JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1983, p. 260–263
0095-1137/83/020260-04$02.00/0

Vol. 17, No. 2

Species Identification and Biotyping of Staphylococci by the API Staph-Ident System

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Received 19 July 1982/Accepted 8 November 1982

The API Staph-Ident system, a commercially available biochemical and chromogenic substrate micromethod, was evaluated as a means for identifying the species and determining the biotypes of human strains of staphylococci routinely encountered in a clinical microbiology laboratory. The species identity of 152 of 188 (80.9%) unique clinical isolates of staphylococci was correctly predicted by this method after 5 h of incubation according to the recommendations of the manufacturer. When results were determined after 24 h of incubation, the overall accuracy of this procedure was 90.4%. The API Staph-Ident system was not an adequate procedure for assessing strain biotypes since the patterns of biochemical reactivity observed with 53 of 54 (98.2%) unique isolates of *Staphylococcus aureus* were identical. Similarly, 58 of 62 (93.6%) different strains of *S. epidermidis* yielded the same biochemical profile.

Definitive species identification and biotyping of staphylococci are of potential usefulness in the clinical microbiology laboratory, particularly in circumstances where knowledge of strain identity is of clinical significance. In 1975, Kloos and Schleifer described an identification scheme which permitted differentiation of most human staphylococci into 10 distinct species and an additional group referred to as *Staphylococcus* sp. (3). Most strains could be identified according to species based on aerobic acid production from 10 different carbohydrates, coagulase activity, hemolysis, and nitrate reduction. However, because of the length of incubation required (up to 3 days) and the complexity of the procedures used in their scheme, it has remained largely a reference laboratory technique. In 1978, Brun and co-workers, in France (1), described a micromethod composed of 19 test substrates which permitted species identification and some biotyping of the same 10 species of staphylococci described by Kloos and Schleifer (3). This method, a modification of a procedure proposed by Peny and Buissiere in 1970 (7), was accurate and easy to use and provided results within 48 h. It is now commercially available in Europe as API ZYM (API Laboratory Products Ltd., Basingstoke, England), but has never been marketed in the United States or Canada.

Most clinical microbiology laboratories in North America continue to divide the human staphylococci into only two groups, *Staphylococcus aureus* and non-*S. aureus* species of staphylococci. This can be readily and accurately accomplished based on the determination of characteristics such as elaboration of extracellular coagulase factor (8), thermostable nuclease production (5), and slide agglutination of rabbit plasma-coated latex particles (2, 6). Definitive identification of non-*S. aureus Staphylococcus* species is rarely attempted.

Recently, a miniaturized conventional biochemical and chromogenic substrate micromethod (API Staph-Ident system; Analytab Products, Plainview, N.Y.) capable of distinguishing all of the human staphylococcal species described by Kloos and Schleifer (3) plus two species of veterinary interest, *S. intermedius* and *S. hyicus*, became available commercially. This system requires 5 h for incubation and permits the determination of 10 biochemical characteristics. When combined with the results of novobiocin susceptibility and coagulase production tests, the API Staph-Ident system has recently been shown to accurately identify 95% of 466 strains of *S. aureus* and 13 non-*S. aureus Staphylococcus* species (4). Since the majority of strains examined in that study were stock cultures, and since many had been recovered from veterinary clinical sources, it was of interest to evaluate the API Staph-Ident system in the setting of a routine clinical microbiology laboratory using strains recovered from humans. The intent of the present study was to determine the accuracy of this system as a means for routinely determining the species identity of human strains of staphylococci and to determine whether this system could be used as a biotyping tool for demonstrating strain identity or dissimilarity

TABLE 1. Identification of staphylococci with the API Staph-Ident system after 5 h of incubation

Organism[a] (no. tested)	No. (%) correctly identified	No. (%) correctly identified
S. aureus (74)	54 (73.0)	20 (27.0)
S. epidermidis (66)	62 (93.9)	4 (6.1)
S. haemolyticus (18)	16 (88.9)	2 (11.1)
S. hominis (10)	3 (30.0)	7 (70.0)
S. warneri (9)	9 (100)	0 (0)
S. simulans (6)	6 (100)	0 (0)
S. xylosus (3)	0 (0)	3 (100)
S. capitis (2)	2 (100)	0 (0)
Total (188)	152 (80.9)	36 (19.2)

[a] Species identity was determined by conventional biochemical tests according to the criteria of Kloos and Schleifer (3).

among different human isolates of the same *Staphylococcus* species.

