Recognition of *Staphylococcus saprophyticus* in Urine Cultures by Screening Colonies for Production of Phosphatase

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Phenolphthalein diphosphate was incorporated into a primary blood agar medium for use in performing quantitative urine cultures. Phosphatase-negative staphylococci, such as *Staphylococcus saprophyticus*, were differentiated from phosphatase-positive species, such as *Staphylococcus epidermidis*, by spot testing colonies on filter paper saturated with 1 N NaOH. Phosphatase-positive colonies turned pink within seconds, and phosphatase-negative colonies showed no color. None of 55 *S. saprophyticus* isolates showed production of phosphatase on this medium. Of 193 consecutive coagulase-negative staphylococci isolated from the urine of 190 adolescent female patients, 84% were phosphatase positive, non-*S. saprophyticus* species; 16% were phosphatase-negative and indicated *S. saprophyticus* (22), *Staphylococcus haemolyticus* (4), *Staphylococcus simulans* (2), *Staphylococcus warneri* (1), and *Staphylococcus hominis* (1). Phosphatase activity was variable in the other flora encountered in the urine cultures. Mixtures of phosphatase-positive and -negative organisms did not cause false-positive reactions.

*Staphylococcus saprophyticus* is a relatively common cause of urinary tract infections in certain populations comprising sexually active female outpatients (2, 5, 7, 11, 22). This organism is second to coliforms as the most common cause of the acute urethral syndrome in women (11, 21).

The laboratory diagnosis of bacteriuria caused by *S. saprophyticus* may be accomplished by biochemical characterization or, presumptively, by demonstrating novobiocin resistance of a coagulase-negative staphylococcal isolate (8). These approaches may require a substantial addition to labor, materials, and time for processing urine cultures, especially when cultures with mixed flora or colony counts of <10^6 CFU/ml are processed. Although commercial identification systems (9, 12) and abbreviated schemes for recognition of *S. saprophyticus* based on growth in novobiocin-containing media (10, 14, 16) or other characteristics (13) have been described, these may not be satisfactory for use in many clinical laboratories because of cost, impracticality, or other factors.

We have developed a practical and inexpensive approach to the incorporation of recovery and identification methods for *S. saprophyticus* into the existing procedures for urine cultures in our laboratory. The principal component was spot-testing colonies of coagulase-negative staphylococci for phosphatase production. We found the majority of phosphatase-negative, coagulase-negative staphylococci from the urine of adolescent females to be *S. saprophyticus* and that all isolates of *S. saprophyticus* were correctly identified by a negative phosphatase spot test and determination of novobiocin resistance.

(Findings of this study have been reported in part previously [D. A. Pickett and D. F. Welch, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 934, 1983].)

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**MATERIALS AND METHODS**

**Urine cultures.** Specimens were obtained by the clean-void method from adolescent females who presented to an adolescent medicine clinic. Specimens not received by the laboratory within 2 h of collection were excluded from study. Primary plating media were Columbia blood agar, phosphate blood agar, and MacConkey agar. Urine was delivered to MacConkey and blood agar plates with 0.001- and 0.01-ml calibrated loops, respectively. Inocula were then streaked for colony isolation.

Phosphate blood agar contained 4% Columbia agar base (BBL Microbiology Systems), 5% defibrinated sheep blood, and 0.05% phenolphthalein diphosphate tetrasodium salt (Sigma Chemical Co.). The phenolphthalein diphosphate solution was sterilized at 0.5% by membrane filtration and added at a 1:10 dilution to the agar base after autoclave sterilization and cooling to 50°C. Culture plates were incubated in room air at 35°C for 24 to 48 h.

**Characterization of isolates.** Colonies appearing on blood agar media and resembling *Staphylococcus* isolates were tested for catalase and for coagulase production (by slide and tube tests) with rabbit plasma-EDTA (Difco Laboratories). The presence of hemolysis or pigment was noted. Coagulase-negative staphylococci were identified to the species level by methods previously described by Kloos and Smith (8). Broth media for carbohydrate utilization tests were incorporated into microtiter plates. Staphylococci were differentiated from micrococi by growth in 1% glycerol containing 0.4 µg of erythromycin per ml. Resistance to novobiocin was assessed by growth in Mueller-Hinton broth containing 2 µg of novobiocin per ml or by a zone size of <17 mm surrounding a 5-µg novobiocin disk (1). Approximately 10^6 CFU were delivered to wells of the microtiter plates with a Dynatech MIC 2000 inoculator, and the plates were incubated at 35°C for 48 h. Phosphatase production for reference testing was determined by a 4-h tube method in 0.5 ml of 0.1% p-nitrophenyl phosphate (Carr-Scarborough Microbiologials) with a heavy inoculum of an organism from...
blood agar. Phosphatase activity resulted in the appearance of a yellow color because of the liberation of p-nitrophenol.

