Five-Hour Novobiocin Test for Differentiation of Coagulase-Negative Staphylococci

BRIAN J. HARRINGTON AND J. MICHAEL GAYDOS
Mercy Hospital, Toledo, Ohio 43624, and The Toledo Hospital, Toledo, Ohio 43606

Received 22 August 1983/Accepted 20 October 1983

A 5-h broth disk test, read visually for growth or no growth to determine resistance of coagulase-negative staphylococci to 1.6 μg of novobiocin per ml, was evaluated as a rapid test for the presumptive identification of Staphylococcus saprophyticus. The correlation with an overnight disk diffusion test was 100%.

Recently, there has been an increase in the awareness that coagulase-negative staphylococci may be cause of urinary tract infections (4, 8, 13, 18). In particular, Staphylococcus saprophyticus has been shown to be a common cause of urinary tract infections in young, sexually active female outpatients (4, 9, 19). Kloos and Schliefer showed that S. saprophyticus, unlike the other commonly occurring coagulase-negative staphylococci in humans, is resistant to novobiocin at 1.6 μg/ml (10). Hovelius and Marsh found that their S. saprophyticus isolates had novobiocin minimal inhibitory concentrations of between 128 and 512 μg/ml (7). Marrie and Kwan reported that the minimal inhibitory concentrations of novobiocin for S. saprophyticus and Staphylococcus cohnii were in the range of 16 to 32 μg/ml, whereas for Staphylococcus epidermidis and other novobiocin-susceptible, coagulase-negative staphylococci, the minimal inhibitory concentrations of novobiocin were in the range of 0.125 to 0.5 μg/ml (14). Kloos and Smith, in the Manual of Clinical Microbiology (11), recommend the use of a 5-μg novobiocin disk on P agar as an overnight disk diffusion technique (10). P agar is a peptone-yeast extract-glucose medium which was not available commercially until recently. Several investigators have reported the use of other, more readily available media for the disk diffusion test. Almeida and Jorgensen (1) compared the results obtained with Mueller-Hinton agar with those obtained with P agar and concluded that Mueller-Hinton agar is a suitable alternative to P agar. Goldstein et al. (6) compared the results obtained with Mueller-Hinton agar, Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with sheep blood, and P agar and found that the results were similar. In addition, other media have been used (19) or not specified (5, 7, 15, 16, 18) by European workers to test for novobiocin resistance. In our study, we compared the results obtained with an overnight disk diffusion technique with those obtained with a broth disk procedure read after 5 h.

(This work was presented at the 83rd Annual Meeting of the American Society for Microbiology, New Orleans, La., 6 to 11 March 1983 [J. M. Gaydos and B. J. Harrington, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C372, p. 373].)

All isolates tested were fresh clinical isolates from urine, blood, or wound cultures, apart from the three stock strains Staphylococcus epidermidis ATCC 14990, S. sciuri ATCC 29060, and S. simulans ATCC 27851. All 35 of the S. saprophyticus isolates and 20 of the S. epidermidis isolates were from urine. The other eight S. epidermidis isolates and the four S. hemolyticus isolates were from wound or blood specimens. Coagulase testing was performed by both slide and tube tests, and only coagulase-negative isolates were tested for novobiocin resistance. Species identification was made by the API Staph-Ident system (Analytab Products, Plainview, N.Y.) or the AutoMicrob system Gram-Positive Identification Card (Vitek Systems, Inc., Hazelwood, Mo.) or both. Disk diffusion susceptibility testing was performed on Mueller-Hinton agar (BBL Microbiology Systems) by the National Committee for Clinical Laboratory Standards Bauer-Kirby method (17), except a 5-μg novobiocin disk was used and the susceptible breakpoint diameter of inhibition was >16 mm. Because it was known from disk diffusion testing that these organisms grow in Trypticase soy broth at 37°C to a distinct turbidity (at least to a 0.5 McFarland standard) within 5 h, this medium was used for the broth disk test. The control tube of broth for this test was the Bauer-Kirby inoculum broth. The test procedure consisted of very lightly inoculating two tubes each containing 3.0 ml of Trypticase soy broth with the organism to be tested, as though both tubes were to be used for the disk diffusion inoculum (17). Immediately after inoculation, one 5-μg novobiocin disk (BBL Microbiology Systems) was added to one of the tubes; the tube then was gently shaken for 5 to 10 s to aid elution of the drug from the disk to give a nominal novobiocin concentration of 1.6 μg/ml of broth. Both tubes were then placed in a 37°C heat block or water bath. The test was read when the control tube showed turbidity comparable to that of a 0.5 McFarland standard.

There was 100% correlation between these two tests with the 70 strains tested. For the 35 S. saprophyticus isolates, the diameters of the zones of inhibition around the 5-μg novobiocin disk ranged from 7.5 to 10.1 mm. For the 28 clinical isolates of S. epidermidis, the diameters ranged from 23.1 to 30.3 mm, and for the S. hemolyticus isolates, the diameters ranged from 20.3 to 28.2 mm. The stock strains of Staphylococcus sciuri, Staphylococcus simulans, and S. epidermidis gave zone diameters of 8.1, 25.8, and 30.5 mm, respectively. In the broth disk test, all strains which were resistant by the disk diffusion method were visibly turbid within 5 h, whereas strains which were susceptible by the disk diffusion method showed no visible growth or turbidity.

* Corresponding author.
after 5 h. After further incubation of these strains overnight, there still was no growth in the presence of 1.6 µg of novobiocin per ml.

From the results of this study, it appears that not only is testing these organisms in Trypticase soy broth an acceptable alternative to disk diffusion, but the broth disk testing has the advantage that the results are available within 5 h. There is at least one commercially available same-day biochemical test identification system for staphylococci which often calls for the determination of resistance and susceptibility to novobiocin as a confirmatory test; this is the API Staph-Ident system. If the broth disk test is set up at the same time as the API Staph-Ident system, the novobiocin result, if needed, would be available at the time that the biochemical system identification is able to be read. If biochemical testing for identification is not performed, a presumptive identification of the organism as S. saprophyticus can be made on the basis of novobiocin resistance at 1.6 µg/ml (3, 11, 19). The use of this broth disk test enables the laboratory to report a presumptive identification of S. saprophyticus within 5 h rather than after 18 to 24 h. Two recent reports of rapid novobiocin susceptibility testing in broth (2, 12) have required the use of automated instruments; however, the method we describe here can be performed without such equipment.

LITERATURE CITED