Urinary tract infections due to *Staphylococcus saprophyticus* biotype 3

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Staphylococcus saprophyticus **biotype 3 (*Micrococcus* subgroup 3 or M3) has usually been shown to be the second commonest cause of urinary tract infections in European women who are not in hospital. It generally causes pyuria and symptoms as severe as those caused by *Escherichia coli*. Unlike *S. epidermidis* it is seldom found as a contaminant in midstream urine specimens, and almost exclusively infects women in their reproductive years. However, *S. saprophyticus* is seldom differentiated from *S. epidermidis* in Canadian clinical laboratories.**

Urinary isolates of *S. saprophyticus* were presumptively differentiated from other coagulase-negative Micrococcaceae by their resistance to novobiocin as demonstrated by a simple disc susceptibility test that misidentified the infecting organism in only 3.4% of specimens. These novobiocin-resistant, coagulase-negative organisms caused similar proportions of the urinary tract infections in young women in York, England and Vancouver — 6.6% and 6.9% respectively. In York these organisms were associated with significantly greater pyuria than novobiocin-sensitive organisms or bile-tolerant streptococci but not *S. aureus* or Enterobacteriaceae. In both communities novobiocin-sensitive, coagulase-negative Micrococcaceae were appreciably more resistant to penicillin than novobiocin-resistant organisms.

Thus, differentiating *S. saprophyticus* from novobiocin-sensitive, coagulase-negative organisms provides information that is clinically useful, particularly for primary care practitioners working in the community or in outpatient clinics.

***Staphylococcus saprophyticus* biotype 3 (*Micrococcus* sous-groupe 3 ou M3) est habituellement considéré comme étant la deuxième plus fréquente cause d'infections urinaires chez la femme européenne non hospitalisée. Il est généralement responsable d'une pyurie et de symptômes aussi graves que ceux qui sont causés par *Escherichia coli*. Contrairement à *S. epidermidis* c'est un rare contaminant des urines du milieu du jet, et il infecte presque exclusivement les femmes pendant leurs années de reproduction. Toutefois, dans les laboratoires cliniques canadiens *S. saprophyticus* est rarement différencié de *S. epidermidis*.**

Les isolats urinaires de *S. saprophyticus* ont été grossièrement différenciés des autres Micrococcaceae coagulase-négative par leur résistance à la novobiocine à l'aide d'un simple antibiogramme par la méthode des disques; une erreur d'identification n'est survenue que pour 3.4% des échantillons. Ces organismes coagulase-négative, résistants à la novobiocine ont été responsables d'un pourcentage similaire d'infections urinaires chez des jeunes femmes de York, Angleterre et de Vancouver, soit 6.6% et 6.9% respectivement. A York ces organismes ont été reliés à une pyurie significativement plus grave que celle causée par les organismes sensibles à la novobiocine ou aux streptocoques résistants à la bile, mais pas celle causée par *S. aureus* ou les Enterobacteriaceae. Dans les deux villes les Micrococcaceae coagulase-négative, sensibles à la novobiocine étaient nettement plus résistants à la pénicilline que les organismes résistants à la novobiocine.

Donc, la distinction de *S. saprophyticus* des organismes coagulase-négative, sensibles à la novobiocine permet d'obtenir une information qui s'avère cliniquement utile, particulièrement pour le médecin de première ligne qui pratique au sein de la communauté ou dans une clinique externe.

Coagulase-negative Micrococcaceae are sometimes grouped together and reported as *Staphylococcus epidermidis* by clinical laboratories. In such reports the designation *S. epidermidis* includes all members (predominantly *Staphylococcus* spp.) of the Micrococcaceae family other than *S. aureus*. Further identification of Micrococcaceae is time consuming and until recently was generally considered impracticable in busy laboratories. The results of a simplified biochemical scheme for identifying clinical isolates of staphylococci[1] were found to have only a 77% correlation[2] with those of a conventional method. A series of changes in the taxonomic position of some members of the Micrococcaceae family[3-5] compounded identification problems in the routine clinical laboratory. Because new names for species have been proposed[5] since the current edition of Bergey's manual[4] was published, we will use the terminology of the particular published articles in referring to them.

