

## Characterization of Coagulase-Negative Staphylococci from Urinary Tract Specimens

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Species of coagulase-negative staphylococci isolated from urine specimens submitted from both inpatients and outpatients to the clinical microbiology laboratory of a teaching hospital were identified with a biotyping system, with species then correlated by clinical features and antimicrobial susceptibility. Of 145 isolates, 102 (70%) were *Staphylococcus epidermidis*, 24 (17%) were *Staphylococcus saprophyticus*, 7 (4.7%) were *Staphylococcus haemolyticus*, 4 (2.8%) were *Staphylococcus hominis*, 3 (2.1%) were *Staphylococcus simulans*, and 5 (3.4%) were other species. Features characterizing persons with bacteriuria with *S. saprophyticus* compared with bacteriuria with any other species included female sex (95% versus 52%), young age (median age, 22 years versus 61 years), ambulatory status (hospital outpatients, 86% versus 23%), and absence of indwelling catheters (4.5% versus 49%). All other coagulase-negative staphylococci were isolated in a setting suggesting nosocomial acquisition, were more frequently resistant to common antimicrobial agents (42% multiply resistant versus 4.2% of *S. saprophyticus*), and were not distinguished by clinical features. Novobiocin susceptibility, with a sensitivity of 100% and specificity of 96%, provided a simple and reliable test for differentiation of *S. saprophyticus* from other coagulase-negative staphylococci and should be routinely used for urinary tract specimens in the clinical laboratory.

The importance of *Staphylococcus saprophyticus* as a cause of acute cystitis in young females has been well documented (1, 4, 7, 21, 24). Recognition of the pathogenicity of this coagulase-negative staphylococcus, together with the increasing significance of *Staphylococcus epidermidis* as a nosocomial pathogen, has highlighted the need for fuller species identification of the coagulase-negative staphylococci. *S. saprophyticus* is characterized by novobiocin resistance, but three other coagulase-negative staphylococcal species (*S. cohnii*, *S. xylosus*, and *S. sciuri*) are also novobiocin resistant (8), and novobiocin-resistant *S. epidermidis* strains have been reported (7, 25). In addition, some authors report the isolation of novobiocin-sensitive *S. saprophyticus* (12, 15, 26). The present study was undertaken to characterize the features of bacteriuria with coagulase-negative staphylococci by correlating species identification by the biotyping schema of Kloos and Schleifer (8) and the International Committee on Systematic Bacteriology Subcommittee on the taxonomy of staphylococci and micrococci (5)

recommendations with antimicrobial susceptibility and clinical features. In particular, the reliability of novobiocin susceptibility alone to distinguish *S. saprophyticus* from other coagulase-negative staphylococci in urinary tract isolates was examined.

### MATERIALS AND METHODS

**Bacterial strains studied.** All gram-positive, catalase-positive, coagulase-negative cocci isolated in single culture in concentrations greater than or equal to 10<sup>4</sup> CFU/ml from urine specimens submitted from both outpatients and inpatients to the Clinical Microbiology Laboratory at the Health Sciences Centre in Winnipeg, Manitoba, from January, 1980, to February, 1981, were included in this study. When possible, clinical data including sex, age, hospital ward, inpatient or outpatient, and midstream or catheterized specimen were recorded for each isolate.

**Microbiology tests.** Urine specimens were plated on split plates of blood agar and MacConkey agar by the quantitative loop technique and incubated for 18 to 24 h. Gram-positive organisms were initially screened in the clinical laboratory for catalase and coagulase production, using standard techniques (11). The ability of the organisms to utilize glucose aerobically or anaerobically (oxidation-fermentation reaction) was tested by the constricted tube method with Facklam's medium formulation (2), with tubes read as oxidative, fermentative,

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tative, or no change after incubation at 37°C for 24 h. Strains which were oxidative or no change and novobiocin susceptible were tested for production of acid aerobically from glycerol in the presence of 0.4 µg of erythromycin per ml (20) to exclude micrococci. Nitrate reduction was tested by a standard technique (11), and phosphatase activity, lysostaphin susceptibility, and hemolysis on bovine blood agar were performed as described previously by Kloos and Schleifer (8). Carbohydrate utilization of nine sugars (sucrose, trehalose, fructose, galactose, mannitol, lactose, mannose, maltose, and xylose), using an agar plate method, was also tested as described by Kloos and Schleifer (8), with the modification of a Steer-Foltz replicator (23) to inoculate the media. For selected isolates, trehalose utilization was further tested, using a standard liquid broth method (11).

