

## Significance of Urinary Isolates of Coagulase-Negative *Micrococcaceae*

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Of 16,347 urine cultures submitted to the hospital laboratory, 68 (0.4%) specimens from 50 patients yielded  $>10^4$  coagulase-negative staphylococci/ml in pure culture. A total of 62 of 63 organisms available for study were staphylococci: 45 *Staphylococcus epidermidis* (predominantly subgroup 1), 15 *Staphylococcus saprophyticus* (subgroup 3), and 2 *Staphylococcus aureus*. Twenty-one patients had "probable" urine infections. Eight patients had two or more positive urine cultures, and all isolates from the same patient were identical (by morphology, antibiotic susceptibility, and hemolytic pattern). Nine (75%) of the 12 isolates of *S. saprophyticus*, which were novobiocin resistant and nonhemolytic on the synergistic hemolysis test, were from patients with probable urinary infection. Eight were young women with acute symptoms and pyuria. Differences in the glucose and mannitol fermentation tests with different media may lead to difficulties in identification. Novobiocin resistance cannot be relied upon to differentiate isolates of *S. saprophyticus* from *S. epidermidis*.

Staphylococci and micrococci both belong to the family *Micrococcaceae* and are classically distinguished on the basis of their ability to utilize glucose anaerobically. This definition has recently been challenged so that some microorganisms formerly classified as *Micrococcus*, subgroups 1 through 4, have now been reclassified on the basis of their deoxyribonucleic acid guanine plus cytosine content as members of the species *Staphylococcus saprophyticus* (3). Thus, staphylococci are now divided into three species (*S. aureus*, *S. epidermidis*, and *S. saprophyticus*) and various subgroups on the basis of biochemical tests. Coagulase production is the most commonly used indicator of pathogenicity; most isolates of *S. aureus* are coagulase positive, whereas all other staphylococci and micrococci are coagulase negative.

Until recently, urinary isolates of coagulase-negative staphylococci were regarded as "contaminants" or, occasionally, as opportunistic pathogens. There are now several reports that implicate these organisms as the causative agent in from 7 to 26% of urinary infections (8, 9). One type, formerly referred to as *Micrococcus* subgroup 3 and now classified as *S. saprophyticus* (subgroup 3), has been reported as having a predilection for the urinary tract. Typically, women aged 16 to 25 years have been affected, and the microorganism produces an acute inflammatory response (1, 9). Others

have claimed that a distinctive feature of this organism is its resistance to novobiocin (10).

In this survey, unlike others: (i) coagulase-negative staphylococci were infrequent urinary isolates; (ii) staphylococci (not micrococci) predominated; and (iii) 2 (13%) of 15 isolates of *S. saprophyticus* (subgroup 3) were novobiocin sensitive. Attention is drawn to some of the technical difficulties in classifying urinary isolates of coagulase-negative staphylococci.

### MATERIALS AND METHODS

**Bacteriological methods.** Members of the *Micrococcaceae* were identified according to the schema of Baird-Parker (3). All organisms were tested for fermentation (anaerobic) of glucose and mannitol using King's oxidation-fermentation medium (13) and in the standard medium recommended by the ICSB subcommittee on the taxonomy of staphylococci and micrococci (11). Aerobic utilization of arabinose, lactose, maltose, and mannitol were determined using the method of Baird-Parker (2). Phosphatase production was determined on 1.5% agar containing 0.5% tryptone and 0.01% sodium phenolphthalein diphosphate. Apparent mannitol fermenters were tested for heat-stable nuclease according to the method of Barry et al. (4). In addition, all organisms were tested on 2% sheep blood agar for synergistic hemolysis by making a single streak of the test organism perpendicular to and 2 to 3 mm distant from a single streak of a  $\beta$ -toxin-producing *S. aureus*. Plates were examined for the presence of hemolysis (synergistically and alone) after 18 to 24 h of incubation at 35 C. Antibiotic susceptibilities were

determined by the disk diffusion method of Bauer et al. (5). A 5- $\mu$ g novobiocin disk was added to the susceptibility plate; resistance to novobiocin was indicated by a zone of inhibition of 9 mm or less. All strains sensitive to novobiocin produced a zone of inhibition equal to or greater than 22 mm; there were no organisms with zones of inhibition of 10 to 21 mm.

The staphylococcal isolates were phage-typed by standard techniques used for the characterization of coagulase-positive staphylococci (6). Concentrated phage at 100 times the routine test dilution was used also. There were no phage lytic reactions observed at either routine or concentrated dilutions.

