapid and accurate identification of CNS species has acquired major importance in the last few years; therefore, clinical isolates should not be disregarded until their possible clinical relevance has been resolved. The characteristics of pathophysiology and description of important clinical outcomes, epidemiological studies, and the antibiotic susceptibility pattern of each clinical isolate should be determined (26).

We found the most common clinical CNS species to be *S. epidermidis* (50.5%), *S. haemolyticus* (18.5%), *S. saprophyticus* subsp. *saprophyticus* (16.0%), *S. lugdunensis* (6.0%), and *S. warneri* (2.5%) (Table 2).

Two commercial systems were compared with the reference method. Of 201 strains studied, 200 (99.5%) were resolved by the method of Kloos and Schleifer; the 200 isolates analyzed were then identified by Staph-Zym (191 isolates; 95.5%) and API-Staph (171 isolates; 85.5%) as indicated. Other researchers obtained similar results with commercial systems (2, 11). Staph-Zym proved to be very effective in the identification of all CNS isolates compared to the reference method. Two *S. sciuri* subsp. *sciuri* strains, rarely found in human specimens, could not be identified because they are not included in the Staph-Zym human index. However, an oxidase-positive test may suggest this species when *S. cohnii* subsp. *cohnii* is presumptively detected.

API-Staph has a large database that includes the genera *Staphylococcus*, *Kocuria*, and *Micrococcus* (16). Even though this system displayed good sensitivity, there were 14 misidentified isolates and 8 unidentified isolates (12.9%) among the species most frequently isolated as *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus* subsp. *saprophyticus* (170 strains).

The simple scheme correctly identified the 104 *S. epidermidis* group isolates, 37 *S. haemolyticus* group isolates, 32 *S. saprophyticus* group isolates, 3 strains of the *S. warneri* group, and 1 strain of the *S. cohnii* group; these isolates were identified to the species level with the additional tests and finally confirmed by the reference method. The simple scheme directly identified to the species level 12 *S. lugdunensis*, 3 *S. simulans*, 2 *S. schleiferi* subsp. *schleiferi*, and 1 *S. capitis* subsp. *capitis* isolates. Three strains of *S. warneri* were misidentified as *S. simulans* because of PYR-positive reactions, which is one of the key components of our scheme. Strains with this characteristic were described by Oberhofer (23). Two strains of *S. sciuri* subsp. *sciuri* were misidentified as *S. cohnii* subsp. *cohnii* because this species is not considered by the simple scheme. Nevertheless, an oxidase-positive test could confirm the identification. One isolate was from a femoral catheter and the other was from a bone biopsy of a diabetic patient; although this organism normally originates in animals, it has been isolated from humans (4, 8, 9, 20, 29).

The isolates identified as belonging to the *S. epidermidis* group, i.e., based on an ornithine decarboxylase-positive test, can be directly identified as *S. epidermidis*. The only isolate of *S. hominis* subsp. *hominis* grew in anaerobic thioglycolate medium, a very uncommon characteristic for this species (7). This strain was β-glucosidase negative and deferoxamine susceptible. The reference method does not include deferoxamine susceptibility, but this test is used in the Staph-Zym for the identification of *S. epidermidis* and *S. hominis* strains (18, 19).

There were differences in the results when synthetic enzyme chromogenic substrates used in commercial systems were compared to conventional methods for β-galactosidase and alkaline phosphatase activities, yielding 9.5 and 12.0% more positive reactions, respectively. Other authors have largely discussed the use of enzyme tests in the characterization and identification of aerobic and facultatively anaerobic gram-positive cocci (2).

With the simple scheme the identification of CNS could be obtained within 24 h when the enzymatic tests were used; meanwhile, up to 72 h was necessary with the growth tests. Simplicity and speed are very important in certain circumstances, e.g., for the identification of CNS isolates from normally sterile body sites such as blood cultures, in which these isolates are the most common cause of nosocomial bacteremia, as well as the most common blood culture contaminants (12, 13). Repeat CNS isolates from patients with invasive diseases should be identified to allow a comparison of the strains. On the other hand, species identification is a prerequisite before typing procedures for epidemiological studies are undertaken. Undoubtedly, CNS species identification will be facilitated and encouraged by the availability of a simple, inexpensive, and accurate procedure, especially in places with limited resources.

In conclusion, our five-test simple scheme showed an excellent correlation with the reference method in the presumptive identification of the CNS species most frequently isolated in clinical specimens. This relatively simple methodology will prove useful in any clinical microbiology laboratory for the presumptive identification of CNS species and species groups.

ACKNOWLEDGMENTS

We thank José Bou Casals for critical reading of the manuscript. We thank Graciela Vazquez for technical assistance.

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