**Antimicrobial susceptibility.** Antimicrobial susceptibility testing was done in the clinical microbiology laboratory by a modified Kirby-Bauer disk method (17). Organisms were grown in brain heart infusion broth for 3 h at 37°C, then diluted to a concentration of 10<sup>8</sup> CFU/ml, inoculated onto Mueller-Hinton agar, and incubated for 18 h at 37°C with antibiotic-containing disks, including penicillin (10 U), oxacillin (1 µg), erythromycin (15 µg), cephalothin (30 µg), gentamicin (10 µg), and clindamycin (2 µg). The diameter of inhibition of growth of the organism was measured, and susceptibility was determined by predetermined breakpoints (11).

For novobiocin susceptibility, a 5-µg disk (Oxoid Ltd., Ottawa, Ontario) was used with a breakpoint zone diameter of 14 mm. All novobiocin-resistant isolates also had minimal inhibitory concentrations (MICs) determined by an agar dilution technique. Organisms were grown to 10<sup>8</sup> CFU/ml in Trypticase (BBL Microbiology Systems) soy broth, diluted to 10<sup>7</sup> CFU/ml, then inoculated, using a Steer-Foltz replicator, onto media containing twofold increasing concentrations of novobiocin. The MIC for the organism was the concentration of antimicrobial agent in the first dilution at which no growth occurred.

## RESULTS

**Characterization of strains.** One hundred and forty-five coagulase-negative staphylococcal strains were identified in urine specimens from 141 patients between January, 1980, and February, 1981. Seventy percent (102 strains) were classified as *S. epidermidis* and 17% (24 strains) as *S. saprophyticus*. These two organisms were reliably differentiated by six characteristics, including the oxidation-fermentation reaction, lysostaphin, phosphatase, NO<sub>3</sub> reduction, novobiocin resistance, colony diameter at 5 days, and utilization of three carbohydrates (Table 1). Only one strain, consistent with *S. epidermidis* by other parameters, was novobiocin resistant. Whereas Kloos et al. (9) found 89 to 96% of *S. saprophyticus* strains (from three different human populations) positive for trehalose utilization, we found only 54.2% of *S. saprophyticus* strains positive for trehalose utilization with our agar technique. However, using the broth tech-

nique, all *S. saprophyticus* strains tested, including all strains negative with the agar technique, were positive for trehalose utilization.

Nineteen strains (13%) could not be classified as *S. epidermidis* or *S. saprophyticus* and were assigned to other coagulase-negative staphylococcal species (Table 2). Identification of these strains by the biotyping system was frequently not clear-cut, with species often assigned on the basis of one characteristic when other characteristics formed an inconsistent pattern. For instance, *S. haemolyticus* was identified when hemolysis was present in strains which were not *S. epidermidis* or *S. saprophyticus* and could not clearly be assigned to any other species; nonhemolytic strains which were mannitol negative were classified as *S. hominis* and, if maltose negative, as *S. simulans*. Four strains (21%) which could not be classified as *S. epidermidis* or *S. saprophyticus* were novobiocin resistant. These novobiocin-resistant strains were 4 of 7 oxidative strains, whereas none of 12 fermentative strains was novobiocin resistant.

**Antimicrobial susceptibility.** All 24 *S. saprophyticus* strains were susceptible to clindamycin, cephalothin, and gentamicin. One strain was resistant to penicillin, three to oxacillin, and four to erythromycin. The single penicillin-resistant strain was the only isolate resistant to more than one antimicrobial agent. In contrast, 73% of *S. epidermidis* strains were resistant to penicillin, 18% to oxacillin, 36% to erythromycin, 56% to

TABLE 1. Characteristics differentiating *S. saprophyticus* and *S. epidermidis*

Characteristics	No. of strains with characteristic (% of strains)	
	<i>S. epidermidis</i> (n = 102)	<i>S. saprophyticus</i> (n = 24)
Oxidation-fermentation of dextrose (% fermentation) <sup>a</sup>	102 (100)	0
Lysostaphin susceptibility	97 (94)	4 (17)
Phosphatase <sup>b</sup>	87 (85)	3 (13)
NO <sub>3</sub> reduction	99 (97)	0
Novobiocin resistance <sup>b</sup>	1 (1)	24 (100)
Colony ≥5 mm at 5 days	2/56 (4)	13/16 (81)
Carbohydrate utilization:		
Galactose	86 (84)	3 (13)
Mannitol <sup>b</sup>	0	21 (88)
Trehalose agar <sup>b</sup>	1 (1)	13 (54)
Trehalose broth	0/23	18/18 (100)

<sup>a</sup> Ability of strains to ferment glucose, using Facklam's media.

<sup>b</sup> Characters recommended by the International Committee on Systematic Bacteriology subcommittee.