The spot test for phosphatase production was performed as follows. A colony was removed from phosphate blood agar and touched to a piece of filter paper saturated with 1 N NaOH. A bright-pink reaction, indicative of free phenolphthalein, was interpreted as phosphatase production by the organism tested.

Quality control of the above tests was performed with one or more of the following appropriate organisms: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, or a strain of S. saprophyticus, Staphylococcus simulans, Staphylococcus haemolyticus, or Staphylococcus hominis (kindly provided by Peter B. Smith, Centers for Disease Control, Atlanta, Ga.).

RESULTS

There was complete agreement between the spot phosphatase and phosphatase tube tests with 130 isolates of S. epidermidis and 55 isolates of S. saprophyticus that were characterized conventionally. A total of 85% of the S. epidermidis isolates were positive and 100% of the S. saprophyticus were negative by both methods. Of 193 strains of coagulase-negative staphylococci consecutively isolated from the urine of 190 adolescent females, 84% were phosphatase positive and 16% were phosphatase negative by spot testing. Of the 30 phosphatase-negative isolates, 22 or 73% were S. saprophyticus, 4 were S. haemolyticus, 2 were S. simulans, 1 was S. hominis and 1 was Staphylococcus warneri. Determination of novobiocin resistance also enabled the correct identification of the 22 S. saprophyticus isolates. None of the eight other phosphatase-negative isolates were novobiocin resistant. Colonial morphology of bacteria growing on Columbia blood agar was not affected by incorporation of phenolphthalein diphosphate. Of the S. saprophyticus isolates, 60% were yellow pigmented and 100% were nonhemolytic. Hemolysis was demonstrated by all isolates of S. haemolyticus, and results of coagulase tests on S. aureus and other staphylococcal species were not affected by incorporation of phenolphthalein diphosphate.

Quantities of S. saprophyticus in urine cultures ranged from $10^3$ to $>10^5$ CFU/ml (Fig. 1). A total of 15 (68%) of these S. saprophyticus isolates were found in quantities of $\geq 10^5$ CFU/ml. Quantities of other coagulase-negative staphylococci were generally lower, ranging from $5 \times 10^2$ to $5 \times 10^4$ CFU of other phosphatase-negative species and from $10^2$ to $10^5$ CFU of phosphatase-positive species per ml of urine. The volume of urine cultured did not permit detection of fewer than $10^3$ CFU/ml. Of the 22 S. saprophyticus isolates, 17 were found in pure culture and 5 were the predominant organism in cultures containing mixtures of various other organisms. With the exception of one isolate, S. hominis, all of the eight other phosphatase-negative isolates were found in mixed culture and were not the predominant organism.

The results of spot tests for phosphatase activity of staphylococci and other organisms encountered in urine cultures are shown in Table 1. In practice, no false-positive reactions were found to occur as a result of testing the colony of a phosphatase-negative organism growing near the colony of a phosphatase-positive organism. It may be noted that phosphate blood agar is satisfactory for isolation and identification of Escherichia coli on the basis of indole spot testing (6).

DISCUSSION

Our results suggest that an approach similar to the indole spot test (6) used by many laboratories for the presumptive identification of E. coli may be applied to the identification of S. saprophyticus in urine cultures. The two approaches differ in the requirement for supplementation of blood agar with the substrate (phenolphthalein diphosphate) in the case of the phosphatase spot test. The mechanism of this test is similar to other methods for detecting phosphatase activity (3). Hydrolysis of the substrate liberates phenolphthalein, which then acts as a pH indicator and becomes pink when alkaline. The plate methods that have been described include the exposure of colonies to ammonia vapors, in
contrast to removal of colonies, for detection of free phenol-
phthalein.

