

Variations in Bacitracin Susceptibility Observed in *Staphylococcus* and *Micrococcus* Species

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Bacitracin susceptibility was evaluated as a laboratory method to differentiate staphylococci from micrococci. A total of 317 staphylococcal isolates and 108 micrococcal isolates were each tested for susceptibility to bacitracin by a disk-diffusion method using disks of three different potencies (0.04, 2.0, and 10.0 U) and a broth dilution method to obtain MICs. When a growth inhibition zone diameter breakpoint of >10 mm was used to establish susceptibility with a 0.04-U disk, all micrococci were bacitracin susceptible and 94.6% of the staphylococci were resistant. Testing with disks of higher potency did not improve the specificity of the disk-diffusion method.

Bacitracin susceptibility (0.04-U disk) was recently described (4) as an accurate laboratory method to distinguish staphylococci (bacitracin resistant) from micrococci (bacitracin susceptible) in the clinical laboratory. Because the Taxo A bacitracin disk is readily available in most clinical laboratories for the routine presumptive identification of group A streptococci, we decided to assess the accuracy of this method for the purpose of differentiating staphylococci from micrococci in our laboratory.

A total of 425 catalase- and gram-positive isolates were included in this investigation. Genus determination was performed by glucose fermentation (3) and the modified oxidase test (1). Staphylococcal species were identified by the schemes recommended by Kloos and Schleifer (6) and Kloos (W. E. Kloos, Clin. Microbiol. Newsl. 4:75-79, 1982). The micrococci were not examined to the species level in this investigation.

The standardized disk-diffusion method described by Bauer et al. (2) was used to determine susceptibility to bacitracin by using disks of different potencies (0.04, 2.0, and 10.0 U; BBL Microbiology Systems). Bacitracin (Sigma Chemical Co.) broth dilution MIC determinations were performed in 1.0 ml of Mueller-Hinton broth (Scott Laboratories, Inc.) as previously described (5) at a concentration between 0.125 and 125 U/ml. Organisms that had an MIC <0.125 U/ml were further tested at a concentration between 0.125 and 0.00015 U/ml. The organism inoculum was adjusted to ca. 10^5 CFU/ml.

The results comparing growth inhibition zone diameters and the bacitracin broth dilution MICs for all organisms tested are shown in Table 1. These results indicate that when a growth inhibition zone diameter of >10 mm was used to establish susceptibility in testing with a 0.04-U bacitracin disk, the method was 100% sensitive but 94.6% specific. Testing with disks of higher potency did not improve the specificity of the disk-diffusion method.

Although the mean growth inhibition zone diameters and broth dilution MICs were considerably different between the staphylococci and micrococci, overlapping was observed. These results are shown in Table 2. Because the specificity of the disk-diffusion method did not improve when disks of

higher potency were used, only the results for the 0.04-U disk are included in Table 2.

The results of our investigation are in disagreement with the report (4) of a previous study, in which when tested with a 0.04-U disk, 100% of staphylococci were completely resistant and showed no zone of growth inhibition. In our investigation, 5.4% of all staphylococci tested had a demonstrable growth inhibition zone diameter (>10 mm). Because most of these bacitracin-susceptible staphylococci were *S. aureus*, it can be argued that this limitation is not significant, because *S. aureus* is not likely to be confused with micrococci in the laboratory. If bacitracin susceptibility was used only to differentiate coagulase-negative species of staphylococci from micrococci, the specificity of the method would increase to 96.8%. However, it is important to note that 4 (15.4%) of the 26 *Staphylococcus hominis* isolates tested had a growth inhibition zone diameter >10 mm with the 0.04-U bacitracin disk and would have been misclassified as *Micrococcus* species. The danger associated with misclassifying a coagulase-negative species of *Staphylococcus* as a *Micrococcus* species correlates well with the differences in pathogenic potential of these organisms. Because micrococci are considered to be nonpathogenic, antimicrobial therapy is generally not administered, whereas proper antimicrobial therapy is required to manage serious infections associated with coagulase-negative staphylococci. Therefore, workers in the laboratory need to exercise care in the proper classification of these organisms.

The difference between the specificity observed in our investigation (94.6%) and that described in a previous report (4) (100%) can be explained by the sampling of organisms in each of the investigations. Many more *S. aureus* isolates ($n = 156$) were included in our investigation than in the previous investigation ($n = 27$). In examining a larger sample of this particular species, we determined that 6.4% of the isolates tested had a significant growth inhibition zone diameter (>10 mm with a 0.04-U disk). Similarly, in examining a larger sample of *S. hominis* isolates ($n = 26$), we determined that 15.4% of the organisms tested also had a significant growth inhibition zone diameter (>10 mm with a 0.04-U disk).

Because the results of the MIC determinations were approximately 100-fold higher for the staphylococci than for the micrococci, we suggest the use of a single-well broth

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TABLE 1. Growth inhibition zone sizes and broth dilution MICs for 425 species of catalase- and gram-positive cocci

Organism	No. of isolates with the indicated zone size or MIC							
	0.04-U disk		2.0-U disk		10.0-U disk		MIC	
	≤10 mm	>10 mm	≤20 mm	>20 mm	≤25 mm	>25 mm	<0.1 U/ml	≥0.1 U/ml
<i>Staphylococcus aureus</i>	146	10	148	8	150	6	2	154
<i>Staphylococcus epidermidis</i>	69	0	69	0	68	1	0	69
<i>Staphylococcus hominis</i>	22	4	21	5	19	7	0	26
<i>Staphylococcus auricularis</i>	15	2	15	2	15	2	2	15
<i>Staphylococcus lentus</i>	17	1	16	2	15	3	0	18
<i>Staphylococcus warneri</i>	9	0	8	1	7	2	1	8
<i>Staphylococcus saprophyticus</i>	7	0	7	0	7	0	0	7
<i>Staphylococcus species</i> ^a	15	0	15	0	15	0	0	15
<i>Micrococcus species</i>	0	108	0	108	0	108	105	3

^a This group includes the following species: *Staphylococcus capitis* (n = 4), *Staphylococcus simulans* (n = 3), *Staphylococcus cohnii* (n = 2), *Staphylococcus haemolyticus* (n = 2), *Staphylococcus xylosus* (n = 1), and three other *Staphylococcus* species that were unidentifiable.

dilution test to determine bacitracin susceptibility. Our results indicate that 312 of 317 (98.4%) staphylococcal isolates would exhibit growth at 0.1 U of bacitracin per ml, whereas 105 of 108 (97.2%) micrococcal isolates would not.

TABLE 2. Breakdown of growth inhibition zone sizes and MICs

Bacitracin MIC (U/ml)	No. of isolates with the indicated growth inhibition zone size (0.04-U bacitracin disk)							
	≤10 mm		>10 ≤ 15 mm		>15 ≤ 20 mm		>20 mm	
	M ^a	S ^b	M	S	M	S	M	S
>0.0001 ≤ 0.001	0	0	3	0	18	1	5	0
>0.001 ≤ 0.01	0	0	11	3	34	0	6	0
>0.01 ≤ 0.1	0	2	10	0	19	0	0	0
>0.1 ≤ 1.0	0	53	0	10	2	1	0	0
>1.0 ≤ 10.0	0	233	0	0	0	2	0	0
>10.0 ≤ 20.0	0	9	0	0	0	0	0	0
32.0	0	3	0	0	0	0	0	0

^a M, *Micrococcus* species.
^b S, *Staphylococcus* species.

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