Identification of Staphylococci Isolated from Clinical Material

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Received for publication 16 February 1970

A total of 350 staphylococci isolated from various clinical sources were examined for bound and free coagulase, fermentation of mannitol, and deoxyribonuclease. The economical coagulase-mannitol-agar method of Esber and Faulcomer was found to be suitable for the detection of free coagulase and mannitol fermentation. A significant number of coagulase- and mannitol-negative staphylococci proved to be deoxyribonuclease-positive.

The slide (bound) and tube (free) coagulase (4, 5, 22) and mannitol-fermentation tests are the conventional tests for the characterization of micrococal isolates as Staphylococcus aureus and S. epidermidis (1, 2, 8, 9, 11-13, 15-17). S. aureus produces coagulase or ferments mannitol, or does both, whereas S. epidermidis is coagulase-negative and does not ferment mannitol. Weckman and Catlin (21) noted almost complete correlation between coagulase and deoxyribonuclease production among staphylococcal isolates, a finding confirmed by others (8, 13). Lately, several laboratories have reported the isolation of coagulase-negative, mannitol-negative, and deoxyribonuclease-positive staphylococci; some of these isolates gave additional reactions characteristic for S. aureus (19, 20; C. H. Zierdt and D. W. Golde, Bacteriol. Proc., p. 72-73, 1969).

This study served to analyze the coagulase, mannitol fermentation, and deoxyribonuclease patterns of 350 clinical isolates of staphylococci, employing a variety of procedures. Specifically, the reliability of the coagulase-mannitol-agar method of Esber and Faulcomer (6) was examined in an effort to reduce the cost of tests for free coagulase and mannitol fermentation. The deoxyribonuclease test of Jeffries et al. (10) was included for purposes of evaluation and determination of the number of deoxyribonuclease-positive, coagulase-negative staphylococcal isolates in our clinical material.

MATERIALS AND METHODS

A total of 350 strains of staphylococci isolated from clinical material were examined during a 3-month period (15 January 1969 through 15 April 1969); one laboratory strain each of S. aureus and S. epi-

dermidis served for control purposes. Each isolate was resubcultured to 5% sheep blood-agar to assure purity. The same pool of carefully controlled, citrated human plasma was used for all coagulase tests, unless otherwise specified. The slide coagulase test was performed by the technique of Cadness-Graves et al. (3). One colony of each isolate was inoculated into tubes containing 3 ml of coagulase-mannitol broth (BBL; 15% added human plasma). Isolates were streak-inoculated onto sectors of coagulase-mannitol agar plates (BBL; 15% added human plasma). Inoculated tubes and plates were read after incubation at 35 C for 18 hr. Tests for deoxyribonuclease were performed by the technique of Jeffries et al. (10). After incubation at 35 C for 18 hr, the plates were flooded with 1 N HCl. A zone of clearing, at least 3 mm in width, surrounding the growth of staphylococci was interpreted as a positive test for deoxyribonuclease.

RESULTS

The deoxyribonuclease, coagulase, and mannitol fermentation reaction patterns of the 350 isolates are listed in Table 1; 186 isolates (53.1%) yielded reaction pattern 1, and 70 isolates (20%) were negative for all tests (reaction pattern 21). A total of 204 isolates were tube coagulase-positive. The same number of isolates was positive for bound coagulase. Twelve strains yielded discrepant results, in that six isolates were positive for bound coagulase but negative for free coagulase, and vice versa. The combination of tube and slide coagulase tests characterized 210 of the 350 isolates as S. aureus. The tube mannitol-fermentation test disclosed an additional four strains of S. aureus. The combination of the two coagulase and the tube mannitol-fermentation tests characterized 219 isolates of S. aureus. A total of 256 isolates produced de-
oxyribonuclease. By designating deoxyribonuclease tests as positive when the zone of clearing was at least 3 mm in width, 27 isolates were scored as negative although they produced zones of clearing 1 to 2 mm in width.

The 350 isolates were examined in parallel with the plate coagulase-mannitol method of Esber and Faulcomer (6) to determine whether this medium yielded a large number of false-positive coagulase tests, as claimed by one group of workers (18) and disputed by others (7). As shown in Table 1, 249 isolates were positive for coagulase. However, 20 of these isolates (reaction patterns 12 and 16) were surrounded by a white-gray zone of opacity 1 to 2 mm in width; mannitol was not fermented, the tube and slide coagulase reactions were negative, and deoxyribonuclease production was variable. Thus, 229 of these 249 isolates were scored as specifically coagulase-positive. The coagulase-mannitol-agar test missed eight isolates (reaction patterns 4, 7, 14, 18, and 19) that would have been detected by the slide or tube coagulase and the tube mannitol-fermentation tests, whereas the coagulase-mannitol-agar test characterized 22 isolates as S. aureus (reaction patterns 11 and 17) that would have remained undetected otherwise.

One hundred additional staphylococci were slide-coagulase tested, by using human citrated plasma and rehydrated rabbit plasma (BBL). No discrepancy was noted; 62 strains were positive for bound coagulase with both substrates.