TABLE 2. Species identification of urinary tract isolates of coagulase-negative staphylococci other than *S. epidermidis* or *S. saprophyticus*

Species	No. (% of all isolates)	Oxidative or no change <sup>a</sup>	Novobiocin resistant <sup>b</sup>
<i>S. haemolyticus</i>	7 (4.8)	3	1
<i>S. hominis</i>	4 (2.8)	2	2
<i>S. simulans</i>	3 (2.1)	0	0
<i>S. capitis</i>	2 (1.4)	0	0
<i>S. cohnii</i>	1 (<1)	1	1
Non-classifiable	2 (1.4)	1	0
Total	19 (13.1)	7	4

<sup>a</sup> Strains which did not ferment glucose with Facklam's media.

<sup>b</sup> Disk diameter  $\leq 14$  mm with a 5- $\mu$ g disk.

clindamycin, and 15% to gentamicin. All strains were susceptible to cephalothin. Thirty-eight percent of strains were resistant to more than one organism. The 19 strains other than *S. epidermidis* or *S. saprophyticus* showed a resistance profile similar to that of *S. epidermidis*, with 32% of strains multiply resistant, 58% penicillin resistant, 21% oxacillin resistant, and 32% erythromycin resistant. The differences between *S. saprophyticus* and *S. epidermidis* in proportions of organisms susceptible to penicillin, clindamycin, gentamicin, and in multiply-resistant strains and the differences between *S. saprophyticus* and other non-*S. epidermidis* coagulase-negative staphylococci in penicillin susceptibility and proportions of multiply-resistant strains were significant ( $P < 0.04$ , chi-square analysis).

The agar dilution MIC for novobiocin was greater than or equal to 16  $\mu$ g/ml for all *S. saprophyticus* isolates. The *S. cohnii* strain and the novobiocin-resistant *S. haemolyticus* and *S. epidermidis* strains had MICs similar to that for *S. saprophyticus*, but the two novobiocin-resistant *S. hominis* had MICs of only 4  $\mu$ g/ml.

**Clinical correlations.** *S. saprophyticus* bacteriuria and bacteriuria with any other coagulase-negative staphylococcus were clearly distinguished by the clinical setting (Table 3). *S. saprophyticus* was isolated from young female

outpatients, whereas other coagulase-negative staphylococci were isolated from older inpatients with no sexual predominance, many of whom had underlying urological abnormalities, as evidenced by the proportion with indwelling catheters (50%), renal transplants (9.6%), or paraplegia (4.3%). These differences in age, proportion of outpatients, and catheter association remain significant when females alone are compared between the two groups.

Whereas the epidemiological features of bacteriuria with coagulase-negative staphylococci other than *S. epidermidis* or *S. saprophyticus* resemble those of *S. epidermidis*, the single *S. cohnii* strain was isolated from a young symptomatic female outpatient, a setting similar to *S. saprophyticus* isolation. Of the other novobiocin-resistant strains, the two *S. hominis* strains were isolated from catheterized inpatients, the *S. haemolyticus* strain was isolated from a young female outpatient who was a renal transplant recipient, and the resistant *S. epidermidis* strain was identified in a 71-year-old female outpatient on long-term prophylactic trimethoprim-sulfamethoxazole for recurrent urinary infections.

## DISCUSSION

At our institution, *S. epidermidis* was the most common coagulase-negative staphylococcal species isolated from urine specimens, followed by *S. saprophyticus*. All other coagulase-negative staphylococci comprised less than 15% of the isolates. This distribution is comparable to that found for 66 strains reported by Marsik and Brake (12), using the same biotyping system. John et al. (6), also using the same biotyping system, did not find *S. saprophyticus* to be a major isolate, a difference likely reflecting different patient populations from whom specimens were submitted. Other reports of coagulase-negative staphylococci species identified in urinary tract isolates from hospital laboratories have used the Baird-Parker typing system (1, 13, 14, 22) or have identified only *S. epidermidis* and *S. saprophyticus* (26), and these reports

TABLE 3. Association of clinical features with coagulase-negative staphylococcal species isolated from urine specimens

Species	Patient age (yr) <sup>a</sup>	No. of isolates (%) associated with the following clinical feature:		
		Female sex	Outpatient	Indwelling catheter
<i>S. saprophyticus</i> (n = 22)	22 (14-64) <sup>b</sup>	21 (96) <sup>b</sup>	19 (86) <sup>b</sup>	1 (5) <sup>b</sup>
<i>S. epidermidis</i> (n = 96)	60 (8-94) <sup>c</sup>	51 (53) <sup>c</sup>	23 (24) <sup>c</sup>	47 (49) <sup>c</sup>
Other (n = 19)	71 (17-92)	10 (47)	4 (16)	11 (58)

<sup>a</sup> Median (range).

