JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1976, p. 192-193

Vol. 4, No. 2
Printed in U.S.A.

SXT and Taxo A Disks for Presumptive Identification of Group A and B Streptococci in Throat Cultures

BRUCE A. GUNN[1]*

Medical Laboratory, Microbiology Service, Department of Pathology (WRAMC), Fort Meade, Maryland 20755

Received for publication 4 March 1976

A bacitracin (0.04 units, BBL)-SXT (trimethoprim, 1.25 mg, plus sulfamethoxazole, 23.75 mg, BBL) susceptibility test was 94% accurate in presumptively identifying streptococci as either group A, B or not group A and B.

Bacitracin susceptibility has routinely been used for the presumptive identification of group A streptococci. Other groups are usually resistant to the antibiotic and are often lumped into the category not-group A. During a recent study it was observed that streptococcal groups vary in their susceptibilities to Taxo A (bacitracin, 0.04 units, BBL) and SXT (trimethoprim [1.25 mg] plus sulfamethoxazole [23.75 mg], BBL) disks. This report concerns the results of that study.

Taxo A, SXT, and methicillin (5 mg; Difco) disks were tested on 305 clinical isolates from throat cultures. Each beta-hemolytic *Streptococcus* was plated onto one-half of a 5% defibrinated sheep blood agar plate (5% sheep blood in Trypticase soy agar, BBL) and inoculated into 10 ml of Todd-Hewitt broth (Difco). In most cases only one colony was used to seed both the broth and the sheep blood agar plate; however, the "minute" streptococci often required several colonies to achieve adequate growth. The three antibiotic disks were placed onto the inoculated surface of the sheep blood agar plates, and the plates were incubated for 18 to 24 h at 37°C in an atmosphere of 5% CO_2 in air. The Todd-Hewitt broth cultures were incubated for 18 to 24 h at 37°C. After incubation the zone sizes on sheep blood agar plates were recorded. The broth cultures were centrifuged at 3,000 rpm for 15 to 20 min, and the supernatants were discarded. The group antigens were extracted from the sedimented cells by the autoclave-Pronase B procedure of Edwards and Larson (2), and the antigens were grouped by the counterimmunoelectrophoresis procedure of Dajani (1). The grouping antisera (A, B, C, D, F, and G) were purchased from Difco Laboratories.

The standard bacitracin differentiation test correctly identified 94.4% (288 out of 305) of the beta-hemolytic streptococci as either group A or not-group A (Table 1). All of the group A strains and 12.2% (17 out of 139) of the not-group A strains were susceptible to bacitracin. In a recent study Moody reported no failures in detection of 656 group A strains with BBL bacitracin disks, but 5% were incorrectly identified with disks purchased from Difco (6). Moody also found that 28% of the not-group A strains were susceptible to BBL bacitracin disks, but only 10% were susceptible to the concentration of bacitracin in Difco disks. The results of that study demonstrates the need for standardization of the routine bacitracin test, as well as the bacitracin-SXT test reported in this paper.

Koch and Burchall reported the medium dependence of trimethoprim's antimicrobial activity (4). Trypticase soy medium was listed as not being suitable for routine testing of the susceptibility of bacteria to trimethoprim. The SXT disks used in this study contained 1.25 mg of trimethoprim (along with sulfamethoxazole, 23.75 mg) and were tested on a sheep blood-fortified Trypticase soy agar base. To confirm medium dependence, one group A streptococcal isolate was tested upon Trypticase soy agar with 5% sheep blood and was found to be resistant to SXT. The same isolate demonstrated a 26-mm zone of inhibition when tested upon Mueller-Hinton agar with 5% sheep blood.

In view of the above information, it should be emphasized that results with the bacitracin-SXT procedure will vary according to the source of the bacitracin disks and the medium used.

As can be seen in Table 2, all susceptibility patterns except the SS pattern acceptably identified the streptococci as either group A, B or not group A and B. (S, Susceptible, R, resistant; the first letter denotes susceptibility to bacitracin, and the last letter denotes susceptibility to SXT.) The SS pattern was composed primarily of groups other than A and B, and was found to constitute only 5% of the 305 cultures. The bacitracin-SXT test was interpre-

[1] Send reprint requests to: Box 205, Walter Reed Army Medical Center, Washington, DC 20012.

