Use of a Novobiocin-Containing Medium for Isolation of
Staphylococcus saprophyticus from Urine

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The use of a novobiocin-containing medium provided little benefit over
observable quantitative growth on blood agar in detecting Staphylococcus sapro-
phyticus in urine cultures.

Recent studies in both Europe and the United
States have established that Staphylococcus
saprophyticus, a novobiocin-resistant, coagu-
lase-negative staphylococcus, is a frequent
cause of urinary tract infections in young women
(1, 3, 5, 7). In these studies, the majority of
infections were characterized by bacterial
counts of \( \geq 10^5 \) per ml of urine. We and others
(1, 2, 9) have reported culturing S. saprophyti-
cus from midstream urine (MSU) in quantities of
\( 10^3 \) to \( 10^4 \) colonies per ml in symptomatic
patients. In these patients, associated findings
such as the presence of pyuria, the absence of
other uropathogens, isolation of S. saprophyti-
cus from suprapubic aspirates, and response to
specific therapy strongly support the etiological
role of the organism despite the low number of
bacteria found in MSU specimens.

S. saprophyticus has a longer generation time in
urine than do members of the Enterobac-
teriaceae (7). This observation provides a possi-
ble basis for the occurrence of infections with
low quantitative counts and suggests that infec-
tions with \( 10^3 \) to \( 10^4 \) S. saprophyticus organisms
may not be uncommon. Furthermore, such in-
fec tions with low bacterial counts in MSU speci-
mens may be overlooked in microbiology labo-
ratories in which sparse growth of gram-positive
organisms is often regarded as contamination.
To determine the proportion of S. saprophyticus
infections characterized by low bacterial counts
in MSU and the ability of our routine microbio-
logical practices to detect these infections, we
incorporated a novobiocin-containing medium
into the microbiological evaluation of urine
specimens obtained from acutely symptomatic
women seen at a university health clinic.

MSU specimens from ambulatory women pre-
senting to the University of Washington Student
Health Clinic, Seattle, with symptoms of dys-
uria, urgency, or frequency of urination were
quantitatively cultured on blood and MacCon-
key agar plates for identification of Enterobac-
teriaceae, Streptococcus faecalis, and staphylo-
occi, as previously described (8). In addition,
from June 1981 through July 1982, MSU speci-
mens were cultured on Trypticase soy agar
(BBL Microbiology Systems) supplemented
with 0.3 g of yeast extract and 1.6 \( \mu \)g of novobi-
cin per ml (3). After overnight incubation at
37°C, blood agar and MacConkey plates were
examined for growth. Colony morphology, Gram
stain, and coagulase tests were used to iden-
tify and characterize staphylococcal isolates
from blood agar plates. As per routine, when
growth of two or more different colony types of
gram-positive organisms was encountered on
blood agar without a predominant organism
identified, specimens were classified as contain-
ing "mixed gram-positive flora," and no further
evaluation was performed. At the same time,
novobiocin-containing plates were examined by
an independent observer without knowledge of
results on the other plates. Colony morphology
and Gram stain were used to identify staphylo-
occi on these plates. In selected instances,
subcultures of isolates to blood agar were per-
formed to enhance differentiation by colony
morphology of staphylococci and enterococci.

Growth of coagulase-negative staphylococci
or novobiocin-containing medium was considered
to be a presumptive indication of S. saprophyti-
cus (6).

A total of 333 MSU specimens were evaluated
in parallel by using routine and novobiocin-
containing media (Table 1). With the standard
medium, 79 specimens were reported as contain-
ing only gram-negative organisms, and 10
showed no growth; S. saprophyticus was not
isolated from any of these specimens on the
novobiocin plates. A total of 244 specimens
contained gram-positive organisms on the blood
agar medium either alone (63 specimens) or
mixed with gram-negative rods (181 specimens).
Cultures of 43 of these 244 urine specimens
revealed coagulase-negative staphylococci as
Gram-negative organisms identified in 27 predominant positive organisms, revealed these containing these organisms (Table 2). Among the 27 specimens containing S. saprophyticus, standard cultures of 25 revealed these to be the predominant gram-positive organism, and in 24 of these 25 (96%), bacterial counts were \( \geq 10^4 \) organisms per ml of urine (Table 2). An additional 18 specimens with predominant growth of coagulase-negative staphylococci failed to grow on novobiocin-containing medium. In contrast to the 25 specimens containing S. saprophyticus, only 5 (28%) of these 18 staphylococci isolates were observed to have bacteria counts of \( \geq 10^4 \) organisms per ml of urine (3.7 \( \times 10^{-7} \) by Fisher’s exact test) (Table 2).

