Rapid, Automated Identification of Novobiocin-Resistant, Coagulase-Negative Staphylococci

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A modified automated method that uses the MS-2 system (Abbott Laboratories, Diagnostics Div., Irving, Tex.) to verify the reaction of coagulase-negative staphylococci to novobiocin is described. This technique permits the testing of a great number of specimens in an average time of 99 min and results in a 100% match with the traditional method of culturing.

The main characteristic used in the recognition of *Staphylococcus saprophyticus* in the simplified system of Kloos and Schleifer (7) is a verification of its susceptibility or resistance to 1.6 μg of novobiocin per ml. The staphylococci that belong to the *S. saprophyticus* group are found in humans and are all resistant to novobiocin. *S. saprophyticus* is the coagulase-negative species most frequently isolated (3) from urine specimens, and the test of resistance to novobiocin can be considered proof of presumptive identification (10).

Various nonautomated techniques have already been described for presumptively identifying *S. saprophyticus* based on its resistance to novobiocin (1, 5, 6, 8, 9).

The automated method described by Almeida and Jorgensen (2) uses the MS-2 system (Abbott Laboratories, Diagnostics Div., Irving, Tex.) with a 5-μg novobiocin disk incorporated into one of the disposable cuvette positions of the antimicrobial drug susceptibility kit (11).

Based on a resistance to 5 μg of novobiocin per ml as a presumptive identification test (1, 5, 10), our objective is to present a rapid, efficient, and economical method that verifies the resistance of coagulase-negative staphylococci to this drug in vitro.

Our method involves the use of ampoules originally intended for testing urine cultures with the automated MS-2 system. One strain is tested in each ampoule; therefore, up to 88 strains can be tested in each module of the apparatus.

The samples studied were from outpatients who were sent to our microbiology service. A total of 95 strains taken at random were used in the comparative study of coagulase-negative staphylococci; these were isolated from 28 first-stream urine specimens, 55 midstream urine specimens, and 12 urethral discharge specimens, from different patients of both sexes. In the comparative study, 26 *S. saprophyticus* strains and 17 *S. epidermidis* strains that were previously identified by the traditional method of Kloos and Schleifer (7) and were already stored in our collection were also used.

A bacterial suspension was prepared from a pure 24-h culture in Columbia blood agar base (Difco Laboratories, Detroit, Mich.) with 5% defibrinated sheep blood. To prepare this bacterial suspension, we homogenized three average-sized colonies in 1 ml of a sterile 0.85% NaCl solution to obtain a concentration corresponding to 0.25 on the MacFarland scale. The cultures were inoculated with 20 μl of the bacterial suspension in Mueller-Hinton agar (Difco Laboratories) containing 5 μg of novobiocin per ml, followed by overnight incubation at 37°C. The MS-2 system was used in parallel; an ampoule of the apparatus containing 1 ml of peptone broth enriched with L-cystine and dextrose was inoculated with 100 μl of the same bacterial suspension and 5 μg of novobiocin. As a positive control, an *S. saprophyticus* strain identified as described in reference 4 was used. *S. epidermidis* ATCC 14990 was used as a negative control. Besides these controls, all the strains that appeared to be susceptible to novobiocin were retested in the apparatus in normal ampoulettes without novobiocin; all showed growth.

Of the 28 first-stream urine specimens, only 2 were resistant to novobiocin. The remainder and the 12 urethral discharge specimens appeared to be susceptible to novobiocin. Of the 55 midstream urine specimens, 32 were resistant and 23 were susceptible to novobiocin. The specimens resistant to novobiocin represented 58% of the total midstream urine specimens isolated, indicating a high level of *S. saprophyticus* infections of the urinary tract.

All the coagulase-negative, novobiocin-resistant staphylococci identified by our method were confirmed by the method of Kloos and Schleifer (7) as being *S. saprophyticus*.

There was 100% correlation between the classic method and ours for the 95 strains tested. Positive results can be obtained in our method in an average of 99 min, with a range of 60 to 130 min. However, negative results were interpreted as such after 5 h, in accordance with the MS-2 standardization.

LITERATURE CITED


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