Differentiation of *Staphylococcus* and *Micrococcus* spp. with the Taxo A Bacitracin Disk

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Taxo A bacitracin disks (BBL Microbiology Systems, Cockeysville, Md.) were evaluated as a test for differentiating staphylococci from micrococci in clinical laboratories. All staphylococci tested were resistant to the disks, with no zone of growth inhibition. Zones of growth inhibition ranging from 10.5 to 25.0 mm in diameter were observed with all micrococci except two isolates which failed to grow on the test medium. These findings were confirmed by minimal inhibitory concentrations to bacitracin. Staphylococci had minimal inhibitory concentrations significantly higher than those of micrococci, with no overlap in the ranges of values.

In recent years there has been an increasing number of reports of infections caused by coagulase-negative staphylococci (1, 5, 7, 8, 11, 13, 15). Thus, in clinical laboratories, it has become more important to accurately differentiate staphylococci from nonpathogenic micrococci.

Numerous methods of differentiating the genera have been proposed (2, 3, 6, 14, 16). Currently recommended methods include resistance to lysostaphin, anaerobic acid production from glucose, growth on Furoxone-Tween 80-oil red O agar, and aerobic acid production from glycerol in the presence of erythromycin. Many of these procedures require special media or may take several days to be easily interpreted. Antibiotic sensitivity using erythromycin (14) has previously been proposed as a differential test. This study presents a new, simple antibiotic sensitivity method of separating staphylococci from micrococci by using a reagent commonly available in clinical laboratories.

A total of 226 catalase-positive, gram-positive cocci were tested (Table 1). These included 126 staphylococci from 15 species and 70 micrococci from 8 species. Of the isolates, 84 were from clinical specimens. The genera were differentiated by anaerobic glucose utilization, growth on Furoxone-Tween 80-oil red O agar (2, 3, 14), and lysostaphin resistance (Remel lysostaphin test; Remel, Lenexa, Kans.). Staphylococci were identified using the STAPH-IDENT system (Analytab Products, Plainview, N.Y.) as previously described (4, 10). Two isolates gave no identification by this method and are listed as *Staphylococcus* spp. Micrococci were not further identified.

In addition, 142 isolates were obtained from various culture collections. A total of 71 staphylococcal strains and 42 micrococal strains were provided by W. E. Kloos of North Carolina State University, Raleigh. Analytab Products provided five staphylococcal isolates; eight micrococal isolates were obtained from Presque Isle Cultures, Presque Isle, Pa. Three isolates of *Staphylococcus aureus* were donated by the Pennsylvania Department of Health, and 13 isolates of *Staphylococcus* spp. were donated by the American MicroScan Co., Hillsdale, N.J. All isolates were transferred to tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants and stored at 2°C.

Disk diffusion sensitivities to bacitracin were tested according to the National Committee for Clinical Laboratory Standards recommended modification of the Kirby-Bauer disk diffusion test (12). A Taxo A disk was applied to each plate. After incubation at 35°C for 18 h, inhibition zones were measured to the nearest 0.5-mm diameter. Organisms with no inhibition zone were interpreted as resistant. Bacitracin (Sigma Chemical Co., St. Louis, Mo.) broth dilution minimal inhibitory concentration (MIC) tests were performed as previously described (17) at two concentration ranges, 0.01 to 2.56 U/ml and 1 to 256 U/ml. Inoculum was prepared in tryptic soy broth (Difco) from 24-h sheep blood agar cultures and adjusted to match a 0.5 McFarland standard. A 40-μl volume of inoculum was added to each tube for a final density of ca. 10^6 CFU/ml. Each organism was first tested against the 0.01- to 2.56-U/ml dilution series; those organisms requiring an MIC of greater than 2.56 U/ml were retested using the higher concentration range.

The results of the Taxo A disk tests are listed in Table 1. All staphylococcal isolates grew well
on Mueller-Hinton medium and were resistant to the bacitracin disk with uninterrupted growth up to the edge of the disk. Of the 70 micrococci tested, two Micrococcus agilis isolates failed to grow at 18 h. The other four M. agilis isolates and all five Micrococcus nishinomiyaensis isolates grew faintly, but with clear-cut zones of inhibition. Of the micrococci which grew on the test plates, 100% exhibited zones of growth inhibition around the disk. The zones of inhibition ranged from 10.5 to 25.0 mm in diameter, with a mean diameter of 17 mm. In our initial investigation of this test, inocula were taken directly from 24-h cultures and streaked onto plates. Although much heavier growth occurred, distinct (albeit proportionally smaller) zones were still observed with all micrococci.

Differential sensitivity to bacitracin was confirmed by the broth dilution MIC results (Table 1). The mean values for each Staphylococcus sp. were at least 10-fold higher than those of each Micrococcus sp. The overall range of MICs was 2.56 to 256 U/ml for staphylococci, whereas that for micrococci was less than 0.01 to 1.26 U/ml. There was no overlap of values, and the extreme values occurred only infrequently: one isolate each of Staphylococcus hyicus and S. aureus required MICs of 2.56 U/ml, and one isolate each of Micrococcus roseus, M. nishinomiyaensis, and a Micrococcus sp. required MICs of 1.28 U/ml.

The standard methods of differentiating Staphylococcus spp. and Micrococcus spp., as previously mentioned, often require special media or procedures. Consequently, attempts at genus identification in hospital laboratories are often limited to Gram stain morphology or colony examination.

Recently introduced antibiotic sensitivity techniques are particularly applicable to clinical microbiology. Antibiotic disks can be added directly to the standard Kirby-Bauer susceptibility testing which accompanies isolation of organisms from critical specimens. Erythromycin and streptomycin (personal communication, W. E. Kloos) have been found to give small zones of growth inhibition with staphylococci and larger zones with micrococci. This study reports another differential sensitivity test using bacitracin. Both genera are sensitive to bacitracin at clinical levels; however, at the concentration employed in the Taxo A disk (0.04 U/ml) a clear separation is made. The Taxo A disk has been commonly used in clinical laboratories to
presumptively identify *Streptococcus pyogenes*. Staphylococci grew with no zone of inhibition, whereas all micrococci tested gave zones ranging from 10.5 to 25.0 mm in diameter. The Taxo A test is also applicable to direct sensitivities from blood culture media which yield heavier growth than those obtained with standardized inocula.

Differential susceptibilities were confirmed by bacitracin MIC results. Moreover, the wide gap between values for staphylococci and micrococci suggests that a disk with a higher bacitracin concentration, for instance, 0.08 or 0.16 U/ml, might give even more definitive zones of inhibition for micrococci without inhibiting staphylococcal growth.

Although *M. nishinomiyaensis* and *M. agilis* isolates exhibited poor growth or failed to grow on the test medium, this presents no problem since *M. agilis* is isolated rarely from human sources (9), and both species can easily be separated from staphylococci by their distinctive pigmentation.

In summary, accurate separation of *Staphylococcus* and *Micrococcus* spp. can be accomplished by application of Taxo A disks to Kirby-Bauer sensitivity plates. This test is easily performed and interpreted and can be incorporated into identification protocols with little expense or effort. The information provided can aid microbiologists in assessing the significance of *Micrococcaceae* isolated from clinical sources.

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**LITERATURE CITED**