MATERIALS AND METHODS

Bacterial strains. During a 3-month period, 188 unique isolates of staphylococci were obtained from a variety of clinical specimens submitted for routine microbiological analysis to the Clinical Microbiology Laboratories of the University of Massachusetts Medical Center. A total of 16 strains were recovered from cultures of blood, 48 from sputum, 59 from wound specimens, 36 from genital specimens, 15 from urine, 8 from normally sterile body fluids, and 6 from upper respiratory tract specimens. A total of 89 isolates were obtained from outpatients; 99 were obtained from inpatients. Among these 188 different clinical isolates, 14 distinct patterns of antimicrobial susceptibility based on the results of the disk diffusion susceptibility were observed. A single well-isolated colony growing on primary medium (either tryptic soy agar with 5% sheep blood [SBA] or enriched chocolate agar) was subcultured to a SBA plate and incubated overnight at 35°C in an atmosphere of 5 to 7% CO_2.

Determination of species by conventional biochemical tests. A single well-isolated colony was transferred from the SBA subculture plate into 3 ml of sterile tryptic soy broth and incubated in ambient room atmosphere for 3 to 5 h at 35°C. This suspension was used to determine the following biochemical characteristics: aerobic acid production from fructose, xylose, arabinose, ribose, maltose, lactose, sucrose, trehalose, mannitol, and xylitol; coagulase activity with citrated rabbit plasma (BBL); and nitrate reduction. Hemolysis was ascertained by an examination of growth on the SBA subculture plate. All tests were performed as described previously (3). Organisms were identified according to the criteria of Kloos and Schleifer (3). In rare cases, determination of anaerobic growth in thioglycollate broth, aerobic acid production from galactose and melizitose, lysostaphin susceptibility, and novobiocin susceptibility were necessary to permit definitive identification of species. These tests were performed and interpreted according to published criteria (3).

API Staph-Ident system. The API Staph-Ident system consists of a cardboard strip with 10 microcupules containing various dehydrated biochemical and chromogenic substrates. Each strip permits the determination of the following characteristics: alkaline phosphatase hydrolysis of *p*-nitrophenylphosphate; urease production; β-glucosidase hydrolysis of *p*-nitrophenyl-β-D-glucopyranoside; mannose, mannitol, trehalose, and salicin utilization; β-glucuronidase hydrolysis of *p*-nitrophenyl-β-D-glucuronide; arginine utilization; and β-galacosidase hydrolysis of 2-naphthol-β-D-galactopuranoside. Strips were inoculated with an inoculum prepared from the SBA subculture plate and incubated for 5 h according to the recommendations of the manufacturer. Reaction patterns were determined, and a four-digit octal code profile was assigned. The strips were then reincubated for an additional 19 h and reexamined. In cases where reaction patterns had changed, a second octal code profile was assigned. The species identity of organisms was ascertained by using the API Staph-Ident profile register. When an octal code profile was not found in the profile register, the identity of the organism was determined by consulting the API computer center.

RESULTS

A total of 152 of 188 clinical isolates of staphylococci (80.9%) were correctly identified by the API Staph-Ident system after 5 h of incubation (Table 1). A total of 54 of 74 isolates of *S. aureus* (73.0%) were correctly identified. Of the 20 strains which were incorrectly identified, 18 were identified as *S. hyicus* based on false-negative mannitol utilization reactions. In all 18 cases, a correct identification of *S. aureus* was obtained when strips were incubated for 24 h, due to conversion of the mannitol result to positive.

A total of 98 of 114 isolates of non-*S. aureus* species of staphylococci (86.0%) were correctly identified after 5 h (Table 1). If it is assumed that identifications obtained by conventional biochemical tests according to the methods of Kloos and Schleifer (3) were correct, then 16 strains of non-*S. aureus* species of staphylococci were incorrectly identified by the API Staph-Ident system. Seven of these strains were identified as *S. hominis* by conventional tests. In all seven cases, the conversion of a single API Staph-Ident reaction (β-glucosidase production in five instances and alkaline phosphatase activity in two instances) from positive to negative would have yielded an identification of *S. hominis*. Among the nine remaining strains, the conversion of more than one API Staph-Ident reaction would have been necessary to achieve concordance with the results of conventional tests.

Among the 152 isolates correctly identified by the API Staph-Ident system after 5 h of incuba-

tion, 126 (82.9%) yielded octal code profiles which were included in the profile register of the manufacturer. The remaining 26 organisms were identified by the API computer center.

