

Abbreviated Scheme for Presumptive Identification of *Staphylococcus saprophyticus* from Urine Cultures

JEFF W. MORGAN

A.K.E. Laboratories, Inc., Lakewood General Hospital, Tacoma, Washington 98499

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A simplified scheme for the presumptive identification of *Staphylococcus saprophyticus* was investigated. Clinical isolates of *Micrococcaceae* were tested in microtiter plates containing phenol red-novobiocin-turanose broth and phenol red-arabinose broth. Standard saline suspensions of test organisms served as inoculum. Identifying features were growth within 18 to 24 h in novobiocin-turanose broth, indicating resistance to 1.6 µg of novobiocin per ml and fermentation of turanose. With this method, all clinical isolates of *S. saprophyticus* tested were correctly identified.

Staphylococcus saprophyticus is a common cause of urinary tract infections in young adult females (2, 9, 11, 12). Isolates suspected to be *S. saprophyticus* may be identified by multiple biochemical testing (4, 6, 7) and by resistance to novobiocin (1, 6, 7). Novobiocin resistance does not identify *S. saprophyticus* specifically (8), and physiological tests are expensive and laborious. We have developed a simplified protocol for identifying urine isolates of *S. saprophyticus* with a high degree of precision and accuracy.

Kloos and Schleifer (7) have presented data which indicate that novobiocin resistance, turanose fermentation, and non-utilization of arabinose may identify *S. saprophyticus* presumptively. This study was undertaken to determine the effectiveness of such a screen.

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Isolates of *Micrococcaceae* were recovered from clinical specimens submitted to the Microbiology Laboratory, Lakewood General Hospital, Tacoma, Wash., during 1982 and 1983. Additional strains of *Micrococcaceae* were provided by T. R. Oberhofer, Microbiology Laboratory, Madigan Army Medical Center, Tacoma, Wash. *Staphylococcus* reference strains *S. aureus* ATCC 12598, ATCC 25923, and ATCC 29212, *S. cohnii* ATCC 29972, *S. epidermidis* ATCC 12228, *S. saprophyticus* ATCC 15305, *S. sciuri* ATCC 29060 and ATCC 29062, and *S. xylosum* ATCC 29966 were obtained from the American Type Culture Collection, Rockville, Md. In all, 279 isolates of *Micrococcaceae* were studied.

Clinical isolates of *Micrococcaceae* were identified as staphylococci or micrococci by Gram stain (10), catalase (10), and lysostaphin

susceptibility (5). Species identification of staphylococci was determined with coagulase (10) and the API Staph-Ident System (Analytab Products, Inc., Plainview, N.J.). There was no attempt made to identify micrococci to the species level.

The media used in the investigation were novobiocin-turanose (N/T) broth and arabinose (Ar) broth. N/T broth was prepared by using phenol red broth base (BBL Microbiology Systems, Cockeysville, Md.) with 1.6 µg of novobiocin (Sigma Chemical Co., St. Louis, Mo.) per ml and 1% D-(+)-turanose (Sigma Chemical Co.). Ar broth consisted of phenol red broth base (BBL Microbiology Systems) with 1% L-(+)-arabinose (Eastman Kodak Co., Rochester, N.Y.).

For each isolate tested, N/T and Ar broths were pipetted in 0.1-ml volumes into each of two separate wells of a sterile microtiter tray (Dynatech Laboratories, Inc., Alexandria, Va.). Organisms were selected from sheep blood agar plates after 18 to 24 h of incubation and suspended in sterile 0.85% sodium chloride to a turbidity equal to that of a McFarland 0.5 standard. The suspension was inoculated into the microtiter test wells with a flame-sterilized platinum loop calibrated to deliver 0.001 ml. This provided a final inoculum density of 1.5×10^5 CFU/ml. The broths were overlaid with sterile mineral oil and incubated at 35°C.

