VALUE AND RELIABILITY OF THE BACITRACIN SCREEN TEST FOR IDENTIFYING LANCEFIELD GROUP-A HAEMOLYTIC STREPTOCOCCI

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S.haemolyticus is one of the most important pathogens of man and many of the bacteriological examinations carried out in a routine diagnostic laboratory are concerned with the identification of this organism.

In examining serial throat swabs of children suffering from scarlet fever or rheumatic fever, it was decided to evaluate Maxted's 'bacitracin screen test' as a means of identifying pathogenic haemolytic streptococci. A Lancefield grouping was carried out in parallel as a control on the bacitracin sensitivity results.

The test orginated with Maxted's observation that the growth of some haemolytic streptococci was inhibited by an adjacent growth of *B.licheniformis*, the inhibiting substance of which is almost identical with the antibiotic bacitracin.

In his test series Maxted showed that group-A streptococci were sensitive to bacitracin while other groups were resistant. He grouped or typed all strains in parallel and stated that as compared with Lancefield grouping, 1·7% appeared to be false sensitive strains and 2·5% to be false resistant.

The present investigation involved screening 520 strains of streptococci obtained from specimens of sputa and pus, nose, eye, vaginal and rectal swabs, and blood cultures.

METHOD

In order to use the least time-consuming technique all swabs were planted on horse-blood agar and the strains grown as surface colonies. It was thus possible to pick off colonies quickly for further investigation. The following morning all suspicious colonies were transferred either directly to fresh-blood plates or subcultured into broth or serum broth. In a few hours sufficient growth had developed in the broth tubes to transfer to a blood-agar plate, which becomes the diagnostic or 'sensitivity' plate carrying 4 strains. One half of each segment of the plate, on which were placed the discs soaked in a bacitracin solution of 5 units per ml., was inoculated heavily, and the other half was inoculated with 1 or 2 light strokes only. This facilitates recognition of contamination. It was essential to pick off at least 2 colonies from each specimen as experience showed that 2 almost identical colonies could give different results with regard to their bacitracin sensitivity.

When a strain produced a doubtful haemolysis it was incubated anaerobically. This usually clarified the type of haemolysis. It occasionally happened that, on the diagnostic plate, a strain showing bacitracin sensitivity changed its haemolytic property, became alpha-haemolytic, and grew as a resistant strain into the area of inhibition. Four such strains were encountered. In one instance the apparent beta-haemolytic streptococcus was in reality alpha-haemolytic for, when apparent alpha- and beta-haemolytic colonies were picked from the same plate and incubated under an atmosphere of CO_2, both types of colony presented alpha-haemolysis which was maintained on subsequent subcultures. In the other 3 cases, however, incubation under CO_2 was followed by the production of pure beta-haemolysis, which also was maintained during 5 subcultures (this phenomenom is at present being investigated).

In carrying out the parallel grouping, group-A serum only was used; cross reactions could therefore not be excluded. The following results were obtained: With the Lancefield grouping 380 strains were identified as group A and 140 strains were not. Of 383 bacitracin-sensitive strains 8, i.e. 2·1%, were found not to belong to group A when tested by Maxted's modification of the Lancefield grouping, and of 137 bacitracin-resistant strains, 5 i.e. 3·5%, proved to be false resistants. These false results show a slightly higher percentage than those published by Maxted.

TABLE I. RESUME OF RESULTS

	No. of Specimens	*Lancefield A Pos. Bacitr. Sensitive**	*Lancefield A Neg. Bacitr. Sensitive*	*Lancefield A Neg. Bacitr. Resistant**	*Lancefield A Pos. Bacitr. Resistant*
Throat swabs	419	326	4	86	3
Sputa	52	19	1	32	0
Pus	36	26	3	5	2
Eye swabs	2	1	0	1	0
Vaginal swabs	6	1	0	5	0
Rectal swabs	3	1	0	2	0
Blood culture	2	1	0	1	0
Total	520	375	8	132	5

* These columns show identical results of the Lancefield grouping and bacitracin sensitivity

DISCUSSION

In requesting bacteriological examination of specimens from the upper respiratory tract, or from foci of infection elsewhere in the body, the clinician is anxious, not only to know the bacterial flora which is present, but also the exact pathogens and their sensitivity to antibiotics. In so far as haemolytic streptococci are concerned, definite tests for classification may be beyond the capacity of the small laboratory. Few small laboratories carry out definitive tests for soluble haemolysin, leucocidin, fibrinolysin or erythrogenic toxin. Even the serological grouping of haemolytic streptococci is seldom available in such a laboratory and, in the absence of facilities for carrying out Lancefield grouping, the laboratory may rely solely on the presence or absence of beta-haemolysis which Schottmueller, as long ago as 1903, suggested as a means of classifying this group of organisms. Haemolysis itself, however, is subject to variation according to the environmental conditions and, even with the greatest care, is sometimes an

unreliable criterion of classification. In addition beta-haemolysis is frequently, but unjustifiably, equated with pathogenicity.

Under these circumstances a simple, reliable test which can be carried out in any laboratory, and which parallels the serological identification of haemolytic streptococci of Lancefield group A with reasonable accuracy, is to be welcomed. Maxted's screening test, using bacitracin, appears to meet this need. It is of no value in so far as anaerobic streptococci are concerned because, as Colebrook has shown, few of them are haemolytic. It might also fail in the presence of haemolytic streptococci acquiring bacitracin resistance owing to therapy with bacitracin but, so far as we know, none of the cases reported in this series had been so treated.

SUMMARY

A report is given of an investigation for bacitracin sensitivity of 520 strains of beta-haemolytic streptococci. The recommended concentration of 5 units of bacitracin per mil. was used with the disc technique. All strains were planted as surface cultures and incubated aerobically. Of 383 bacitracin sensitive strains 8 (2·1%) were false sensitive and of 137 bacitracin resistant strains 5 (3·5%) were false resistant.

I wish to thank Mr. P. Roux, of the bacteriological department of the South African Institute for Medical Research, for the encouragement I received from him when discussing this paper, and Mr. R. Robinson of the same department for his technical advice.

REFERENCE

Maxted, W. R. (1953): J. Clin. Path., 6, 224.