A total of 170 MSU specimens were reported as containing “mixed gram-positive flora” on blood agar. Only two (1%) of these had S. saprophyticus on the novobiocin-containing medium (bacteria counts of \( 10^3 \) and \( 10^2 \) per ml of urine, respectively).

In this study, culturing MSU specimens on a novobiocin-containing medium did not significantly increase the isolation rate of S. saprophyticus. In this patient population, the quantities of these organisms encountered in MSU specimens made their detection by routine microbiological practices quite possible. Furthermore, S. saprophyticus was infrequently found hidden within the classification “mixed gram-positive flora.” Thus, the method routinely used in our laboratory, inoculation of urine on blood agar plates, proved to be an equally sensitive technique for recognizing S. saprophyticus infection.

Characteristically S. saprophyticus is resistant to novobiocin (4). Other coagulase-negative staphylococci share this property, but their occurrence in urinary specimens is so infrequent that demonstration of resistance to novobiocin by a coagulase-negative staphylococci isolated from urine is strong presumptive evidence for its identification as S. saprophyticus (6). In our laboratory, we now determine novobiocin susceptibility by using sensitivity disks containing 5 \( \mu g \) of novobiocin. This provides a simple method for prompt identification of these infections in urine specimens containing a predominant growth (usually \( \geq 10^6 \) organisms per ml) of coagulase-negative staphylococci.

### TABLE 1. Recognition of coagulase-negative staphylococci by using standard isolation and novobiocin-containing medium

<table>
<thead>
<tr>
<th>Pattern of growth on standard isolation (n)</th>
<th>No. (%) of organisms containing coagulase-negative staphylococci</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Standard isolation</td>
</tr>
<tr>
<td>Gram-negative organisms only (79)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gram-positive organisms only (63)</td>
<td>29 (46)</td>
</tr>
<tr>
<td>Gram-positive and gram-negative organisms (181)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>No growth (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (333)</td>
<td>43 (13)</td>
</tr>
</tbody>
</table>

the predominant gram-positive organism growing on blood agar. S. saprophyticus was identified in 27 of these 244 specimens with the novobiocin plate. Among the 27 specimens containing S. saprophyticus, standard cultures of 25 revealed these to be the predominant gram-positive organism, and in 24 of these 25 (96%), bacterial counts were \( \geq 10^4 \) organisms per ml of urine (Table 2). An additional 18 specimens with predominant growth of coagulase-negative staphylococci failed to grow on novobiocin-containing medium. In contrast to the 25 specimens containing S. saprophyticus, only 5 (28%) of these 18 staphylococci isolates were observed to have bacteria counts of \( \geq 10^4 \) organisms per ml of urine (3.7 \( \times 10^{-7} \) by Fisher’s exact test) (Table 2).

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### TABLE 2. Correlation of growth of coagulase-negative staphylococci and mixed gram-positive flora on novobiocin-containing medium with quantitative growth on blood agar plates

<table>
<thead>
<tr>
<th>Growth on blood agar</th>
<th>Growth on novobiocin-containing medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td>No. of organisms per ml of urine</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>( \geq 10^5 )</td>
</tr>
<tr>
<td>(coagulase-negative)</td>
<td>( 10^2-10^4 )</td>
</tr>
<tr>
<td></td>
<td>( &lt;10^2 )</td>
</tr>
<tr>
<td>Mixed gram-positive flora</td>
<td>( \geq 10^3 )</td>
</tr>
<tr>
<td></td>
<td>( 10^2-10^4 )</td>
</tr>
<tr>
<td></td>
<td>( &lt;10^2 )</td>
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### LITERATURE CITED