DIFFERENTIATION OF GROUP A FROM OTHER BETA HEMOLYTIC STREPTOCOCCI WITH BACITRACIN[1]

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In the study of upper respiratory infections, nose and throat cultures for beta hemolytic streptococci[3] are essential. Since group A streptococci are implicated in human respiratory infections more than other groups, it is important that streptococci recovered on culture be identified as belonging to group A or not. The finding of streptococci which belong to groups other than A in the nose or throat usually is interpreted as being of little or no clinical or epidemiological importance.

The only satisfactory means for differentiation of group A streptococci has been a serological method. The supply of necessary antisera has been limited, and the method requires considerable skill and technical experience if any high degree of accuracy is to be obtained. In epidemiological studies in large populations during epidemics of streptococcal infections, the number of streptococci isolated may be large and the serological identification burdensome and costly.

Maxted (1953) reported a method for differentiating streptococci belonging to group A from other beta hemolytic streptococci by their sensitivity to bacitracin. Filter paper squares were soaked in the antibiotic and rapidly dried. The strain of streptococcus to be tested was streaked on the surface of a blood agar plate and a bacitracin impregnated filter paper square placed on the inoculated area. After 24 hours' incubation the plate was examined for zones of inhibition. Group A streptococci were found to be inhibited by filter paper squares impregnated with 5 units per ml while streptococci of other groups were resistant to this concentration. Over 3,000 strains were tested by Maxted. The method was found to accurately differentiate 99.9 per cent of group A strains and 95.3 per cent of strains that were not group A.

In view of the obvious importance of this work to the laboratory in which it is not feasible to use serological methods for differentiation of streptococci and to those conducting large scale epidemiological investigations on respiratory disease, further investigation and confirmation of Maxted's results were desirable. Minor modifications in technique were necessary, but the method was found to be highly accurate.

METHODS AND MATERIALS

Preparation of discs. Filter paper discs, 6.4 mm in diameter, were cut with an ordinary paper punch. These were autoclaved and then saturated with a saline solution of the desired concentration of bacitracin. They were then placed in a narrow mouthed pyrex bottle, frozen in a dry ice-methyl cellosolve mixture, and dried under a vacuum on a Flosdorf-Mudd Cryochem apparatus. When dry, the discs were emptied into a sterile petri dish and stored in a freezer at −4 C. Discs prepared in this manner could be stored for at least two months without loss of potency.

Concentration of bacitracin[4] used. In preliminary tests, a concentration of 5 units per ml appeared to be excessive. This is demonstrated in the results of three series of tests shown in table 1. Each of these strains was freshly isolated in

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[2] The opinions and assertions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or of the Naval Service at large. Usage of a commercially available product in connection with this study cannot be construed as an endorsement of such product.

[3] The use of the terms streptococcus or streptococci hereafter refers only to strains causing beta hemolysis on blood agar plates unless otherwise specified.

[4] The bacitracin used in this study was kindly supplied by Chas. Pfizer and Co., Inc., New York, through the courtesy of Dr. Harry Seneca.

TABLE 1

Sensitivity of beta hemolytic streptococci other than group A to differing concentrations of bacitracin

No. of Non-group A Strains	No. Sensitive or Resistant to Bacitracin in Concentration of:									
	1.0 u/ml		1.5 u/ml		2.0 u/ml		2.5 u/ml		5.0 u/ml	
	S	R	S	R	S	R	S	R	S	R
21									17	4
13							10	3	11	2
15	3	12	5	10	5	10				

connection with concurrent epidemiological studies. Concentrations lower than 5 units per ml resulted in a decreasing proportion of non-group A strains which were inhibited by bacitracin. All group A streptococci tested in preliminary studies were sensitive to a concentration of 1 unit per ml, and this concentration was selected as optimum.