Some Micrococcaceae are resistant to novobiocin and may be differentiated by a simple disc susceptibility test.[6-8] Most urinary isolates of these novobiocin-resistant organisms are now officially classified as *S. saprophyticus* biotype 3.[4] Alternative designations include *Micrococcus* subgroup 3, or M3,[3] and possibly Micrococcaceae groups Ciii and Civ.[5]

In Europe this organism has usually been found to be the second commonest cause of urinary tract infection in women who are not in hospital, to infect more young than older women, and to cause pyuria and symptoms as severe as those caused by *Escherichia coli*.[6,7,9-15]

We wished to test the hypothesis that novobiocin-resistant and novobiocin-sensitive, coagulase-negative Micrococcaceae are two distinct groups of organisms. As pyuria is one host response to organisms that implies pathogenicity, the quantitative urine leukocyte counts of specimens obtained from patients in York, England and Vancouver infected with a variety of organisms were compared to assess the relative patho-

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genicity of coagulase-negative Micrococcaceae and other bacteria. The antibiotic susceptibility patterns of novobiocin-resistant and novobiocin-sensitive, coagulase-negative organisms were compared to obtain further evidence that they are distinct urinary pathogens.

We have been able to find only two reports[16,17] of the isolation of *S. saprophyticus* in the United States and none in Canada.* The epidemiology of staphylococcal urinary tract infections in Vancouver was therefore studied to explore the possibility that infections with this pathogen may occur in North America.

Materials, methods and patients

Culture and microscopy of urine specimens

Most specimens were refrigerated immediately after being collected and were examined within 18 (usually 4) hours. Specimens from inpatients at one hospital, though unrefrigerated, were received at the laboratory within 2 hours of collection and so were accepted for this study. Significant bacteriuria was arbitrarily defined as the presence of more than 10^5 colony-forming units per millilitre of a single midstream urine specimen or any colony-forming units in catheter or suprapubic specimens. The cut-off point for the midstream urine specimens, originally intended for replicate urine specimens containing Enterobacteriaceae, was extended to other organisms because separate definitive criteria have not been established. However, for *S. saprophyticus* only 1 specimen out of 99 at York District Hospital contained between 10^4 and 10^5 organisms per millilitre, and none contained fewer than 10^4 organisms, as counted by the semiquantitative calibrated loop technique. No attempt was made to exclude multiple specimens from the same patient, although no recurrent infections or duplicate specimens were noted from patients infected with Micrococcaceae.

The York survey was limited to midstream urine specimens from females of a mean age of 24.9 years (standard deviation 7.9 years); during 1975 the specimens were submitted by general practitioners to York District Hospital with a request for culture. The leukocytes in an uncentrifuged specimen of urine were counted quantitatively in a Helber counting chamber to measure the patient response to each organism. Urine was cultured on both blood agar (5% volume per volume) and MacConkey agar (without crystal violet). Organisms from patients with significant infection were identified as follows: lactose-fermenting Enterobacteriaceae and bile (MacConkey medium)-tolerant streptococci were not identified further; non-lactose-fermenting Enterobacteriaceae were identified by the API 20E system (API Laboratory Products Ltd., Farnborough, England); Micrococcaceae were subdivided by the tube coagulase test (with rabbit plasma) into *S. aureus* and coagulase-negative strains; and coagulase-negative strains were differentiated by both the novobiocin disc sensitivity test and the Baird-Parker classification.[3]

In Vancouver urine specimens were obtained during a 2-month period in 1979 from females of a mean age of 25.9 years (standard deviation 9.8 years): some were hospital outpatients or general practice patients at Lions Gate Hospital; others were general practice patients whose specimens were sent to Metropolitan Clinical Laboratories. Additional specimens were obtained during the same period from inpatients at Vancouver General Hospital. Urine leukocyte counts were not available at any of the Vancouver microbiology laboratories participating in this study. Organisms were counted semiquantitatively on blood agar and MacConkey agar (with crystal violet). The methods were generally similar to those at York District Hospital except that Micrococcaceae were subdivided only on the basis of the tube coagulase and novobiocin disc tests.

Differentiation tests

Antibiotic disc sensitivity tests: The antibiotic susceptibility patterns of all organisms isolated at York were determined by the comparative disc test following method A of the Association of Clinical Pathologists[18] with Wellcotest agar (Burroughs Wellcome Ltd., Beckenham, England) and discs containing 2 U of penicillin. The susceptibility patterns of organisms isolated at Vancouver were determined by the Kirby–Bauer test[19] with Mueller–Hinton agar and discs containing 10 U of penicillin.

