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Comparison of the Staph-Ident System with a Conventional Method for Species Identification of Urine and Blood Isolates of Coagulase-Negative Staphylococci

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The Staph-Ident system (Analytab Products) for species identification of coagulase-negative staphylococci was compared with the conventional method of Kloos and Schleifer (21). A total of 101 clinical isolates from urine cultures and 95 clinical isolates from blood cultures were studied: overall agreement between the two methods was 86%. We concluded that the Staph-Ident system is a practical test for most clinical microbiology laboratories and that results obtained from this rapid test are comparable to those obtained from the more cumbersome conventional method. Additional investigations are needed to determine the clinical relevance of such species identification.

Because coagulase-negative staphylococci are ubiquitous skin commensals, the isolation of these organisms is often discounted as resulting from contamination or is considered innocuous. These organisms may, however, be the causative agents of severe infections (1, 12, 32), with some species associated more than others with specific infections. However, identification of these staphylococci has not been feasible for most clinical laboratories because of laborious methodology.

Recently, a commercial micromethod with 10 biochemical tests was introduced. This method, the Staph-Ident system (Analytab Products), has the potential to make identification of coagulase-negative staphylococci practical. We examined species identification of urinary tract and blood isolates of coagulase-negative staphylococci and compared the results obtained from this system with the results obtained from the conventional method of Kloos and Schleifer (21).

MATERIALS AND METHODS

Organisms. Test strains were obtained from clinical specimens and stored frozen at −70°C until used. A total of 101 urine culture isolates were selected with one of the following criteria: a pure isolate with a colony count of $>10^5$ organisms per ml obtained from a clean-catch specimen or a pure isolate with any colony count obtained from a suprapubic aspiration of urine. A total of 95 blood culture isolates were also selected. The following quality control strains were used: *Staphylococcus epidermidis* ATCC 14990, *S. saprophyticus* ATCC 15305, *S. haemolyticus* ATCC 29970, *S. hominis* ATCC 27844, *S. capitis* ATCC 27840, *S. xylosus* ATCC 29971, *S. warneri* ATCC 27836, *S. cohnii* ATCC 29974, *S. simulans* ATCC 27848, and *S. aureus* ATCC 25923.

Microbiology. Coagulase-negative staphylococci were identified by testing for free coagulase by the tube method (22) with rabbit plasma (BBL Microbiology Systems). Species were identified in duplicate, both by the reference method of Kloos and Schleifer (21) and by the Staph-Ident system.

Conventional species identification. The conventional procedures for determining key characteristics for species identification were done as originally described (21). These characteristics were coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from D-fructose, D-xylose, D-arabinose, D-ribose, D-maltose, D-lactose, D-sucrose, D-trehalose, D-mannitol, and D-xylitol. Results for each strain were tabulated, and identification was made with the criteria of Kloos and Schleifer (21). Discordant results in duplicate runs were resolved by retesting the isolates. Additional characteristics were anaerobic growth in thioglycolate medium, lysostaphin susceptibility, phosphatase activity, and novobiocin susceptibility.

Staph-Ident species identification. Staph-Ident strips were used according to the directions supplied by the manufacturer. Each strip consisted of 10 microcupules which contained either dehydrated substrates or nutrient medium or both and which used either a miniaturized conventional test or a miniaturized chromogenic test. The strips tested phosphatase activity, urea utilization, β-glucosidase hydrolysis, β-glucuronidase hydrolysis, β-galactosidase hydrolysis, arginine utilization, and aerobic acid production from D-mannose, D-mannitol, D-trehalose, and D-salicin. Each strip was inoculated, and after 5 h of incubation at 35°C, biochemical profiles were derived for each isolate. Four-

TABLE 1. Species identification of urine and blood isolates of staphylococci by two methods

Staphylococci	No. of isolates identified			
	Blood		Urine	
	Conventional	Staph-Ident	Conventional	Staph-Ident
S. epidermidis	82	62	51	49
S. saprophyticus	0	2	23	23
S. haemolyticus	5	2	13	11
S. hominis	5	23	4	5
S. simulans	0	0	7	8
S. cohnii	0	2	2	2
S. capitis	3	4	0	1
S. warneri	0	0	1	1
S. xylosus	0	0	0	0
S. aureus (coagulase negative)	0	0	0	1

digit numerical profiles obtained from these results and from the Staph-Ident profile register were used to determine the species of each staphylococcal isolate. Isolates that had profile numbers with more than one identification choice were tested again before a final determination was made. Additional characteristics used to separate biochemically similar species were novobiocin susceptibility, coagulase activity, and aerobic acid production from D-xylose and D-arabinose.