Strains of streptococci tested. A total of 1,163 strains of streptococci isolated from the nose and throat of Navy recruits with respiratory infections or in carrier studies during the winter and spring of 1953–1954 were tested. Streptococcal infections were prevalent in the population during a part of the period, and types 19, 18, and 14 were predominant. The cultures were obtained on dry cotton swabs and then either streaked directly on a blood agar plate or incubated in 2 ml of brain heart infusion broth containing 3 per cent sheep red blood cells for 4 hours before streaking. The blood agar plates were prepared by adding 40 g blood agar base (Difco), 10 g bacto-agar (Difco), and 500 ml Todd-Hewitt broth (1932) to 1 L of distilled water. The pH was adjusted to 7.2. After sterilization, 40 ml of sheep red blood cells were added.

Plates were incubated for 18 hours after streaking and examined against a strong light for streptococcal colonies exhibiting beta hemolysis. These were subcultured until a pure culture was obtained. All strains were serologically identified by the method of Swift, Wilson, and Lancefield (1943) and are shown in table 2.

Strains were tested by placing the disc over the heavily inoculated area of a blood agar plate. Three or four strains could be tested on a single blood agar plate. After 18 to 24 hours' incubation at 37 C the plate was examined for zones of inhibition. In determining inhibition, Maxted's description was followed as closely as possible.

TABLE 2

Number of serologically classified strains of beta hemolytic streptococci tested for bacitracin sensitivity

Group and Type	Number	Per Cent
A	819	70.5
Type 19	314	
Type 18	83	
Type 14	82	
Type 6	56	
Type 28	50	
Type 1	33	
Type 5	29	
Type 12	23	
Other*	45	
Untypable†	114	
B	56	4.8
C	67	5.8
G	126	10.8
F, H, or K	6	0.5
Not A, B, C, F, G, H, or K	89	7.6
Total	1,163	100.0

* Types 2, 3, 11, 15, 26, and 44.

† Did not type with available antisera.

If any colony grew up to the edge of the disc, the strain was recorded as resistant. In most cases the result was unquestionable, but in a few instances the opinions of several workers might vary. If there was any doubt, the test was repeated. Sensitive and resistant control strains were tested with each series of cultures. Figure 1 illustrates sensitive and resistant strains of streptococci.

RESULTS

Bacitracin sensitivities of 1,163 strains tested are summarized in table 3. A total of 866 strains were found to be bacitracin sensitive, and of these, 807 or 93.2 per cent were group A. Fifty-nine (59) or 6.8 per cent did not belong to group A.

Two hundred ninety-seven (297) strains were not inhibited by this concentration of bacitracin. Of these, 285 or 96 per cent were not group A. On the basis of initial tests, a total of 46 resistant strains were listed as belonging to group A. Only 34 of these were available for retesting, and all proved to be either sensitive to the bacitracin or not group A. The remaining 12 strains could not be retested and still appear in table 3 as resistant strains although the validity of this is doubtful.

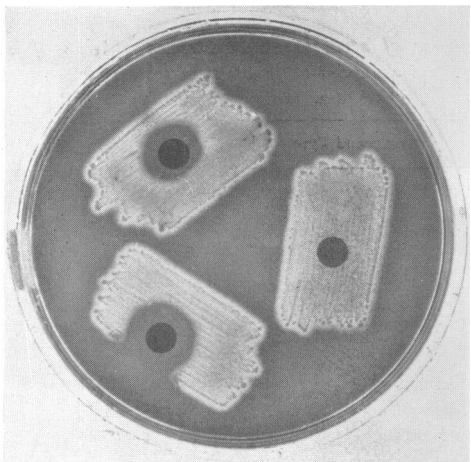

Figure 1. Blood agar plate showing sensitive and resistant reactions of beta hemolytic streptococci to bacitracin discs.

All sensitive strains of streptococci identified serologically as belonging to groups other than group A were also retested and confirmed.

Of the 819 strains of group A streptococci isolated, 807 or 98.5 per cent were found to be sensitive to a concentration of 1 unit per ml of bacitracin, while 82.9 per cent of the 344 nongroup A strains were resistant to this concentration.

During the course of the investigation a series of 25 strains found resistant to 1 unit per ml was tested against discs impregnated with solutions containing 2.5 and 5.0 units per ml. None of these were serologically identified as group A streptococci. Two strains proved to be sensitive to 2.5 units per ml, and another two were sensitive to 5.0 units per ml. This again demonstrates that a higher concentration of bacitracin was likely to increase the error of the test.