TABLE 1. *Susceptibility of beta-hemolytic streptococci to methicillin, bacitracin, and SXT*

Group	No. of strains	Methicillin	Bacitracin	SXT	Susceptibility pattern[a]
A	165	S(21)[b]	S(12)[b]	R(0)[b]	
B	1	S(15)	S(11)	R(0)	SR
G	1	S(24)	S(12)	R(0)	
A	1	S(18)	S(8)	S(18)	
C	9	S(20)	S(10)	S(22)	SS
F	3	S(13)	S(13)	S(20)	
G	3	S(21)	S(10)	S(15)	
B	33	S(15)	R(0)	R(0)	RR
G	1	S(20)	R(0)	R(0)	
C	25	S(18)	R(0)	S(19)	
F	13	S(16)	R(0)	S(22)	
F	1	R(0)	R(0)	S(12)	RS
G	32	S(21)	R(0)	S(19)	
Other[c]	17	S(21)	R(0)	S(21)	

[a] S, Susceptible; R, resistant. The first letter denotes susceptibility to bacitracin and the last letter denotes susceptibility to SXT.

[b] Numbers in parentheses indicate the mean diameter of the zones of inhibition for all tested strains. Susceptibility to the antibiotics is defined as any size zone of inhibition for bacitracin, zones greater than 9 mm for methicillin, and zones greater than 10 mm for SXT.

[c] Beta-hemolytic streptococci of groups other than A, B, C, F, or G.

ted to include within the group A streptococci both SR and SS susceptibility patterns, as found in Table 2. It should be noted, however, that 98.8% of the strains with the SR pattern and only 6.3% with the SS pattern were actually group A. It remains to be seen whether or not the SS pattern can be acceptably removed from the group A streptococci, thereby strengthening the usefulness of the bacitracin-SXT test over the routine bacitracin procedure.

Methicillin was included in this study because of the noted resistance of the group D enterococci to this antibiotic (3). There were no group D streptococci isolated during this study of 305 throat culture isolates. Of these 305 strains only one was found to be resistant to methicillin. The resistant isolate was a group F strain, a group that has been reported to be occasionally methicillin resistant by Matsen and Coghlan (5). Two group D enterococci were isolated from urine cultures by this laboratory and were found to be resistant to methicillin and of the RR susceptibility pattern.

TABLE 2. *The use of bacitracin-SXT to report the presumptive identification of group A and B streptococci*

Susceptibility pattern[a]	Presumptive identification	% Accuracy
SR	Group A	(165/167) 98.8%
SS	Group A	(1/16) 6.3%
RR	Group B	(33/34) 97.1%
RS	Beta-hemolytic streptococci not group A or B	(88/88) 100.0%

[a] The first letter denotes susceptibility to bacitracin and the last letter denotes susceptibility to SXT.

The data reported here demonstrate the usefulness of the bacitracin-SXT test for presumptively identifying beta-hemolytic streptococci as either group A, B or not group A and B.

LITERATURE CITED

1. **Dajani, A. S.** 1973. Rapid identification of beta hemolytic streptococci by counterimmunoelectrophoresis. J. Immunol. 110:1702-1705.
2. **Edwards, E. A., and G. L. Larson.** 1973. Serological grouping of hemolytic streptococci by counter-immunoelectrophoresis. Appl. Microbiol. 26:899-903.
3. **Facklam, R. R.** 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl. Microbiol. 26:138-145.
4. **Koch, A. E., and J. J. Burchall.** 1971. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. Appl. Microbiol. 22:812-817.
5. **Matsen, J. M., and C. R. Coghlan.** 1972. Antibiotic testing and susceptibility patterns of streptococci, p. 189-204. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.
6. **Moody, M. D.** 1972. Old and new techniques for rapid identification of group A streptococci, p. 177-188. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.