RESULTS

Table 1 shows that a variety of species of coagulase-negative staphylococci can be isolated from urine and from blood. *S. epidermidis* was the predominant organism isolated from both types of specimens. Both *S. saprophyticus* and *S. haemolyticus* (20 and 10%, respectively) were isolated more frequently from urine than from blood. One isolate of coagulase-negative *S. aureus* was identified by the Staph-Ident system.

Identification by both methods agreed for 93% of the urine isolates and for 79% of the blood isolates; overall agreement was 86%. Neither method was considered more accurate than the other. Table 2 shows the types and frequencies of discrepancies observed. The most frequent discrepancy between the two methods was the identification of an isolate as *S. epidermidis* by the Kloos and Schleifer method and as *S. hominis* by the Staph-Ident system. This disagreement occurred in 16 of the 27 discrepancies. A key reaction in the Staph-Ident system for *S. hominis* is negative phosphatase activity. The conventional method included phosphatase activity in the battery of tests performed, but the results were not part of the identification system. Of the 16 strains identified differently, 14 showed negative phosphatase activity in the conventional method and were perhaps more apt to be identified as *S. hominis* than as *S. epidermidis*. If these isolates are identified as *S. hominis*, the overall agreement between the two systems becomes 93%. Of these 16 isolates, however, 15 showed good anaerobic growth in thioglycolate broth within 24 h of inoculation, suggesting that they were *S. epidermidis*.

Novobiocin susceptibility testing was performed on all strains and used as adjunct testing for identification in both methods. Except for strains identified differently, all strains of *S. saprophyticus*, *S. cohnii*, and *S. xylosus* were resistant to novobiocin.

TABLE 2. Types and frequencies of discrepancies in identification of isolates obtained from blood and urine by the Staph-Ident system and by the conventional method

Species identified by:		No. of isolates identified differently by each method	
Staph-Ident system	Conventional method	Urine	Blood
S. capitis	*S. epidermidis*	1	0
S. simulans	*S. saprophyticus*	1	0
S. saprophyticus	*S. hominis*	1	0
S. hominis	*S. haemolyticus*	2	1
S. hominis	*S. epidermidis*	1	15
S. cohnii	*S. epidermidis*	0	1
S. saprophyticus	*S. haemolyticus*	0	2
S. cohnii	*S. haemolyticus*	0	1
S. aureus (coagulase negative)	*S. hominis*	1	0

The Staph-Ident system identified 95.1% of the isolates without repeat testing, whereas the conventional method identified 91.3% of the isolates after initial testing. The reproducibility of the methods was determined with American Type Culture Collection controls. Each new batch of medium for the conventional method or series of strips for the Staph-Ident system was tested with each of the American Type Culture Collection species. *S. epidermidis* ATCC 14990 and at least one other American Type Culture Collection strain were included in each run to identify the unknown strains. In all cases, the control strains (each tested at least three times) yielded the same reactions and identification.

DISCUSSION

Coagulase-negative staphylococci are now recognized as capable of causing a wide variety of infections. *S. saprophyticus* has been reported to cause urinary tract infections in women (2, 14, 17, 19, 28, 37). The observation that *S. saprophyticus* has a marked ability to attach to urethral cells (26) and has been implicated as a causative agent of nongonococcal urethritis (18) agrees with these reports. The isolation from blood cultures of coagulase-negative staphylococci, although frequently present because of skin contaminants (35), may represent septicemia (8, 24, 25) or endocarditis or both (5, 20); endocarditis is particularly likely in patients with prosthetic heart valves (7, 9, 34). In reference to prosthetic valve endocarditis, one study showed that some strains of coagulase-negative staphylococci produce a mucoid substance that facilitates adherence of the organisms to synthetic materials (3). Hence, these organisms may be particularly able to colonize and later infect a prosthetic device. Coagulase-negative staphylococci clearly have a tendency to colonize and infect synthetic materials (3, 7, 9, 11, 15, 23, 25, 31, 33, 34, 38, 39), and it would not be surprising if the production of a mucoid substance were involved.