After 18 to 24 h of incubation, the N/T broth was read for novobiocin resistance as evidenced by a visible button of growth. Turanose and arabinose were observed for fermentation by a pH shift to acid and subsequent yellow color. Susceptibility to ≤ 1.6 µg of novobiocin per ml was presented by no visible growth in N/T broth, and failure to acidify carbohydrates in

TABLE 1. Results of staphylococci and micrococci in novobiocin-turanose and arabinose broths

Organism	No. of strains	% of strains with positive reactions		
		Novobiocin resistant ^a	Turanose fermented ^b	Arabinose fermented
<i>S. aureus</i>	48	0	0	0
<i>S. capitis</i>	15	0	0	0
<i>S. cohnii</i>	7	100	0	0
<i>S. epidermidis</i>	52	0	0	0
<i>S. haemolyticus</i>	5	0	0	0
<i>S. hominis</i>	18	0	0	0
<i>S. intermedius</i>	2	0	0	0
<i>S. saprophyticus</i>	79	100	100	0
<i>S. sciuri</i>	3	100	33	100
<i>S. simulans</i>	13	0	0	0
<i>S. warneri</i>	4	0	0	0
<i>S. xylosus</i>	5	100	100	100
<i>Micrococcus</i> spp. ^c	28	36	32	4

^a Novobiocin reactions were considered positive when a visible button of growth was apparent after incubation for 18 to 24 h at 35°C.

^b Carbohydrate reactions were considered positive when acid production was apparent after incubation for 18 to 24 h at 35°C.

^c Includes *Micrococcus* spp. and other gram-positive and catalase-positive cocci (not identified to species).

N/T and Ar broths was considered a negative test.

A limited study was performed to determine the effect of novobiocin on turanose fermentation when simultaneously tested in N/T broth. Thirty-six turanose-fermenting coagulase-negative staphylococci were evaluated in phenol red-turanose broth with and without added novobiocin, using the methods described above. Novobiocin susceptibility was determined by the method of Almeida and Jorgensen (1).

Results of testing 279 isolates of *Micrococccaceae* are listed in Table 1. *S. saprophyticus* was unique in that all strains tested exhibited growth in N/T broth, fermented turanose, and failed to ferment arabinose. All other staphylococci in the investigation failed to react in the above pattern. Twenty-nine percent (8/28) of the micrococci studied reacted in the pattern described for *S. saprophyticus*.

Addition of novobiocin to turanose broth proved to inhibit turanose fermentation by novobiocin-susceptible strains of coagulase-negative staphylococci. All 15 novobiocin-susceptible strains tested failed to ferment turanose in N/T broth, but did ferment the carbohydrate in the absence of the antibiotic. When novobiocin-resistant strains of coagulase-negative staphylococci were tested, no demonstrable effects on turanose fermentation were noted as all 21 isolates fermented turanose equally well in N/T broth and phenol red-turanose broth.

The simplified scheme presented presumptively identified *S. saprophyticus* with 100% specificity and 100% sensitivity. Occasional strains of *S. cohnii*, *S. hominis*, and *S. epidermidis* may be novobiocin resistant and turanose

positive (7). However, this scheme will provide more presumptive information than will novobiocin testing alone.

The volumes of broth and final inoculum densities described here allow this scheme to be easily adapted to standardized minimum inhibitory concentration testing, using either in-house or commercially prepared plates. The addition of 0.1-ml volumes of N/T and Ar broths to vacant wells of the minimum inhibitory concentration plate and routine inoculation technique will obviate the need to inoculate with a platinum loop.

The carbohydrate reactions of staphylococci we encountered paralleled those reported by Kloos and Schleifer (7) despite the differences in carbohydrate test media and duration of incubation. Novobiocin was added to turanose broth to selectively inhibit the reported occasional fermentation of turanose by isolates of coagulase-negative staphylococci which have novobiocin minimum inhibitory concentrations of <1.6 µg/ml.

The simplified scheme with N/T and Ar broths was found to be of practical value in providing cost-effective, accurate presumptive identification of *S. saprophyticus* when tested with urine isolates.

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