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Evaluation of Staph ID 32 System and Staph-Zym System for Identification of Coagulase-Negative Staphylococci

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The purpose of the investigation was to evaluate two commercially available identification systems: a new modification of the Staph-Zym system (Rosco, Tåstrup, Denmark) and the Staph ID 32 API system (API System, BioMérieux, Paris, France). A local standard method to be used in routine laboratories was also evaluated. A total of 200 staphylococcal isolates, including strains from both the American Type Culture Collection and the Czechoslovak Collection of Microorganisms as well as 89 clinical isolates, were used in tests of all three identification systems. The Staph ID 32 API system identified from 50 to 100% of the reference strains and 82.1% of the clinical isolates correctly. The Staph-Zym system identified from 90 to 100% of the reference strains and 82.1% of the clinical isolates correctly. Most misidentifications were of minor importance, but in both systems major failures appeared (*Staphylococcus aureus* was identified as a coagulase-negative staphylococcus). Both systems needed backup from a reference laboratory to determine if two isolates were of the same strain.

Coagulase-negative staphylococci, which are part of the normal skin flora, are now established as important in the pathogenesis of infections acquired in the hospital (2, 16, 17). They are associated with the presence of foreign bodies such as prosthetic valves, cerebrospinal fluid shunts, orthopedic prostheses, as well as intravascular, urinary, and dialysis catheters (10). Clinical coagulase-negative staphylococcal isolates should not be disregarded until their possible clinical relevance has been resolved. As an indicator of the clinical relevance of isolated bacteria, it is important to identify the strains both with respect to species and in some cases with respect to biotype (1, 3, 6).

A variety of methods have been proposed for use in the identification of coagulase-negative staphylococcal species that are important in human medicine. The methods include conventional identification (4, 5, 7) as well as commercial identification systems (8, 9, 12–14). The conventional methods, i.e., those of Kloos and Schleifer (7), are relatively cumbersome for routine laboratory use and take up to 5 days of incubation to obtain a result (6). The commercially produced test systems for the identification of coagulase-negative staphylococci usually share the same problem: lack of sufficient strains in the accompanying databases to permit accurate identification of various strains (some systems). Only a few systems include a representative panel of strains for quality control of the test (6, 15). The present investigation was done in order to evaluate two commercially available systems for the identification of coagulase-negative staphylococci: a new modification of the Staph-Zym system (Rosco, Tåstrup, Denmark) and the Staph ID 32 API system (API System, BioMérieux, Paris, France). A local standard method to be used in routine laboratories was also tested.

MATERIALS AND METHODS

Strains. A total of 200 staphylococcal isolates were identified; of those, 111 were control strains and 89 were isolates from blood specimens taken in the period from January 1992 to December 1993. The control strains previously identified by the conventional methods outlined by Kloos and Schleifer (7) included 10 *Staphylococcus cohnii*, 10 *Staphylococcus hominis*, 10 *Staphylococcus haemolyticus*, 10 *Staphylococcus warneri*, 10 *Staphylococcus lugdunensis*, 5 *Staphylococcus schleiferi*, 5 *Staphylococcus xylosus*, 4 *Staphylococcus cohnii* subsp. ureolyticum, 5 *Staphylococcus capitis* subsp. *urealyticus*, 5 *Staphylococcus capitis*, 5 *Staphylococcus auricularis*, 1 *Staphylococcus caprae*, 5 *Staphylococcus simulans*, 6 *Staphylococcus saprophyticus*, and 20 *Staphylococcus epidermidis* organisms. Both American Type Culture Collection (ATCC) as well as Czechoslovak Collection of Microorganisms (CCM) reference strains were included among the control strains, as follows: *S. cohnii* ATCC 29974 and CCM 2736, *S. hominis* CMM 2448, CCM 2449 and CCM 3474, *S. haemolyticus* ATCC 29970 and CCM 2737, *S. warneri* CCM 2730, *S. lugdunensis* ATCC 43809, *S. schleiferi* ATCC 43808, *S. capitis* CCM 2734, *S. caprae* CCM 3573, *S. auricularis* ATCC 33753, *S. simulans* ATCC 27848 and CCM 2705, *S. saprophyticus* ATCC 15305 and CCM 883, and *S. epidermidis* ATCC 14990. All isolates were stored in nutrient broth (Statens Seruminstitut, Copenhagen, Denmark) with 15% glycerol at −80°C and were recovered for the present study on 5% blood agar (Statens Seruminstitut, Copenhagen, Denmark) incubated at 37°C for 18 h.

