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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 saprophyticus* in urine cultures.

Recent studies in both Europe and the United States have established that *Staphylococcus saprophyticus*, a novobiocin-resistant, coagulase-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. saprophyticus* 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. saprophyticus* 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 *Enterobacteriaceae* (7). This observation provides a possible basis for the occurrence of infections with low quantitative counts and suggests that infections with 10^3 to 10^4 *S. saprophyticus* organisms may not be uncommon. Furthermore, such infections with low bacterial counts in MSU specimens may be overlooked in microbiology laboratories 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 microbiological 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 presenting to the University of Washington Student Health Clinic, Seattle, with symptoms of dysuria, urgency, or frequency of urination were quantitatively cultured on blood and MacConkey agar plates for identification of *Enterobacteriaceae*, *Streptococcus faecalis*, and staphylococci, as previously described (8). In addition, from June 1981 through July 1982, MSU specimens were cultured on Trypticase soy agar (BBL Microbiology Systems) supplemented with 0.3 g of yeast extract and 1.6 μg of novobiocin 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 identify 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 containing "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 staphylococci on these plates. In selected instances, subcultures of isolates to blood agar were performed to enhance differentiation by colony morphology of staphylococci and enterococci. Growth of coagulase-negative staphylococci on novobiocin-containing medium was considered to be a presumptive indication of *S. saprophyticus* (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 containing 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

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

Pattern of growth on standard isolation (*n*)	No. (%) of specimens containing coagulase-negative staphylococci	
	Standard isolation	Novobiocin-containing medium
Gram-negative organisms only (79)	0 (0)	0 (0)
Gram-positive organisms only (63)	29 (46)	20 (32)
Gram-positive and gram-negative organisms (181)	14 (8)	7 (4)
No growth (10)	0 (0)	0 (0)
Total (333)	43 (13)	27 (8)

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 ($P = 2.7 \times 10^{-6}$ 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).

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

Growth on blood agar		Growth on novobiocin-containing medium	
Organism	No. of organisms per ml of urine	Present	Absent
Staphylococci (coagulase-negative)	$\geq 10^5$	22	1
	10^2–10^4	2	12
	$<10^2$	1	5
Mixed gram-positive flora	$\geq 10^5$	0	14
	10^2–10^4	2	142
	$<10^2$	0	12

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 μg of novobiocin. This provides a simple method for prompt identification of these infections in urine specimens containing a predominant growth (usually $\geq 10^4$ organisms per ml) of coagulase-negative staphylococci.

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