The novobiocin disc test: A 5-μg novobiocin disc was employed with the standard test methods used at York[18] and Vancouver.[19] Organisms with an inhibition zone diameter of less than 14 mm were judged resistant.[8]

Statistical analysis of data

Urine specimens containing more than 1000 leukocytes per cubic millimetre (1×10^9/l) were not quantitated further in the York study. The significance of differences between leukocyte counts was therefore compared by the chi-square two-tailed *t*-test of medians rather than means. The age distributions of the two patient populations were compared by a chi-square test of the means, assuming normal distribution only.

Results

Significant bacteriuria was found in 1488 urine specimens submitted by general practitioners in York. The median (and quartile) quantitative urine leukocyte counts for each organism are shown in Table I; the corresponding counts in 826 urine specimens submitted for culture from patients without significant bacteriuria (fewer than 10^4 organisms per millilitre) were only 10/mm^3 (6 and 40/mm^3). There was no significant difference in the patients' response to infection by novobiocin-resistant, coagulase-negative Micrococcaceae and lactose-fermenting Enterobacteri-

*Since this article was accepted, *S. saprophyticus* has been reported as an important urinary pathogen by another North American centre (JORDAN PA, IRAVANI A, RICHARD GA, BAER H: Urinary tract infection caused by *Staphylococcus saprophyticus*. *J Infect Dis* 1980; 142: 510–515).

aceae (P = 0.355), *Proteus* spp. (P = 0.13) or *S. aureus* (P = 0.87). Infections due to novobiocin-resistant, coagulase-negative Micrococcaceae were associated with higher urinary leukocyte counts than those due to either novobiocin-sensitive Micrococcaceae (P = 0.030) or bile-tolerant streptococci (P < 0.001).

The novobiocin-resistant Micrococcaceae from York were identified as M2 in 5 specimens and M3 (*S. saprophyticus* biotype 3) in 93. The novobiocin-sensitive organisms were identified as S1 (*S. aureus*) in 10 specimens, S2 in 15, S5 in 6, S6 in 8, M1 in 1 and M2 in 9. Thus, the novobiocin disc test misidentified only 5 of the 147 infecting Micrococcaceae.

Of the 729 specimens from Vancouver outpatients and general practice patients with significant bacteriuria, novobiocin-resistant, coagulase-negative Micrococcaceae were cultured from 6.9%, novobiocin-sensitive, coagulase-negative Micrococcaceae from 2.1% and *S. aureus* from 0.4%. Though none of 1382 significant urinary tract infections in hospital inpatients in Vancouver were due to novobiocin-resistant, coagulase-negative Micrococcaceae, the hospitals in the study had relatively few obstetric inpatients, a group that sometimes becomes infected with these organisms. There was no significant difference (P = 0.41) in the age distribution of patients infected with these organisms in York and Vancouver (Table II). All such infections in the two cities occurred in females, whereas the infections with novobiocin-sensitive, coagulase-negative Micrococcaceae occurred in both sexes.

The penicillin susceptibility patterns of the coagulase-negative Micrococcaceae isolated from patients in the community were similar in the two cities: no novobiocin-resistant isolates were resistant to penicillin and 9% were of intermediate sensitivity to penicillin; by contrast, 43% of the novobiocin-sensitive isolates were fully resistant to penicillin. The novobiocin-sensitive Micrococcaceae from hospital inpatients were even more resistant to penicillin, 78% of the isolates being fully resistant.

Discussion

Most British and European studies have shown that the majority of acute urinary tract infections in young women from Micrococcaceae are due to novobiocin-resistant isolates of *S. saprophyticus* biotype 3. The similarities in both age distribution and location (in hospital or outside) of the York and Vancouver patients infected by these organisms raise the possibility that infections due to *S. saprophyticus* biotype 3 may be under-reported in patients outside of hospital in North America.