Conventional identification. The 111 strains (including 18 reference strains) were previously identified to the species level by the following modified conventional scheme (7). The first-level tests included catalase test; slide test for clumping factor; tube coagulase test; susceptibilities to furazolidone (50 μg), polymyxin (colistin 150 μg), and novobiocin (5 μg); and inhibition zones of >16 mm in diameter. Furazolidone-resistant strains were identified as *Micrococcus* species. Polymyxin-resistant strains were further identified by a test for thermostable nuclease, Tween 80-splitting enzyme, urease production, and aerobic and anaerobic acid production from mannitol. Strains susceptible to novobiocin, furazolidone, and polymyxin were tested for aerobic acid production from trehalose, mannitol, maltose, and lactose and for urease production. Strains resistant to novobiocin and susceptible to both furazolidone and polymyxin were tested for aerobic acid production from sucrose, raffinose, xylose, and turanose as well as urease production and nitrate reduction. The carbohydrates were all tested in Hugh & Leifson's O/F medium for up to 5 days. All strains were identified with the Staph-Zym system (Rosco, Copenhagen, Denmark) and the Staph ID 32 API system (BioMérieux).

Identification with the Staph-Zym system. The identification procedure includes both fermentation tests and antibiograms. The Staph-Zym system consists of a rigid, transparent plastic strip with 10 upright minitubes containing dehydrated chromogenic and modified conventional substrates. Included in the new Staph-Zym system are tests for β-glucosidase, β-galactosidase, β-glucuronidase, trehalose, maltose, urease, arginine dihydrolase, nitrate reduction, pyrrolidonyl-aminopeptidase, and alkaline phosphatase. Susceptibilities to furazolidone (50 μg), polymyxin (colistin, 50 μg), novobiocin (5 μg), and deferoxamine (250 μg) were determined. All drugs were supplied as Neo-Sensitabs from Rosco.

Procedures were performed as directed by the manufacturer. Briefly, test organisms were removed from a blood agar plate with a sterile inoculator (Nunc, Copenhagen, Denmark). The inoculator was agitated in 3 ml of physiological saline until the turbidity produced by dispersed cells was equivalent to a no. 2 McFarland standard. Approximately 0.25 ml of the bacterial suspension was

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TABLE 1. Flow and identification chart for coagulase-negative staphylococci

Step	Result	Result
Step 1: gram-positive cocci in clusters	Catalase positive; go to step 2	Catalase negative and α-hemolytic = *Aerococcus* or *Stomatococcus*
Step 2		
Slide test negative	Tube coagulase negative	Coagulase-negative staphylococci; go to step 3
Slide test positive	Tube coagulase positive	*S. aureus*
Slide test negative[a]	Tube coagulase positive[a]	*S. aureus*
Slide test positive[a]	Tube coagulase negative[a]	Coagulase-negative staphylococci; go to step 3
Step 3: furazolidone (50 μg)	Resistant (zone size, ≤16 mm)	*Micrococcus* sp.
	Susceptible (zone size, >16 mm)	Go to step 4
Step 4: novobiocin (5 μg)	Resistant (zone size, ≤16 mm)	*S. saprophyticus* group[b]
	Susceptible (zone size, >16 mm)	Coagulase-negative staphylococci[c]
Step 4: polymyxin B (50 μg)	Resistant (zone size, ≤10 mm)	*S. epidermidis* group; go to step 5
	Susceptible (zone size, >10 mm)	Coagulase-negative staphylococci[c]
Step 5: thermostable nuclease	*S. epidermidis* negative	*S. hyicus* positive
	S. lugdunensis negative	*S. schleiferi* positive
Step 5: pyrrolidonyl-acryomidase activity	*S. epidermidis* negative	*S. hyicus* negative
	S. lugdunensis positive	*S. schleiferi* positive

[a] In situations in which there is a discrepancy between the slide test and the tube test, DNase and lipase activities should also be tested.
[b] *S. saprophyticus*, *S. cohnii*, *S. xylosus*, *S. sciuri*, or *S. lentus.*
[c] All species which do not belong to the *S. epidermidis* or *S. saprophyticus* groups.

dispensed into each of the 10 tubes, 3 drops of sterile oil was added to the arginine dihydrolase tube, and the strip was incubated at 37°C for 18 to 24 h.

Prior to reading, appropriate reagents were added to the pyrrolidonyl-aminopeptidase and nitrate reduction tubes. Positive reactions were recorded and converted to four-digit numbers. A fifth digit was generated from the results of the novobiocin, polymyxin, and deferoxamine susceptibility tests. Susceptibility tests were performed on Mueller-Hinton agar (Oxoid, Copenhagen, Denmark) by standard methods. A zone size of ≥16 mm (confluent or semiconfluent growth) was considered for susceptibility to polymyxin and novobiocin. For deferoxamine any zone size was considered to indicate susceptibility. The five-digit number provided an identification selection in the Staph-Zym system's human profile index. Possible tests were suggested when needed for the delineation of species with the same profile number.