<sup>b</sup>  $P < 0.005$  (chi-square analysis) when tested against values for *S. epidermidis* or values for other.

<sup>c</sup> No significant difference (chi-square analysis) when tested against value for "other species."

cannot be compared with our own (16).

The two most commonly isolated species, *S. epidermidis* and *S. saprophyticus*, were clearly differentiated by several characteristics. In our hands, trehalose utilization by the agar method was not a satisfactory differentiating test, although the broth technique did reliably differentiate these two species. Oeding and Digranes (18) have also reported difficulty with trehalose utilization on solid media. The reasons for this variation are not clear. One factor may be inoculum size, as a Steer-Foltz replicator would deliver a smaller inoculum than the streak plate method reported by Kloos and Schleifer (8), with subsequent difficulty in identifying weak positives. The other coagulase-negative staphylococci isolated from urine were seldom as clearly identified as *S. epidermidis* or *S. saprophyticus* by the biotyping system, a difficulty recognized by certain other investigators (12, 18) examining other clinical isolates. Some of these difficulties in identification may be eliminated by the use of the recently introduced commercial test systems for the identification of staphylococci (10), although these require further assessment in the clinical laboratory.

We found novobiocin susceptibility for *S. saprophyticus* identification to have a sensitivity of 100%, a specificity of 96%, and a positive predictive value of 83%. Other investigators (4, 22) have also found novobiocin susceptibility to be a reliable differentiating characteristic. If novobiocin resistance alone were used as a presumptive test for identification of *S. saprophyticus*, 5 of 29 isolates among our strains would have been misidentified as *S. saprophyticus*. However, these novobiocin-resistant, coagulase-negative staphylococci which are not *S. saprophyticus* were, except for the *S. cohnii* isolate, identified in a clinical setting which permits their being distinguished from *S. saprophyticus*. We identified only one (1%) novobiocin-resistant *S. epidermidis* strain, a proportion similar to that reported by Hovelius et al. (4) for urinary tract isolates, although other reports suggest novobiocin-resistant *S. epidermidis* are isolated more frequently from blood or pus (4, 25).

Williams et al. (26) reported the identification of novobiocin-susceptible urinary tract isolates of *S. saprophyticus*, but did not identify their isolates beyond *S. saprophyticus* and *S. epidermidis*. Novobiocin-resistant *S. saprophyticus* strains were identified in all of the patients with symptomatic infection in their study, and the novobiocin-susceptible *S. saprophyticus* may, in fact, have been other species. Marsik and Brake (12) also reported 2 of 18 *S. saprophyticus*, at least one of which was a urinary tract isolate, to be novobiocin susceptible, but do not enlarge on

this observation, and its significance is difficult to interpret. Other reports (3, 22) substantiate our observation that urinary tract isolates of *S. saprophyticus* are uniformly novobiocin resistant. Thus, identification of a novobiocin-susceptible, coagulase-negative staphylococcus in the urine reliably excludes *S. saprophyticus*.

*S. saprophyticus*, as we and other investigators (1, 4, 7, 21, 24) have shown, occupies an epidemiological niche distinct from all other coagulase-negative staphylococci. That is, it is isolated from young females with symptomatic urinary infection, who are otherwise healthy. The general susceptibility of this species to standard antimicrobial agents has also been reported by other investigators (7, 19). Non-*S. saprophyticus* coagulase-negative staphylococci, by contrast, when isolated from the urine, are found in a nosocomial setting and are frequently multiply resistant. Individual species do not appear to be distinguishable by clinical features or antimicrobial susceptibility profile. Some infrequently isolated species such as *S. cohnii*, may, like *S. saprophyticus*, be true pathogens in the urinary tract, but further clinical correlation will be necessary to clarify this association. As bacteriuria with species other than *S. saprophyticus* is associated with common clinical features, routine identification of coagulase-negative staphylococcal urinary isolates for the clinical microbiology laboratory, beyond the identification of *S. saprophyticus*, would not appear to be clinically useful. Further identification of other species would, however, be of value in some circumstances, such as epidemiological investigations.

In summary, coagulase-negative staphylococci are isolated from urine specimens in two distinct settings, acute cystitis in young female outpatients, invariably due to *S. saprophyticus*, and in patients with underlying urological abnormalities or nosocomial acquisition for all other species. Novobiocin susceptibility provides a simple and reliable means for differentiation of *S. saprophyticus* from other coagulase-negative staphylococci in urine specimens. Further species identification as a routine procedure for urine specimens in the clinical microbiology laboratory would not appear to be clinically useful.

#### ACKNOWLEDGMENTS

This work was supported in part by grant MA5973 from the Medical Research Council of Canada. L.E.N. was a recipient of a Medical Research Council of Canada Research Fellowship.

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