All of the 54 isolates of *S. aureus* that were correctly identified within 5 h had one of four different octal code profiles: 7740, 5740, 5700, or 6700. The majority of these strains (36) yielded a single profile, 7740. Incubation of strips from the remaining 18 isolates for a total of 24 h yielded this same 7740 profile in 17 cases, due to positive conversion of negative urease reactions in 12 instances, positive conversion of negative arginine utilization reactions in 4 cases, and conversion of 1 alkaline phosphatase reaction. Similarly, for the 62 strains of *S. epidermidis* correctly identified, the following four different octal code profiles were obtained: 7040 (21 strains), 7000 (18 strains), 3000 (14 strains), and 3040 (9 strains). After 24 h of incubation, 37 of the 41 strains with octal code profiles other than 7040 yielded the 7040 profile. Positive conversion of negative reaction results obtained at 5 h with the arginine utilization and β-glucosidase production tests were responsible for all changes in octal code profiles at 24 h.

DISCUSSION

A reliable, technically nondemanding method for determining the species identities and biotypes of human clinical isolates of staphylococci would have great value in circumstances where knowledge of species identity or biotype is of clinical relevance. Examples include multiple blood culture isolates of staphylococci from the same patient; non-*S. aureus* strains of staphylococci recovered from wound or body fluid specimens in patients with devices such as vascular and hip prostheses, ventricular shunts, intracranial pressure monitors, central nervous system reservoirs, or indwelling vascular catheters; and repetitive isolates of non-*S. aureus* species of staphylococci recovered from sites not normally colonized with these organisms.

The intent of the present investigation was to determine the accuracy of a new, rapid, miniaturized biochemical system, API Staph-Ident, as a means for identifying the species of isolates of staphylococci routinely encountered in clinical microbiology laboratories. In addition, it was of interest to know whether this procedure could be used to determine strain identity or dissimilarity based on patterns of biochemical reactivity.

The overall accuracy of the API Staph-Ident system was 80.9% when 188 strains of staphylococci were tested. The accuracy among strains of *S. aureus* (73.0%) and strains of non-*S. aureus* species (86.0%) was comparable. False-negative mannitol utilization reactions accounted for 18 of 20 (90.0%) incorrect identifications of *S. aureus* when strips were incubated for 5 h according to the recommendations of the manufacturer. In all 18 cases, conversion of the mannitol test result to positive occurred after incubation for 24 h, thus providing a correct identification. False identification among strains of non-*S. aureus* staphylococci were often the result of false-positive results obtained with a relatively small number of test substrates. Although explanations for these false-positive results are not immediately obvious, if the problem of false-negative mannitol utilization reactions could be corrected, possibly by increasing the inoculum size, lengthening the incubation period, or increasing the amount of substrate present in the mannitol microcupule, then the overall accuracy of the API Staph-Ident system would be increased to 90.4%. The accuracy for identifying *S. aureus* would be 97.3%.

Even in cases where a correct identification was obtained after 5 h of incubation, the API Staph-Ident system should not be considered a rapid method routinely capable of providing same-day identification results. In most cases, an overnight subculture of organisms recovered on primary plates was necessary to achieve an inoculum size large enough to permit performance of the test.

Because of the variety of substrates incorporated into this system, the possibility exists that patterns of biochemical reactivity (i.e., biotypes) might be used to determine strain identity or dissimilarity among isolates of the same species. Among 54 strains of *S. aureus* correctly identified after 5 h of incubation, 36 (66.7%) yielded the same 7740 octal code profile. Incubation of strips from the remaining 18 isolates for 24 h yielded this same 7740 profile in 17 cases. These results are consistent with the observations of Kloos and Wolfshohl, who found that the majority of strains of *S. aureus* that they tested yielded a 7740 profile after 5 h of incubation (4). It would be of interest to know whether 24-h incubation of those strains which yielded different profiles in their study would have achieved this same 7740 reaction pattern.

Similarly, among 62 strains of *S. epidermidis* correctly identified within 5 h, the 7040 octal code profile was observed most frequently (21 strains, or 33.9%). After 24 h of incubation, this same profile was obtained with 37 of the remaining 41 strains. This profile was not common among the strains of *S. epidermidis* examined by Kloos and Wolfshohl (4). Again, it would be of interest to know what profiles would have been obtained after 24 h of incubation.

The results of the present study demonstrated, therefore, that among the two species recovered in numbers large enough to permit a mean-

ingful analysis (*S. aureus* and *S. epidermidis*), the large majority of strains had the same biochemical reactivity pattern, at least after 24 h of incubation. Given the heterogenicity of specimen types from which these isolates were recovered, the fact that many were obtained from outpatients, and the diversity of antibiograms exhibited by the organisms examined in this study, it is unlikely that they represented one or a few endemic hospital strains. It would seem unlikely, therefore, that the API Staph-Ident system could be of value in determining strain identity or dissimilarity among human clinical isolates of the same staphylococcal species.

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