Identification with the Staph ID 32 API system. The Staph ID 32 system strip consists of 32 cupules, 26 of which contain dehydrated biochemical media for colorimetric tests. The tests included acid production from glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, raffinose, sucrose, *N*-acetylglucosamine, turanose, ribose, arabinose, and cellobiose; decarboxylation of arginine and ornithine; production of urease, β-glucuronidase, β-galactosidase, acetoin, alkaline phosphatase, arginine arylamidase, and pyrrolidonyl-arylamidase; hydrolysis of esculin; reduction of nitrate; and susceptibility to novobiocin. The manufacturer's recommended procedures (API System, BioMérieux) were followed. Briefly, the bacterial suspensions were prepared from overnight cultures on blood agar plates (5% horse blood). They were standardized to a no. 0.5 McFarland standard in 6 ml of sterile distilled water and were distributed into the wells of the strip by repetitive delivery of 135 μl per well with a pipette. After an incubation period of 24 h at 37°C, reagents were added for the nonspontaneous tests. Strain profiles were read and identified with Automatic Testing Bacteriology equipment and were interpreted with APILAB software. This software gives the probability of the identification result in a range of 10 to 100%. Possible tests were suggested when needed for the delineation of species.

The routine laboratory tests. A simplified routine laboratory test system included a catalase test; slide and tube coagulase tests; determination of resistance to furazolidone (50 μg), polymyxin (50 μg), and novobiocin (5 μg); determination of phenotype characteristics; and thermostable nuclease and pyrrolidonyl-arylamidase tests. The different levels and time schedule for the test procedures are given in Table 1.

Intra- and interassay variations. Ten isolates giving no identification problems in any of the systems were tested three times (blinded) in each system. Furthermore, all strains which gave identification problems in the first test in any of the systems (no name or several suggestions of species) were retested twice.

RESULTS

The two commercial test systems were used to test all isolates. First, the two systems were validated by testing 111 staphylococcal strains previously identified by a conventional test system (7). Second, the results obtained with the two systems were compared by testing 89 clinical isolates with the routine laboratory test system (modified conventional test).

In Table 2 the result of the identification of the 111 control strains is presented. Twenty-two of these strains resulted in identification problems for one or both of the two commercial tests. The Staph-Zym test identified all strains except one strain of *S. warneri*; the system could not provide any name for the organism. The Staph ID 32 API system was considered problematic if the probability of the species identification was less than 85%. In 22 cases there were identification problems. In 13 of these cases the correct species was suggested, but the probability of identification ranged from 67 to 82%. Two strains of *S. auricularis* were identified as *Micrococcus luteus*, one *S. capitis* strain was identified as *S. epidermidis*, two *S.*

TABLE 2. Identification of 111 coagulase-negative staphylococcal strains by the Staph ID 32 API and Staph-Zym systems

Organism	No. (%) of isolates:		
	Tested	Identified correctly	
		API ID 32 Staph	Staph-Zym
S. auricularis	5	3 (60)	5 (100)
S. capitis	10	7 (70)	10 (100)
S. caprae	1	1 (100)	1 (100)
S. cohnii	14	7 (50)	14 (100)
S. epidermidis	20	17 (85)	20 (100)
S. hominis	10	6 (60)	10 (100)
S. haemolyticus	10	10 (100)	10 (100)
S. lugdunensis	10	10 (100)	10 (100)
S. saprophyticus	6	6 (100)	6 (100)
S. schleiferi	5	2 (40)	5 (100)
S. simulans	5	5 (100)	5 (100)
S. warneri	10	8 (80)	9 (90)
S. xylosus	5	4 (80)	5 (100)

cohnii and two *S. klosii* strains were identified as *S. hominis*, one *S. hominis* strain was identified as *S. saprophyticus*, one *S. schleiferi* strain was identified as *S. caprae*, and one *S. warneri* strain could not be named.

