Identification of Coagulase-Negative Staphylococci with the API Staph System

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Received 5 April 1982/Accepted 18 August 1982

A kit for the identification of staphylococci based on the biochemical criteria proposed by Kloos and Schleifer (W. E. Kloos and K. H. Schleifer, J. Clin. Microbiol., 1:82–88, 1975) is now available commercially. The system was used to identify 100 strains of coagulase-negative staphylococci isolated from various body sites as the primary etiological agent of clinical infection. The increasing importance of staphylococci and their resistance to antibiotics provided the rationale for such an investigation. Over 90% of the Staphylococcus isolates were easily identified as to their species on the basis of their reaction profile to 19 biochemical tests included in the kit. The remainder, which showed minor variations, could also be assigned to the various species. Identification of the isolates was as follows: S. epidermidis, 54; S. haemolyticus, 5; S. simulans, 2; S. hominis, 1; S. capitis, 4; S. cohnii, 2; S. warneri, 2; S. xylosus, 8; and S. saprophyticus, 22. Antibiotic sensitivity patterns were determined for each of the isolates. Novobiocin resistance was detected in strains of S. saprophyticus and S. xylosus, a property hitherto recognized in Micrococcus sp. type 3 causing bacteriuria in young women. Resistance to penicillin was widespread among strains of several species, whereas resistance to tetracycline was mainly confined to strains of S. epidermidis. General resistance to sulfamethoxazole and nalidixic acid was found among all strains, with almost uniform sensitivity to the other drugs tested.

Over the last 10 years there has been an increasing number of reports describing the incidence of coagulase-negative staphylococci in clinical infections, and these have been recently reviewed (4, 7). Such infections include those of the urinary tract (11, 13, 20, 21, 27, 32, 35), subacute bacterial endocarditis (16), bacteremia after ventriculoatrial shunt implantation (12), and miscellaneous infections (26, 29). The presence of a greater degree of resistance to a variety of antibiotics among such organisms (15, 19, 30) has fostered interest in their biotyping.

Among the earliest schemes for classification of staphylococci and micrococi was that of Baird-Parker (2, 3), which remained the method of choice until that of Kloos and Schleifer (18) with the identification of several new species of Staphylococcus (31). This latter typing scheme has been applied to clinical isolates of coagulase-negative staphylococci in numerous studies (5, 7, 14, 24, 25).

In each case, a relatively large number of biochemical and physiological tests were used. The present study attempted to evaluate the efficacy of a commercial kit of 19 tests which can be easily interpreted within 24 h, based on the scheme of Kloos and Schleifer (18), to identify 100 clinically significant coagulase-negative staphylococci isolated from a variety of infections.

MATERIALS AND METHODS

Source of isolates. A group of 86 isolates was sent from the Institute of Hygiene, Cologne, West Germany (courtesy of G. Pulverer), of which 20 were obtained as significant blood cultures, 27 from miscellaneous infections, and 30 from urinary tract infections. Another 14 were obtained from the Western Infirmary, Glasgow, Scotland, from patients with urinary tract infections. All isolates were tested for the production of coagulase and protein A (6).

Species identification. Each isolate was grown overnight on 10% horse blood agar plates to check for purity, and suspensions containing 5 × 10⁸ staphylococci per ml were made in a standard liquid medium [5 g of yeast extract, 2 g of (NH₄)₂SO₄, 5 g of sodium chloride, 10 ml of trace elements solution, and distilled water to 1 liter]; equal portions were used to aseptically inoculate each cupule of the gallery of tests enclosed within a rigid plastic tray (API Laboratory Products Ltd., Basingstoke, England). The galleries were incubated in a moist chamber for 24 h at 37°C before reading and interpretation of the results.

The biochemical reactions tested included the fermentations of glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, xylitol, melibiose, raffi-
nose, xylose, sucrose, α-methyl glucoside, and N-acetyl glucosamine, the reduction of nitrate, the hydrolysis of arginine and urea, phosphatase activity, and the formation of acetoin from pyruvate.

To demonstrate alkaline phosphatase activity, acetoin formation, and nitrate reduction, certain reagents were added to the respective cupules at the end of the incubation period. They were as follows: alkaline phosphatase, detection of hydrolysis of α-naphthyl phosphate by addition of (i) lauryl sulfate in Tris-hydrochloride and (ii) fast blue BB in 2-methoxyethanol; acetoin formation, detection by addition of (i) 40% potassium hydroxide and (ii) 6% α-naphthol in ethanol; nitrate reduction, detection by addition of (i) 0.8% sulfanilic acid in 5 N acetic acid and (ii) 0.6% dimethyl-1-naphthylamine in 5 N acetic acid.

Drug sensitivity tests. Each isolate was tested for its sensitivity to a variety of antibacterial agents by the method of Stokes and Ridgeway (34), using S. aureus Oxford as the control organism. The antibacterial agents included in this study were ampicillin (25 μg), cephalixin (30 μg), clindamycin (2 μg), fusidic acid (10 μg), gentamicin (10 μg), mexitillin (5 μg), nalidixic acid (30 μg), nitrofurantoin (200 μg), penicillin (1 IU), sulfamethoxazole (100 μg), tetracycline (30 μg), and trimethoprim (2.5 μg). Strains were designated as sensitive if the zone of inhibition was ≥6 mm smaller than that around the control organism. Strains were designated as resistant if the zone diameter was ≤12 mm when each sensitivity disk measured 6 mm.

RESULTS AND DISCUSSION

The interpretation of the various biochemical tests based on a distinctive color change occurring within 24 to 48 h after the initiation of incubation proved to be quite satisfactory as a basis for discrimination between different staphylococcal strains; the identification based on the codes provided by the manufacturers is shown in Table 1. A computer-assisted identification list was used to convert the codes to the most likely species.