In three patients, attempts to localize the site of urinary infection were undertaken by determining the presence of antibody coating the bacteria (12).

**Clinical methods.** Whenever possible, patients were seen and examined by one of us (D.N.W.) and were questioned with regard to both present symptoms and past history of urinary tract infection, bladder instrumentation, and previous antimicrobial therapy. A patient was regarded as having a "probable" urinary infection if two or more consecutive clean-voided or a single urethral catheter (refers to catheterization for diagnostic purposes and excludes those patients with long-standing catheters *in situ*) specimen contained equal to or greater than  $10^4$  organisms in pure culture per ml of urine. In addition, patients with acute urinary symptoms, pyuria, and a single clean-voided specimen with  $10^4$  or more organisms/ml were regarded as having "probable" infection. Pyuria counts were made after centrifugation for 5 min at 2,000 to 2,500 rpm and graded as: 1+ (4 to 15 leukocytes/high power field [HPF]); 2+ (15 to 40 leukocytes/HPF); and 3+ (greater than 40 leukocytes/HPF).

## RESULTS

During the 9-month study period, of 16,347 urine cultures routinely submitted to the Diagnostic Microbiology Laboratory of the University of Minnesota Hospitals, 68 (0.4%) specimens, from 50 patients, yielded equal to or greater than  $10^4$  coagulase-negative staphylococci/ml in pure culture. In 42 isolates the colony count was equal to or greater than  $10^5$  organisms/ml. Five patients had two morphologically different isolates of coagulase-negative staphylococci in the same urine specimen. In three of these patients, the isolates were identical on the basis of three tests (fermentation reactions, antibiotic susceptibility, and pattern of synergistic hemolysis with a beta-toxin-producing isolate of *S. aureus*). In one patient, the two isolates were different in all three respects, whereas in the other patient the two isolates differed only in the hemolytic pattern. Those organisms from a given culture that were identical in all respects (with the exception of minor differences in colonial mor-

phology) were considered to be the same organism.

Sixty-three organisms from 48 patients were available for study; 62 were staphylococci and one was a *Micrococcus*. Of the 62 staphylococci, 45 were *S. epidermidis*, 15 were *S. saprophyticus*, and 2 were *S. aureus*. All the *S. saprophyticus* isolates belonged to subgroup 3. They fermented glucose and mannitol by King's method but were negative in the ICSB method. The *S. epidermidis* and *S. aureus* fermented the appropriate sugars in both methods. The two coagulase-negative *S. aureus* isolates produced heat stable nuclease. None of the mannitol-fermenting organisms (by King's method) were phage typable at the routine test dilution.

**Coagulase-negative, mannitol-fermenting (King's method) organisms.** There were 17 isolates of mannitol-fermenting organisms (15 *S. saprophyticus* and 2 *S. aureus*) from 17 patients. Twelve isolates (all *S. saprophyticus*) had identical hemolytic patterns (no hemolysis either alone or synergistically) and were novobiocin resistant. All isolates occurred in women, and the 10 patients with adequate clinical documentation were acutely symptomatic. Pyuria was documented in nine of these women. Eleven women were aged between 19 and 33 years, the exception being a 61-year-old woman. The other five mannitol-fermenting organisms included two isolates of *S. aureus*, and all had different hemolytic patterns. Only one of these isolates was resistant to novobiocin. Three were isolated from males, and none of the patients presented with acute symptoms or with pyuria.

**Multiple urinary isolates from the same patient.** Eight patients had equal to or greater than  $10^4$  coagulase-negative staphylococci/ml isolated from urine specimens collected on 2 or more different days. Five patients had two, and one each had three, four, and five, isolates of coagulase-negative staphylococci in pure culture. Isolates from each patient were identical.

**Localization studies.** One of the three patients (all infected with *S. saprophyticus*) in whom the site of urinary infection was determined had an "upper" (or renal) focus, whereas the remaining two had a "lower" (or bladder) focus.

**Clinical data.** Fifty patients, with an age range from 1 week to 82 years, were studied. There were 31 females (mean age, 30 years) and 19 males (mean age, 43 years). Clinical data was in some way incomplete in 13 patients. Twenty-three (62%) of the 37 remaining patients gave a past history of urinary tract infection, 24 (65%) of 37 patients gave a past history

of urinary tract instrumentation, and 23 (59%) of 39 patients were acutely symptomatic. A diagnosis of probable urinary tract infection was made in 21 patients (Table 1). Nine of the 10 patients presenting with acute symptoms and pyuria were young females infected with *S. saprophyticus*. The degree of pyuria was usually 2+ or 3+. All the remaining patients with probable urine infection yielded *S. epidermidis* subgroup 1. Ten patients (20%) had documented evidence of previous significant urinary isolates of coagulase-negative staphylococci within the preceding 2 years. Two of these patients were currently infected with *S. saprophyticus*, and all the remainder were infected with *S. epidermidis* subgroup 1. Seven of these 10 patients had probable urine infection.

