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Comparison of Dio-Bacit, Bacitracin-Trimethoprim/ Sulphamethoxazole and Latex Agglutination in the Diagnosis of Group A Beta-Hemolytic Streptococci

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Not only is Group A beta-hemolytic Streptococcus (GAS) the most frequent cause of bacterial pharyngitis, it is also the culprit in various skin and systemic infections, acute rheumatic fever, post streptococcal glomerulonephritis, and other disorders and complications. A new, ready-to-use media, Dio-Bacit, in a two section plate containing 5% sheep blood agar on one side and sheep blood agar with bacitracin (2 μg/ml) on the other was compared for its efficiency in identifying GAS with bacitracin and bacitracin + sulphamethaxazole / trimethoprim disk tests applied after isolation of beta-hemolytic colonies. We also used the latex-agglutination test as the gold standard method for differentiating GAS from streptococci belonging to other groups. Compared with the latex-agglutination test, we found the sensitivity and specificity of the Dio-Bacit method to be 92.0% and 96.9%, respectively. Dio-Bacit plates provide an easy and very useful way to identify GAS within one day, saving time, labor, and money for routine diagnostic microbiology laboratories.

Key Words: GAS, Dio-Bacit, bacitracin-trimethoprim / sulphamethoxasole, latex-agglutination

INTRODUCTION

Group A Beta Hemolytic Streptococcus (GAS) is the most frequent cause of bacterial pharyngotonsillitis. Although group C and G beta hemolytic streptococci are, on rare occasions, the causative agents of upper respiratory infections, beta-hemolytic streptococci other than group A are not considered as pathogens in general when they are identified in the upper respiratory system. Therefore, it is important to determine whether beta hemolytic streptococci identified in throat cultures belong to group A.[1,2]

The bacitracin disk test is commonly used to identify the group in which the streptococci belong.[2,3] To apply this test, colonies showing beta hemolysis in throat cultures are subcultured onto blood agar plates, and a paper disk containing bacitracin is placed. After 18 to 24 hours of incubation, the presence of an inhibition zone around the disk shows that the streptococci belong to group A. To overcome the need for a subculture and one more day of incubation, a bacitracin containing media, Dio-Bacit, has been introduced. The Dio-Bacit culture plate consists of a two-section plate containing 5% sheep blood agar on one side and the same medium with 2 μg/ml of bacitracin on the other. If beta hemolytic colonies are observed in the section without bacitracin and not in the section with bacitracin, this indicates the presence of Group A Streptococci. If beta hemolytic colonies are observed on both sides, they are considered not to belong to Group A. By eliminating the need for subculturing and the bacitracin disk test, Dio-Bacit reduces the time needed for GAS diagnosis.

This study was performed to identify the efficiency of Dio-Bacit in identifying GAS by comparing it with classical bacitracin, bacitracin + trimethoprim/sulphamethoxazole disk tests and latex agglutination.

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MATERIALS AND METHODS

Study population

Throat culture specimens were obtained from 490 children and adults (277 male, 213 female) who were admitted to the Hospital of Afyon Kocatepe University, School of Medicine, between October and December 2001 and were inoculated to Dio-Bacit plates.

Microbiological studies

The samples obtained by sterile swabs were first streaked on the side of the Dio-Bacit plates without bacitracin, then on the other side containing bacitracin (Diomed Biotechnology Products, Istanbul, Turkey). Then, the single colony streaking technique was applied to both sides using a wireloop. After overnight incubation in an atmosphere containing 5% CO_2, the plates were examined for the presence of beta-hemolytic streptococci. The presence of beta-hemolytic colonies on the side without bacitracin and their absence on the side with bacitracin suggested the presence of GAS. The presence of beta-hemolytic colonies on both sides indicated that these did not belong to group A.

Colonies with beta-hemolysis on 5% sheep blood agar but no growth on the side with bacitracin were subcultured, and disks containing 0.04 U Bacitracin (B) and 1.25 mg Trimethoprim & 23.75 mg Sulphamethoxazole (SXT) (Oxoid) were onto this subcultured plate. The plates were incubated overnight at 37℃. Colonies with beta hemolysis were classified according to their susceptibility to B (any zone) and resistance to SXT (no zone) as GAS. Additionally, all beta-hemolytic colonies were serologically classified by latex-agglutination (Streptococcal Grouping kit, Oxoid).

Statistical analysis Statistical comparisons between groups were performed by the McNemar test and the difference was considered to be significant when $p<0.05$.

RESULTS

Throat specimens obtained from 490 children and adults (277 male, 213 female) belonging to different age groups. Of the 490 inoculated Dio-Bacit culture plates, 71 (14.5%) contained beta-hemolysis in only the section without bacitracin. When the 71 strains, classified as GAS by Dio-Bacit, were subcultured to 5% sheep blood agar and tested for B and SXT susceptibility by disks, 69 were identified to be susceptible to bacitracin, while 59 were also resistant to SXT. These 71 strains were also evaluated by the latex agglutination test and 58 of them were defined as GAS (Table 1). Compared with the latex-agglutination test, the sensitivity and specificity of Dio-Bacit were 92% and 96.9%, respectively, and statistical significance was not observed ($\chi^2=3.12$, $p>0.05$) (Table 2).