The pathogenic properties of *S. saprophyticus* biotype 3 have not been explained. Its growth in urine is relatively slow,[20] and attempts to demonstrate that it can adhere to bladder epithelial cells have been unsuccessful.[13] Although the majority of isolates of this organism and a minority of other Micrococcaceae are urease positive, the contribution of this enzyme to pathogenicity is unclear.[10,21] There is conflicting evidence for venereal spread or an association with promiscuity.[10,13,14,22] Infection with *S. saprophyticus* biotype 3 provoked a median leukocyte count similar to that provoked by *E. coli* in a small study that made no statistical comparisons.[10] In another study[23] pyuria was found to be significantly more common with staphylococcal than coliform infections, and with novobiocin-resistant than novobiocin-sensitive, coagulase-negative staphylococci (pyuria was determined by counting the cells in a high power field in centrifuged urine). These previous studies support the current findings from York, which also provide a probable rank order of the inflammation-producing potential of other urinary pathogens. Mitchell, and subsequently others, noted that while *S. saprophyticus* biotype 3 causes cystitis in otherwise healthy women, other micrococci and staphylococci are generally secondary pathogens in patients in hospital or in individuals with anatomic abnormalities.[7,9,14,15,24] Furthermore, novobiocin-resistant Micrococcaceae are seldom found as contaminants in urine and are therefore more likely to be significant than novobiocin-sensitive organisms.[14]

We are not aware of comparative trials of treatment regimens for infections with novobiocin-resistant Micrococcaceae, although the value of sulfonamides was questioned in a small clinical trial;[10] it showed that all isolates were moderately or fully sensitive to penicillin and could be expected to respond to urinary concentrations, but that the response to sulfonamides may be unsatisfactory. There is some evidence that most of these infections respond to a 5- to 7-day course of

Table I—Urine leukocyte counts in young women with bacteriuria in York, England

Infecting organism	No. (and %) of specimens	Median urine leukocyte count/mm³ (and quartiles)
Lactose-fermenting Enterobacteriaceae	1148 (77.2)	160 (20, 1000)
Novobiocin-resistant, coagulase-negative Micrococcaceae	98 (6.6)	300 (60, 640)
Novobiocin-sensitive, coagulase-negative Micrococcaceae	39 (2.6)	45 (0, 360)
Staphylococcus aureus	10 (0.7)	105 (35, 320)
Proteus spp.	97 (6.5)	480 (43, 1000)
Bile-tolerant streptococci	83 (5.6)	10 (0, 100)
Miscellaneous	12 (0.8)	-

Table II—Age of the patients with urinary tract infections due to novobiocin-resistant, coagulase-negative staphylococci in York and Vancouver

Age (yr)	% of patients in each age group: Vancouver	York
≤ 15	2.0	2.0
16–20	24.0	36.7
21–25	36.0	27.6
26–30	24.0	14.3
31–35	4.0	12.2
> 35	10.0	7.1
Mean ± standard deviation	25.9 ± 9.8	24.9 ± 7.9

ampicillin or penicillin; a telephone survey at York (by J.D.A.) showed that 55 of 60 patients were asymptomatic at both 3 to 5 and 12 to 15 days after the start of therapy. This tentative conclusion has not yet been rigorously tested, and localization of infection in the renal tract has not been established.

The novobiocin disc susceptibility test provides an attractive method of differentiating novobiocin-resistant Micrococcaceae from other coagulase-negative Micrococcaceae isolated from urine. The misidentification rate of 3.4% in this investigation is comparable to that in other studies — 2%,[25] 4%[2] and 7%.[24] The conclusion from a small American study[16] that novobiocin resistance cannot be relied upon to differentiate *S. saprophyticus* and *S. epidermidis* seems atypical.

The clear differences in epidemiology, pathogenicity and antibiotic susceptibility patterns between novobiocin-resistant and novobiocin-sensitive, coagulase-negative Micrococcaceae provide a strong case for differentiating these organisms in all clinical laboratories. Novobiocin-resistant urinary isolates may be reported as "presumptive *S. saprophyticus*" by laboratories that do not wish to perform rigorous identification. Differentiation is likely to be particularly helpful for women not in hospital, in whom novobiocin-resistant organisms are almost always truly pathogenic and not contaminants.

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Origin of "kidney"

The origin of the word kidney is uncertain. According to Skeat, kidneer or kidnere originated in Iceland, where kid was a corruption of the Icelandic word for womb — kid, quid, quith or koithr. The form "kidenei" first appeared in the early 14th century.