On the basis of the above findings, the following procedure was adopted for the routine identification of staphylococcal isolates in our laboratory. Each isolate, if present in sufficient quantity on the primary blood or chocolate-agar plates, is tested for bound coagulase. If positive, the strain is designated and reported as "Staphylococcus, coagulase positive." If negative, or if present in insufficient quantity, the isolate is subjected to the coagulase-mannitol-agar and deoxyribonuclease tests. If coagulase- and deoxyribonuclease-positive, the isolate is reported as "Staphylococcus, coagulase-positive, deoxyribonuclease-positive"; if negative for deoxyribonuclease, this is indicated on the report. Those isolates that are negative for both enzymes or produce deoxyribonuclease only are signed out as "Staphylococcus, coagulase-negative, deoxyribonuclease-negative (deoxyribonuclease-positive)." The noncommittal designations

### Table 1. Reaction patterns obtained with 350 clinical isolates of staphylococci

<table>
<thead>
<tr>
<th>Reaction pattern</th>
<th>Deoxyribonuclease production</th>
<th>Tube coagulase production</th>
<th>Slide coagulase production</th>
<th>Tube mannitol fermentation</th>
<th>Plate coagulase</th>
<th>Plate mannitol</th>
<th>No. of strains yielding respective pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>186</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>350</td>
</tr>
<tr>
<td>21</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>350</td>
</tr>
</tbody>
</table>

* Of 350 isolates, 241 strains were designated S. aureus, whereas 109 strains (reaction patterns 6, 12, 16, and 21) were identified as S. epidermidis.
**TABLE 2. Coagulase/mannitol and deoxyribonuclease reaction patterns of 500 additional staphylococcal isolates as related to clinical sources**

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Designation of isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> (coagulase-mannitol-positive, deoxyribonuclease-positive)</td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Ear</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Nose</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Eye</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Sputum</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Bronchial washing</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Blood</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Stool</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Urethra, vagina, cervix, lochia</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Wounds, abscesses</td>
<td>77</td>
<td>3</td>
</tr>
<tr>
<td>Catheter tips, Holter valves</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Joint, pleural, peritoneal fluid</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Urine</td>
<td>Catheterized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10^4 to 10^5 organisms/ml</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt; 10^5 organisms/ml</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Clean voided</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10^4 to 10^5 organisms/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>&gt; 10^5 organisms/ml</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>9</td>
</tr>
<tr>
<td>Per cent</td>
<td>45.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Isolates were examined with coagulase-mannitol-agar and deoxyribonuclease tests.

were chosen so as to not influence the clinical interpretation of laboratory reports. An additional 500 staphylococci (from 500 patients) that were characterized by the coagulase-mannitol-agar and deoxyribonuclease tests are listed in Table 2 according to their clinical sources. Nearly one-half of the isolates proved to be coagulase-mannitol- and deoxyribonuclease-positive; 1.8% of the *S. aureus* isolates were negative for deoxyribonuclease. One-third of the isolates were negative for all three tests. However, 84 of the isolates (16.8%) were coagulase- and mannitol-negative but deoxyribonuclease-positive.

**DISCUSSION**

The results obtained confirm the data of Graber et al. (7), namely that the coagulase-mannitol-agar method of Esber and Faulcomer (6) is suitable for the detection of free coagulase; however, 20 of 350 isolates produced a zone of nonspecific gray-white opacity on this medium. Similarly, Graber et al. (7) had reported 24 false-positive reactions among 400 strains tested, but it was not stated what the zones of nonspecific opacity, if any, appeared like. Originally, Esber and Faulcomer (6) had emphasized that a yellow zone of opacity surrounding the staphylococcal growth was indicative of coagulase production, whereas a canary yellow zone (apparently transparent) resulted from mannitol fermentation. Jenkins and Metzger (11) found that tube coagulase tests, performed with fresh human plasma, yielded significantly fewer positive results than slide coagulase tests utilizing the very same substrate, an observation at variance with our data.

The findings listed in Table 1 allowed one to adopt the coagulase-mannitol-agar method of Esber and Faulcomer to test for free coagulase and mannitol fermentation, even though occasional nonspecific reactions were encountered. Up to 12 isolates could be tested simultaneously...
per plate with ease, resulting in greatly reduced media and substrate expenses.

Branson (1) reported a lack of correlation between coagulase production and fermentation of mannitol by \textit{S. aureus}. Our data agree with those of Kimler (12, 13) and Person et al. (17). An unexpected finding was that a significant number of our coagulase- and mannitol-negative staphylococcal isolates proved deoxyribonuclease-positive. A small number of these isolates were derived from respiratory tract, blood, and cerebrospinal fluid specimens; the majority were isolated from wound and genitourinary tract specimens. Person et al. (17) pointed out that a number of their staphylococci gave false-positive and false-negative deoxyribonuclease tests, although no specific criteria were given.

It is known that individual colonies of \textit{S. aureus}, derived from single coagulase-positive colonies, may vary in their coagulase behavior (14). Likewise, Burns and Holtman (2) detected variants among strains of \textit{S. aureus} that were coagulase- or deoxyribonuclease-negative, or both. Thus, one should pick several colonies of staphylococci for coagulase, mannitol, and deoxyribonuclease tests, a practice easily achieved through the use of several sectors of coagulase-mannitol and deoxyribonuclease-agar plates, respectively.

ACKNOWLEDGMENTS

This study was supported by a grant from the United Medical Research Foundation of North Carolina.

We thank Pat Reich for her competent assistance.

LITERATURE CITED