### DISCUSSION

This study differs from other reports in several respects. Not only is the frequency of urinary isolates of coagulase-negative organisms lower than previous surveys, but, in addition, staphylococci rather than micrococci predominated. These differences may be due to a number of factors, such as geographical considerations (most previous studies on the significance of coagulase-negative staphylococci were from European countries), the nature of the patients studied, and differences in laboratory techniques. Maskell, using very similar criteria for inclusion as a "positive" urine culture (equal to or greater than  $10^4$  organisms/ml of urine in pure culture) found the prevalence rate in domiciliary practice in southeastern England to be about 1.7% (175 of 10,065 specimens submitted; 9) compared with 0.4% in this hospital-based survey.

The predominance of staphylococci in this survey is, in part, due to differences in definition and the transfer of the old *Micrococcus* subgroups 1 through 4 to the genus *Staphylococcus*. These organisms, currently referred to as *S. saprophyticus*, do present technical difficulties in identification to the routine labora-

tory. By the standard ICSB test for glucose and mannitol fermentation they appear to be non-fermenters, whereas they do ferment these sugars by the more sensitive test of King, Corse and Williams (7), who used a nonstandard fermentation test, reported 16 strains of coagulase-negative, mannitol-fermenting staphylococci very similar to those reported here. The inadequacy of the standard fermentation test is recognized (3, 4), but a completely satisfactory substitute test has not been defined. It has been suggested that novobiocin resistance may identify these organisms in the laboratory (10). However, our finding of 2 (13%) of 15 apparent *S. saprophyticus* (subgroup 3) sensitive to novobiocin and Corse and Williams' findings of resistant *S. epidermidis* argue against using this test as the criterion for presumptive identification of these organisms.

A combination of tests seems indicated for the presumptive identification of isolates of *S. saprophyticus*. These organisms form a homogeneous group. All nine implicated in probable urinary tract infections were novobiocin resistant, were nonhemolytic in the synergistic hemolysis test, and fermented both glucose and mannitol in King's oxidation-fermentation medium but not in the standard medium of the ICSB Subcommittee. One of the three patients infected with *S. saprophyticus* in whom the site of urinary infection was determined had an upper (or renal) focus. Subsequent localization studies of two patients with equal to or greater than  $10^5$  *S. saprophyticus*/ml of urine revealed one upper and one lower focus (D. N. Williams, J. E. Lockey, and L. Reye, unpublished data). Of the remaining six isolates of *S. saprophyticus*, three shared all of these characteristics, whereas the remaining three were clearly different, in that they produced synergistic hemolysis, and two of the three were novobiocin sensitive. Thus, 9 (75%) of 12 isolates of *S. saprophyticus* showing a pattern of nonhemolysis on the hemolysis test and novobiocin resistance were from patients with probable urinary tract infection. Both coagulase-negative isolates of *S. aureus* showed synergistic hemolysis and novobiocin sensitivity.

Twelve (27%) of the 45 isolates of *S. epidermidis* were from patients with probable urinary tract infections. With two exceptions, isolates of *S. epidermidis* were characterized as subgroup 1. A diagnosis of probable urinary infection was made on the basis of multiple isolates from the same patient, and, unlike the situation with *S. saprophyticus*, there was no uniform clinical pattern.

In this study, three simple criteria (colonial morphology, antibiotic susceptibility, and pat-

TABLE 1. Probable urinary tract infection (21 patients)

Parameter	No. of patients
Symptomatic	
With 1 positive culture and pyuria	10 <sup>a</sup>
With 1 positive catheter culture	1
With 2 or more positive cultures	7
Asymptomatic	
With 1 positive catheter culture	2
With 2 positive cultures	1

<sup>a</sup> Nine *S. saprophyticus*.

tern of synergistic hemolysis) were found to be useful in differentiating between isolates in the same patient. A previous study has confirmed the validity of this approach, using phage typing as the definitive criterion (14). It would appear, therefore, that coagulase-negative staphylococci can indeed produce urinary infection but not with the frequency previously reported.

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