In this fairly limited survey of clinical isolates of coagulase-negative staphylococci, 54 were identified as S. epidermidis and 22 were S. saprophyticus. Few of the other seven species of coagulase-negative staphylococci described by Kloos and Schleifer (18) were identified among the isolates. When the various species were separated according to their clinical source, S. epidermidis and S. saprophyticus accounted for most of the organisms isolated from the urinary tract (Table 2).

These results are broadly in keeping with the results of others (14, 24, 28) using biochemical tests and the same classification scheme and of Brun et al. (5) using a prototype miniaturized biochemical test system. However, Nord et al. (24) identified many more S. saprophyticus in their study, probably due to the fact that young women outpatients were featured in his population of patients. Papapetropoulos et al. (25) examined 120 strains of coagulase-negative staphylococci and attempted to classify them. They found that two strains elaborated a heat-stable DNase and were recognized as S. aureus. Of the others, S. epidermidis was isolated most frequently (76 of 118); S. saprophyticus (10 of 118), S. hominis (10 of 118), and the other species recognized by Kloos and Schleifer (18) were recognized much less often. In this study, 10 of 120 isolates could not be classified clearly.

Since little attempt hitherto has been made to monitor the antibiotic susceptibility patterns of the different species of coagulase-negative staphylococci, the 100 strains were tested against a range of antistaphylococcal drugs, including those used specifically in the treatment of urinary tract infections. The results are illustrated in Table 3.

In our study we have demonstrated that strains of S. epidermidis are more resistant than those of S. saprophyticus to tetracycline (24 versus 4.5%), penicillin (48 versus 36%), and nalidixic acid (90 versus 50%). With respect to the source of these species, nalidixic acid resistance is seen primarily in S. epidermidis and not in S. saprophyticus isolated from urinary tract infections. Equally worth noting, sulfamethoxazole resistance is more prevalent among S. saprophyticus than among S. epidermidis strains.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. isolated</th>
<th>No. of isolates from:</th>
<th>Endocarditis (blood cultures)</th>
<th>Urinary tract infections</th>
<th>Miscellaneous infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>54</td>
<td>12</td>
<td>30</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>22</td>
<td>4</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. simulans</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. hominis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. capitis</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>S. cohnii</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. warneri</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. xylosus</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
isolated from the urinary tract. Of 120 isolates investigated by Papapetropoulos et al. (25), about one-half were resistant to penicillin G, streptomycin, and tetracycline. Most were sensitive to methicillin, cephalaxin, cephapirin, gentamicin, and cotrimoxazole.

Hitherto, several groups (1, 17, 19, 36) have examined drug resistance patterns in *S. "albus*" without further resolution into species or due regard to clinical etiology. Sabath et al. (30) examined 35 *S. epidermidis* isolates for sensitivity to 65 antimicrobial agents and showed that the median minimal inhibitory concentrations of the penicillins, cephalosporins, and aminoglycosides were higher than those against *S. aureus* strains.

Estimates of methicillin resistance of from 15 to 40% of all coagulase-negative staphylococci isolated in a clinical situation have been reported (17, 33). Disk methods of testing coagulase-negative staphylococci probably underestimate the incidence of both methicillin resistance and resistance to cephalosporins (19), and cross-resistance between methicillin and cephalosporins among coagulase-negative staphylococci has been seen (15). None of the coagulase-negative staphylococci included in the present study were methicillin resistant.

When our strains were analyzed according to source, little difference in antibiotic susceptibility was seen among organisms isolated from blood (usually from cases of endocarditis or infected prosthetic heart valves) or from the urinary tract or miscellaneous skin infections (see Table 3).

This study has shown that clinically significant isolates of coagulase-negative staphylococci can be identified as to species within 24 h by using the API Staph system. Differences were recognized in the antibiotic susceptibility patterns of the isolates that may be relevant to the clinical condition from which they were isolated. In light of earlier discoveries that coagulase-negative staphylococci produce many of the toxins and enzymes more synonymous with *S. aureus* (7-10) and can initiate experimental infections in mice per se (10, 22, 23), there is merit in subjecting clinical isolates to more precise identification with this biochemical test system. Antibiotic susceptibility patterns will also yield valuable information regarding therapy of the underlying infection and have potential use as markers for hospital-acquired strains.

**ACKNOWLEDGMENT**

We thank API Laboratories, La Balme les Grottes, France, for donating the API Staph galleries used in the study.

**LITERATURE CITED**


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### TABLE 2. Antibiotic resistance patterns of coagulase-negative species

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. isolated</th>
<th>No. resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Novobiocin</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>S. capitis</em></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>S. cohnii</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>S. xylosus</em></td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>22</td>
<td>4</td>
</tr>
</tbody>
</table>

*a* Uniform sensitivity to the other drugs tested (methicillin, fusidic acid, clindamycin, gentamicin, cephalaxin, and nitrofurantoin) was seen with all 100 isolates.

### TABLE 3. Resistance patterns of coagulase-negative staphylococci classified according to source

<table>
<thead>
<tr>
<th>Isolates from:</th>
<th>No. isolated</th>
<th>% Resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Novobiocin</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>53</td>
<td>5.7</td>
</tr>
<tr>
<td>Miscellaneous infections</td>
<td>27</td>
<td>22.2</td>
</tr>
</tbody>
</table>

*a* Uniform sensitivity to the other drugs tested (methicillin, fusidic acid, clindamycin, gentamicin, cephalaxin, and nitrofurantoin) was seen with all 100 isolates.