In addition to urinary tract infections and endocarditis, coagulase-negative staphylococci have caused or have been implicated in a number of other infections, including vascular graft infections (23), infections associated with intravenous cannulae (25, 33), prosthetic joint infections (29, 38, 39), skin abscesses (40), peritonitis in patients receiving peritoneal dialysis (30), foodborne gastroenteritis (6), infections in immunosuppressed patients (4), and eye and ear infections (10, 18, 36). This diversity of infections is somewhat surprising considering the previously presumed lack of pathogenicity of these organisms. One factor that may have accounted for this lack of pathogenicity was the belief that coagulase-negative staphylococci produced neither coagulase nor the wide variety of extracellular enzymes formed by *S. aureus*. Gemmell and Roberts, however, showed that coagulase-negative staphylococci isolated from human infections do produce a limited number of toxins and enzymes (13). These investigators developed a technique in which a semisolid agar overlaying a monolayer of skin fibroblasts is used to assay the toxigenicity of these strains. They found that cytopathogenic strains were primarily *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus* biotypes. This theoretically greater pathogenicity for these biotypes has been confirmed in several clinical reports (27, 32).

Coagulase-negative staphylococci are clearly able to cause infections. There is also evidence that *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus* may be more pathogenic than other staphylococcal species. Thus, routine identification of species of coagulase-negative staphylococci is a potentially useful procedure in clinical microbiology laboratories. Certainly routine species identification is necessary to learn more about the epidemiology and virulence of these strains. Unfortunately, species identification of coagulase-negative staphylococci has been a tedious procedure requiring several days for results. The introduction of a rapid commerical method incorporating micromethods for biochemical identification of these staphylococci makes species identification more practical for clinical laboratories. The cost (<$2.00 per test) would not be prohibitive if the method were comparable to a conventional method and if the results were clinically relevant. Accordingly, we compared these two methods.

We found agreement to be very high (86% overall) between the conventional method and the Staph-Ident system. With regard to discrepancies in identification, neither of the methods was considered more accurate than the other. The only way such discrepancies can be settled is through DNA hybridization studies with prototype strains. Since different tests and indicators are used in each method, a direct correlation between the methods cannot be made. Such differences among dissimilar methods is not unusual. Nevertheless, we include comments on the most common of the discrepancies, in which 16 isolates were identified by the Staph-Ident system as *S. hominis* but by the conventional method as *S. epidermidis*. A comparison of common reactions of both methods showed most isolates to be phosphatase negative, mannitol negative, and trehalose negative. Results for another common reaction, mannose, varied between the methods. Thus, the negative phosphatase reaction appeared to be heavily weighted in favor of identifying the strains as *S.*

hominis in the Staph-Ident system. Conversely, the results of the thioglycolate tests suggested that the strains were *S. epidermidis*.

Regarding ease of performance, the Staph-Ident system is superior: it takes much less time to set up and is read after 5 h, resulting in same-day identification. The conventional method takes considerable time to set up, and readings continue for up to 72 h. Identification of organisms with the commercial strip is made with the sum of 10 reactions, thereby statistically weighing all 10 reactions together. On the other hand, reactions occurring in the conventional method are read one at a time, so that one aberrant reaction could lead to totally incorrect identification. Also, the reaction chart used in the conventional method is somewhat confusing, because the abundance of ± reactions may result in more subjective assessments of reactions than would result in the Staph-Ident system.

We did not attempt to evaluate the clinical relevance of the species biotypes. The biotypes in blood and urine cultures were found in frequencies similar to those reported by others (24, 27, 32). The most important finding is that the Staph-Ident system is a convenient, rapid method for species identification of coagulase-negative staphylococci gives results comparable to those from the conventional method. Thus, additional information concerning the clinical relevance of species identification could be obtained by routine use of such a method. Such information is needed before routine species identification of coagulase-negative staphylococci can be recommended.

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