TABLE 3

Sensitivity of 1,163 strains of beta hemolytic streptococci to filter paper discs impregnated with a solution of bacitracin containing 1 unit per ml

Groups				Not groups A, B, C, or G	Total
A	B	C	G		
Bacitracin sensitive					
807	12	7	23	17	866
Bacitracin resistant					
12	44	60	103	78	297

DISCUSSION

These studies confirm the observations made by Maxted as to the high degree of accuracy with which group A and other groups of streptococci can be differentiated with bacitracin discs. In the present series, if the serologic results are wholly accurate, the resultant differentiation by bacitracin would have been in error in 6.8 per cent of the sensitive strains and 4.0 per cent of the resistant strains. These discrepancies are slightly greater than were found by Maxted, but in both studies the largest error among the nongroup A strains was in groups B and G and less in group C. In this study a greater number of groups B and G strains were isolated, while Maxted tested a greater number of group C strains. This accounts for the greater error among sensitive strains reported in this study. If the distribution of Maxted's groups B, C, and G were made comparable to that reported in this study, there would be no significant difference in the resultant error. In any event, the error seems to be small and little more than will occur using serological methods on a large number of strains. The error is probably less than would be encountered in the use of serological methods by inexperienced technicians. Of the 819 strains of group A streptococci studied, 1.5 per cent were reported as resistant. It is likely that none was actually resistant as the 1.5 per cent represents strains which could not be retested. These results compare favorably with those of Maxted, and it is probable that few strains of group A streptococci will prove to be resistant to a concentration of 1 unit per ml of bacitracin unless resistant strains result from the clinical use of bacitracin. As yet none has been reported, and it appears unlikely that this will ever occur.

The largest source of error would seem to lie in the erroneous classification of streptococci other than group A. Had we used Maxted's original concentration of 5 units per ml, a fairly large proportion of these strains would have been recorded as sensitive and classified as group A. Even with a concentration of 1 unit per ml, 59 strains which were inhibited by bacitracin proved not to belong to group A. During an epidemic of streptococcal infections this error would be negligible since a high percentage of all strains recovered is group A.

In nonepidemic periods, however, when a large percentage of the strains recovered may not belong to group A, this potential source of error may be a more serious problem. As the over-all accuracy of differentiation was better than 90 per cent, the problem would not be serious except where exact identification of all strains is required or where the diagnosis of a particular case may hinge on the classification of the organism. It is felt, however, that any strain resistant to bacitracin may be categorically discounted as belonging to group A.

A certain amount of caution need be exercised in estimating the results of this method. Maxted has emphasized that it applies only to beta hemolytic streptococci and that some strains of nonhemolytic streptococci are sensitive. It was noted in connection with the resistant strains in this series that some exhibited inhibition of hemolysis, but not of growth, in the zone surrounding the disc. When this phenomenon occurs, it must be ascertained that the culture is pure and not a mixture of a sensitive group A strain and a resistant streptococcus which is not beta hemolytic before the growth is accepted as indicating a resistant beta hemolytic streptococcus.

The paper punch offers an easy method of preparing discs of uniform size and is easier than cutting squares. The freeze-drying technique is efficient and appears to have an advantage over the phosphorus pentoxide method used by Maxted since the discs retained their potency for a period of at least two months whereas Maxted reports that the average life of his squares was three weeks. Commercially available discs have not, as yet, been tested but may offer a convenient source of discs.

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SUMMARY

The observation by Maxted that beta hemolytic streptococci belonging to group A can be easily differentiated from nongroup A strains by the use of bacitracin impregnated filter paper on the surface of inoculated blood agar plates has been confirmed.

Growth of all or nearly all strains of group A streptococci is inhibited by a concentration of 1 unit of bacitracin per ml. This concentration will also inhibit about 7 per cent of strains belonging to other groups.

The method is simple and has few sources of error compared to potential sources inherent in methods for serological differentiation.

It is recommended for use in laboratories where serological methods cannot be carried out and will be of help to those laboratories which need to differentiate large numbers of strains.

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