*S. saprophyticus* has been associated with 7 to 42% of
urinary tract infections in young women (2, 5, 7, 11, 22).
Though not specifically addressed in our study, the inci-
dence of *S. saprophyticus* in our center has been studied
and is consistent with the range noted in other studies (D.
Am. Soc. Microbiol. 1982, C139, p. 294). In contrast, adult
inpatients of general hospitals (15) and in- or outpatients
of Veterans Administration hospitals (20) rarely become in-
fected with *S. saprophyticus*. The higher incidence rates
tend to be associated with sexually active patients and with
patients in an age group of 16 to 25 years. The adolescent
population of our hospital may be similarly characterized,
although the maximum age was 21 years.

The major difficulty in evaluating urine cultures that
contain coagulase-negative staphylococci concerns the fre-
quency with which these organisms are recovered. During
our prospective study of cultures from 190 adolescent fe-
male isolates, 193 staphylococcal isolates were recovered in
quantities of >10 CFU/ml of urine. Even in quantities of
>10^4/ml of urine, coagulase-negative staphylococci may
represent contamination because of heavy colonization of
the anterior urethra or delayed processing of the specimen.
On the other hand, incidental contamination of urine by low
numbers of coagulase-negative staphylococci occurs very
commonly and is practically unavoidable. In this context,
the differentiation of true bacteriuria associated with low
colonies from non-bacteriuria becomes complicated for the
laboratory. We therefore evaluated an approach to the
recognition of *S. saprophyticus* that would be simpler
than biochemical characterization or tests of novobiocin
resistance of all coagulase-negative staphylococci encoun-
tered in urine cultures.

A major advantage of the phosphatase spot test was the
elimination of *S. epidermidis*, which is phosphatase positive
and usually not clinically significant. Correct identification
of 22 *S. saprophyticus* isolates was achieved on the basis of
a negative phosphatase spot test and novobiocin resistance.

None of eight other phosphatase-negative isolates was re-
sistant to novobiocin. However, others have noted occa-
sional discrepancies between novobiocin resistance and other
methods for identification of *S. saprophyticus* (4). The use of
a 5-μg novobiocin disk as an additional test for accurate
identification of *S. saprophyticus* does not detract from the
potential usefulness of the phosphatase spot test as a screen-
ning method. A significant cost savings is involved by avoid-
ing the use of novobiocin testing of all phosphatase-positive

The predictive value of the negative phosphatase spot test
alone was 73%. When cultures of phosphatase-negative staphylococci containing <10^4 CFU/ml were disregarded,
the predictive values for identification of *S. saprophyticus*
increased to 87.5%. We have been unable to identify any

### TABLE 1. Phosphatase spot test reactions of organisms
encountered in urine cultures

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. positive/ no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>111/130 (85)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12/12 (100)</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>0/75 (0)</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>0/3 (0)</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>0/6 (0)</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>0/14 (0)</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>2/14 (14)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2/11 (18)</td>
</tr>
<tr>
<td><em>Klebsiella</em> sp.</td>
<td>7/13 (54)</td>
</tr>
<tr>
<td><em>Citrobacter</em> sp.</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>1/8 (13)</td>
</tr>
<tr>
<td><em>Virdans streptococci</em></td>
<td>0/7 (0)</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>0/4 (0)</td>
</tr>
<tr>
<td><em>Gardnerella</em> sp.</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td><em>Lactobacillus</em> sp.</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td><em>Serratia</em> sp.</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td><em>Enterobacter</em> sp.</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td><em>Colonibacterium</em> sp.</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td><em>Candida</em> sp.</td>
<td>0/11 (0)</td>
</tr>
</tbody>
</table>

LITERATURE CITED

1. Almeida, R. J., and J. H. Jorgensen. 1982. Use of Mueller-
Hinton agar to determine novobiocin susceptibility of coagu-

2. Anderson, J. D., A. M. Clarke, M. E. Anderson, J. L. Isaac-
Renton, and M. G. McLoughlin. 1981. Urinary tract infections

In C.-G. Héden (ed.), Principles of biochemical tests in diag-

biochemical identification of coagulase-negative staphylococci.

Tuckwell, and A. L. Hilton. 1978. Urinary tract infection in
young women, with special reference to *Staphylococcus*.