DISCUSSION

GAS are the most frequent cause of bacterial pharyngotonsillitis, with the sequels named post-streptococcal syndromes, as in acute rheumatic fever and acute post streptococcal glomerulonephritis.[4] Seasonal changes and age may also contribute to these variations. Overcrowding, poor nutrition, and inadequate respiration give rise to epidemics of tonsillitis. Isolation rates of GAS vary depending on the growth plate used atmospheric conditions, incubation period, and description techniques.[1,2]

Although there are many other microbiological and biochemical tests for GAS, bacitracin susceptibility is the technique that is most widely used. Although very sensitive, but not completely specific, the bacitracin test is one of the most preferred methods for its convenience and low cost.[2] However, bacitracin resistance is reported to correlate variously to GAS in different studies. While Kiraz and Aksit[5] reported all GAS to be sensitive to bacitracin, Pallock and Dahlgren[6] reported bacitracin resistance to be 0.5%. Yajko, et al.[7] reported sensitivity and specificity rates of GAS to bacitracin to be 100%.

A study done in Eskişehir/Turkey showed that the prevalence of GAS was 13.16% in the student population of a primary school.[8] Takeuchi and Kawakita[9] isolated GAS in 12.2% of the 41,373 primary school students. In another study made

Table 1. Comparison of B, B-SXT, Latex Agglutination and Dio-Bacit Results in Throat Isolates, Assuming the Results of Latex Agglutination as the Gold Standard

		Dio-Bacit			
		GAS		Non-GAS	
		N	%	N	%
Bacitracin Disk test	S*	69	97.2	2	0.5
	R†	2	2.8	417	99.5
Bacitracin−SXT Disk test	SS‡	11	15.5	13	3.1
	SR	59	83.1	8	1.9
	RR	1	1.4	398	95
Latex-agglutination test	GAS	58	81.7	5	1.2
	Non-GAS	13	18.3	414	98.8

*S, Susceptible; †Resistant; ‡SS, Susceptible to both B and SXT.

Table 2. Comparison of Latex-Agglutination and Dio-Bacit Results in Throat Isolates

		Latex agglutination		Total
		GAS	Non-GAS	
Dio-Bacit	GAS	58	13	71
	Non-GAS*	5	414	419
Total		63	427	490

*Including colonies out of Streptococci.
Dio-Bacit sensitivity: 58 / 63: % 92.0, Dio-Bacit specificity: 414 / 427: % 96.9.

in our region, the incidence of GAS was reported to be 17.0% among primary school students.[10] The rate found in that study (17.0%), which was higher than that of other investigations, may have been due to the crowded classrooms in this region. Accordingly, it was reported that the increased frequency of GAS recurrence occurred from increased exposure in crowded day-care centers and schools. These authors also pointed out that the peak months of occurrence of GAS tonsillopharyngitis was late fall to early spring (November to March).[10] The present study was done in this period and could also explain the high incidence of GAS.

We detected 71/490 (14.5 %) GAS by Dio-Bacit and 63 by latex agglutination. Out of 63 GAS determined by latex agglutination, but 5 of them were accepted out of Streptococci. Dio-Bacit's sensitivity and specificity were 92% and 96.9%, respectively (Table 2).

Many factors, like disc diffusion technique, inoculation technique, incubation period, antibiotic concentration, type of growth plate, and amount of inoculated bacteria affect the sensitivity and specificity of GAS diagnostic tests.

The latex agglutination test, which is based on specific antigen-antibody reaction, is more sensitive and specific than bacitracin-SXT tests, but sometimes cross-reaction could be occurred. However, its high cost prevents routine laboratory application.[11]

Bacitracin susceptibility is 10% among group-C, F, and G streptococci, and 5% in group-B. For that reason, bacitracin testing together with SXT was suggested. Group-C and G streptococci are usually susceptible to SXT, while group A and B are

resistant. Since GAS can be resistant to both bacitracin and SXT, serological confirmation is necessary.[10] Kiraz, et al.[5] reported that among 118 GAS they tested, 97% were resistant to SXT.

The bacitracin disk test is preferred for defining GAS in daily routine tests since it is inexpensive, easy-to-use, and has a high rate of sensitivity (99%), although it does not have complete specificity. The tests give higher false positive results than false negative. Bacitracin-SXT test has a higher specificity than bacitracin alone. Durmaz, et al.[12] reported the specificity and sensitivity of Bacitracin-SXT test as 97% and 84%, respectively. The sensitivity and specificity determined in this study were 92.0% and 96.9%, respectively. The disadvantages of these tests are their requirements of subculturing to a new fresh blood agar plate, placement of paper disks containing bacitracin and SXT, and most of all an additional overnight incubation. On the other hand, obtaining individual beta-hemolytic colonies among the crowded bacterial colonies belonging to normal flora for making a pure subculture is sometimes very difficult. Dio-Bacit provides the opportunity to eliminate subculturing, additional culture plates, and disks containing bacitracin. Best of all, it saves one day for diagnosing GAS in routine laboratories currently using the bacitracin disk test. Overall savings may be more pronounced if the elimination of antibiotics unnecessary for treatment is considered. The addition of SXT to the section without bacitracin of the Dio-Bacit plate may increase the sensitivity of this test plate in the detection of GAS.

Serological diagnosis of GAS is very important in patients with immune disorders, tonsillopharyngitis epidemias, and serious infections, such as menengitis, osteomyelitis and endocarditis, and in determining penicillin tolerance.[1,2,13-16] We suggest the use of the latex-agglutination test in such cases due to its higher sensitivity and specificity compared to the other tests in addition to its easy use and short duration.

We conclude that Dio-Bacit plates provide an easy, very useful way to screen GAS within one day; saving time, labor, and money for routine microbiological laboratories that currently use the bacitracin disk test.

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