Results of identification of 89 clinical isolates with the Staph ID 32 API system, the Staph-Zym system, and a routine laboratory test system (BBH-Staph-test) were as follows: for the Staph ID 32 API system, correct identifications, 73 strains (82.1%); problem identifications, 16 strains (17.9%); for the Staph-Zym system, correct identification, 73 strains (82.1%); problem identifications, 16 strains (17.9%); for the BBH-Staph-test, correct identification, 87 strains (97.7%); problem identifications, 2 strains (2.3%). The identities obtained by the routine laboratory method (modified conventional identification system) were considered to be the correct ones. However, if both of the commercial systems identified the strain to be another species, all three tests were repeated. The identification provided by the Staph ID 32 API system was considered unsatisfactory if the probability of species identification was less than 85%. For both the Staph ID 32 API and the Staph-Zym tests 16 strains were found to have been unsatisfactorily identified. This gives a rate of correct identification of 81.8%. The routine laboratory identification method identified 97.7% of the strains correctly. For the Staph ID 32 API test three major problems were found: in one case an *S. epidermidis* strain and an *S. haemolyticus* strain were each found to be an *S. aureus* strain, and in one case an *S. capitis* strain was found to be an *M. luteus* strain. The Staph-Zym system had one major problem in which an *S. haemolyticus* strain was found to be an *S. aureus* strain. The routine test did not show any major problems.

The reidentifications of the 10 nonproblematic strains were 100% correct with all three test systems. When a strain gave problems in some of the reactions, however, different suggestions of species came up each time (three times) that the strains were examined in any of the three test systems.

DISCUSSION

Hospital-acquired infections caused by coagulase-negative staphylococci are a serious problem in many developed countries. Most infections are caused by *S. epidermidis* (75% of all coagulase-negative staphylococci isolated from blood), but species such as *S. haemolyticus* (7%) and *S. hominis* (5%) are also of importance (3). Lately, *S. lugdunensis* has been described as a cause of serious staphylococcal infections (11). Therefore, identification of staphylococci is important for determination of the characteristics of pathophysiology and description of important clinical outcomes, as well as epidemiological studies (3, 7, 11). Two commercial tests and one routine conventional test system for the identification of coagulase-negative staphylococci were compared: the Staph ID 32 API system and the Staph-Zym system as well as the laboratory's modified conventional system. The two commercial test systems performed differently in the identifications of 111 control strains: the Staph ID 32 API system identified 86% of the strains correctly, whereas the Staph-Zym system identified 99.1% correctly. For routine use it is disturbing that these systems in some cases operate with two or more suggestions for identification with a comparable safety level, i.e., *S. epidermidis* and *S. hominis* are suggested with a safety level of 60 or 65% for the same isolate at the same time. One suggestion or no suggestion of a species is relevant. The Staph ID 32 API system frequently suggests several species as well as possible tests for the delineation of species, which is important for the reference laboratory or when two isolates are compared.

In the identification of the clinical isolates, the two commercial test systems were equal in performance, with 82.1% of the strains correctly identified. However, both systems gave a lower percentage of correctly identified strains than the modified routine test system, which had 97.7% correct identifications. Both commercial tests systems also had major misidentifications (the Staph ID 32 API system had three and the Staph-Zym system had one), identifying coagulase-negative staphylococci as *S. aureus* or micrococci. In a clinical setting such misidentifications could be very important. In this respect it is also a concern that upon retesting of problem strains the commercial systems gave new species suggestions each time that they were tested (three times).

Practical handling of the systems. The Staph ID 32 API system can, according to the manufacturer, be used manually both for inoculation and for reading. We found that because of the amount of time consumed and the risk of failures when the system was handled manually, use of automatic inoculation and reading by an optical instrument are preferable. For the Staph-Zym system, which uses fermentation tests as well as antibiograms, it is easy to perform inoculation and read the results without any instrumental help. Occasionally, however, there were problems deciding whether some tests were weakly positive or negative. The addition of dextrophosphamine increased the safety of species identification. All three tests require at least 24 h from the time of inoculation to the time that a result can be obtained. This time period is acceptable for coagulase-negative staphylococci, bacteria that usually do not cause rapidly fatal infections.

Economy. The cost to identify each isolate by the two commercial test systems was $5.40 for the Staph ID 32 API system and $4.80 for the Staph-Zym system. The cost for the routine laboratory system was $1.80 per isolate.

In conclusion, the Staph ID 32 API system appeared to be inadequate for the identification of both reference strains and clinical isolates. The Staph-Zym test system appeared to be the more reliable system and processing was easier with this system. It was also the less sophisticated of the two commercial systems. It showed reasonably decent performance in the identification of reference strains, but it showed low specificity in the identification of clinical isolates. Neither of the two commercial systems surpassed the simplified routine identification system with respect to identification quality or time savings. Furthermore, if a laboratory which uses the Staph ID 32 API system or the Staph-Zym system needs to know whether several independent coagulase-negative staphylococcal isolates do in fact represent the same strain, it is recommended that all isolates be forwarded to a reference laboratory where ribotyping, further biotyping, and, for example, lectin typing can be performed. We therefore find that for routine laboratories, simple test systems should be used.

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