

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 $<10^5$ 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].)

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 micrococci 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 10^4 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 Microbiologicals) with a heavy inoculum of an organism from

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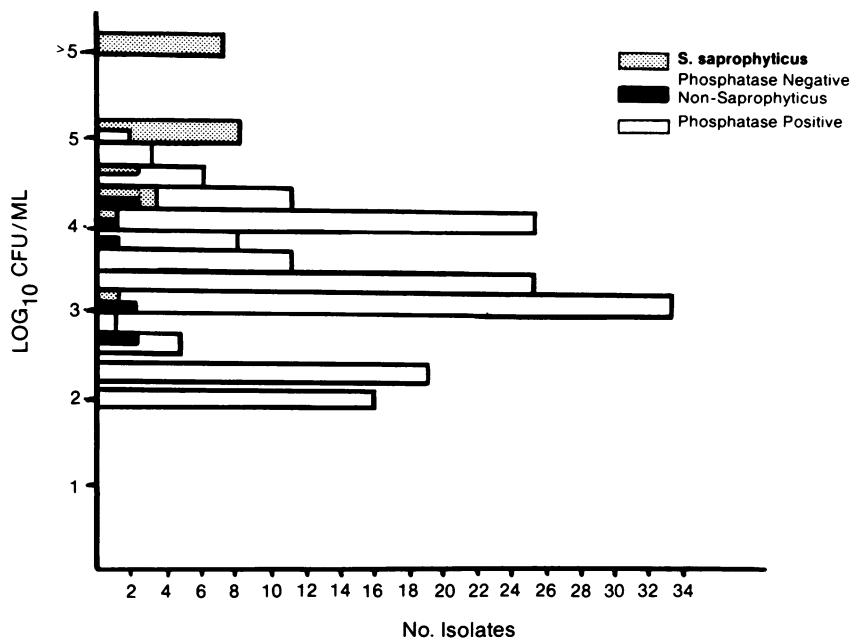


FIG. 1. Quantitation of coagulase-negative staphylococci in urine cultures of adolescent females.

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 5×10^2 to 5×10^4 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 10^2 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

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

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

contrast to removal of colonies, for detection of free phenolphthalein.

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 incidence of *S. saprophyticus* in our center has been studied and is consistent with the range noted in other studies (D. Pickett, P. J. Rettig, and D. F. Welch, Abstr. Annu. Meet. 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 infected 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 frequency with which these organisms are recovered. During our prospective study of cultures from 190 adolescent females, 193 staphylococcal isolates were recovered in quantities of >100 CFU/ml of urine. Even in quantities of >10⁴/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 colony counts 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 encountered 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 resistant to novobiocin. However, others have noted occasional discrepancies between novobiocin resistance and other methods for identification of *S. saprophyticus* (4). The use of a 5- μ g 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 screening method. A significant cost savings is involved by avoiding the use of novobiocin testing of all phosphatase-positive staphylococci.

The predictive value of the negative phosphatase spot test alone was 73%. When cultures of phosphatase-negative staphylococci containing <10⁴ CFU/ml were disregarded, the predictive values for identification of *S. saprophyticus* increased to 87.5%. We have been unable to identify any phosphatase-positive strains of *S. saprophyticus*, but the percentage of phosphatase-positive *S. saprophyticus* isolates that has been cited in other studies ranges from 0% (19) to 13% (17). The explanation for the variability of this characteristic among different studies is unclear, but it may be noted that the substrate phenolphthalein diphosphate is relatively unstable. Degradation, which may occur in media or the original substance if not stored at -20°C, apparently results in disassociation of the phenolphthalein and phosphate and results in false-positive reactions. Media prepared in our laboratory are stable for at least 3 weeks. The phosphatase test results might also depend on the method used (18) or might be influenced by interpretative difficulties.

S. saprophyticus was usually (68%) found in numbers of $\geq 10^5$ CFU/ml of urine. Other phosphatase-negative staphylococci were found in smaller numbers. However, one patient, whose urine contained only 10³ CFU of *S. saprophyticus* per ml of urine, was assessed clinically as having a urinary tract infection. Therefore, all coagulase-negative staphylococci should be spot tested for phosphatase production, and then only the phosphatase-negative staphylococci need to be tested for novobiocin resistance.

Columbia sheep blood agar supplemented with 0.05% phenolphthalein diphosphate is useful as a differential medium for screening staphylococcal isolates. Most phosphatase-negative staphylococci recovered from the urine of adolescent females are *S. saprophyticus*. Our laboratory now uses phosphate blood and MacConkey agar plates as primary isolation media for most urine cultures. The combination of these two media permits rapid and inexpensive presumptive identification of the two most common urinary tract pathogens, *E. coli* and *S. saprophyticus*, in young adult females.

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