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Distribution of Streptococcal Groups in Clinical Specimens with Evaluation of Bacitracin Screening

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During a 2-year period, 4,968 strains of beta-hemolytic streptococci were examined for the clinical source distribution and bacitracin sensitivity of each group. In the upper respiratory tract, groups A (51.7%) and C (20.4%) accounted for most of the isolates, and in wounds and exudates group A (79.1%) made up most of the isolates. Group B (71.2%) was the major component of isolates from the genitourinary tract and, while composing 29.3% of the lower respiratory tract isolates, competed with group A (18.8%) and the nongroupables (22.8%) for supremacy. Bacitracin screening showed that 0.5% of group A streptococci were resistant, and sensitive non-group A isolates were group B (2.6%), group C (6.0%), group G (8.0%), and the nongroupables (2.2%). It was found that those groups which were most predominant in wounds and the upper respiratory tract gave the highest rate of false positives with bacitracin, whereas the predominant group of the genitourinary tract gave the lowest rate of false positives.

Infections caused by beta-hemolytic streptococci have long been a primary diagnostic concern. The complications of rheumatic fever and glomerulonephritis have made the need to distinguish group A streptococci from other groups an important charge of the clinical laboratory. Bacitracin sensitivity and fluorescent antibody (FA) methods have been rapid screening tools used for this purpose. Although group A streptococci appear to predominate in upper respiratory tract and wound infections, more recent reports indicate that other groups may be involved in the infectious process in other parts of the body (4, 6, 10, 11). Over the years, a number of reports have evaluated the bacitracin sensitivity of various streptococcal groups. Those which evaluated the bacitracin results for each group either used homemade strips containing more than 0.02 μm of bacitracin (5), a 1:5,000 dilution of the drug (8), or the number of non-group A strains studied were small (3, 4, 9). The reported false-positive reactions in the latter studies were extremely high.

We were interested in determining the group distributions from different clinical sources and what effect these might have on the reliability of the bacitracin screening technique as used in the clinical laboratory.

MATERIALS AND METHODS

Specimens. The specimens were from routine cultures submitted to the microbiology laboratories at the Harborview Medical Center from 15 December 1969 to 17 December 1971. All of the specimens were plated on blood agar base (BBL) containing 5% sheep blood and incubated anaerobically at 35 C. Other media were included in the protocol as dictated by the type of specimen.

Bacitracin sensitivity. A representative of each colony type was streaked to a whole blood agar plate and a bacitracin disk (0.02 μm, BBL) was placed at the junction of the first and second quadrant. After overnight incubation, the zone of inhibition was measured with a microcalipers. Any zone of inhibition extending beyond the edge of the disk was considered sensitive.

FA. All of the above organisms were screened by FA methods for their presence or absence in group A. Fluorescein-tagged antibody (BBL) was used according to the recommendations of the manufacturer. All organisms giving a negative bacitracin and FA or having a disagreement between the two tests were examined by a precipitin test for their Lancefield grouping.

Lancefield grouping. The streptococcal groupings were performed, by the capillary precipitin technique, using the rapid enzyme extraction method described by Maxted (7). An enzyme preparation from *Streptomyces albus* (Lytase; BBL) was used to extract the carbohydrate for grouping.

RESULTS

More than 4,000 streptococci were studied. Streptococcal isolates are tabulated by group and clinical source in Table 1. Only the clinical sources representing the majority of the isolates are included; thus, 263 isolates from ears, eyes,

TABLE 1. *Occurrence of common Lancefield groups in major clinical sources*

Source	Streptococcal groups				
	A	B	C	G	NG[b]
Upper respiratory	916 (51.7)[c]	153 (8.6)	363 (20.4)	134 (7.6)	207 (12.8)
Lower respiratory	143 (18.8)	222 (29.3)	95 (12.5)	94 (12.5)	203 (22.8)
Genitourinary	29 (4.3)	479 (71.2)	55 (8.2)	39 (5.8)	71 (11.5)
Wounds and exudates	1,188 (79.1)	65 (4.3)	60 (4.0)	110 (7.3)	79 (5.9)
Total	2,276 (48.4)	919 (19.9)	573 (12.2)	377 (8.0)	560 (11.5)

[a] Specimens (263) from ears, eyes, skin, blood, and spinal fluid are not included.
[b] NG, Nongroupable.
[c] Numbers in parentheses are percentages.

skin, blood, and spinal fluids are not found here. Group A streptococci composed 51.7% of the isolates from the upper respiratory tract, with group C and the nongroupable (NG) streptococci accounting for 20.4 and 10.4%, respectively. Wounds and exudates showed a greater prevalence of members of group A, which composed 79.1% of these isolates, whereas group G and NG streptococci made up 7.3 and 5.9%, respectively. On the other hand, group B predominated in the two other major categories of specimen types. These organisms accounted for 71.2% of the genitourinary tract isolates and 29.3% of the isolates from the lower respiratory tract. These specimens were predominantly sputa and tracheal aspirates in which NG streptococci composed 22.8% of the organisms isolated, group A composed 18.8%, and groups C and G accounted for 12.5% of these isolates each. NG streptococci and group C organisms were the next most frequent isolates behind group B in the genitourinary tract specimens. These composed 9.0 and 8.2% of the isolates, respectively.

An examination of the bacitracin sensitivity of all isolates, including those omitted from Table 1, demonstrated that some groups had a greater tendency to give false-positive reactions with bacitracin than did others (Table 2). Of 4,968 strains examined, 2,468 were group A and the remainder were members of other groups. Of these, group B accounted for 943 isolates, 581 were members of group C, 404 were members of group G, 501 were NG streptococci, and 71 members of group F were identified in the last 2,550 streptococci examined. Groups C and G had the highest rate of bacitracin sensitivity for non-group A streptococci. Of the group C organisms, 35/581 or 6.0% of them were sensitive. In group G 30/404 or 8.0% were sensitive, in group B 24/943 or 2.6% were sensitive, and in NG 11/501 or 2.2% were sensitive. There were no bacitracin-sensitive members among the group F isolates. Most of the bacitracin-sensitive streptococci in each group demonstrated inhibition zones of 12 to 16 mm by the technique used. Only 11/2,468 group A streptococci were resistant to bacitracin, giving 0.5% false negatives.

TABLE 2. *Bacitracin sensitivity of different streptococcal groups*

Group	Sensitive	Resistant	Discrepancy (%)
A	2,457	11	0.5
B	24	919	2.6
C	35	546	6.0
F[a]	0	71	0.0
G	30	374	8.0
NG	11	490	2.2

[a] Based on 2,550 isolates.

DISCUSSION

During a 2-year period, 4,968 beta-hemolytic streptococci were examined. The cultures have been presented as they occurred, with no judgment being made as to the clinical significance of the isolates. As would be expected from other studies, members of group A accounted for a major portion of the isolates from wounds and throats. The majority of the isolates from the lower respiratory tract and the genitourinary tract were members of group B. In this study, the rate of false-negative reactions was 0.5% compared with 4.1% reported by Ederer et al. (3), and the varying results depending on disk source reported by others (2, 9). The number of false-positive reactions observed in this study, by the bacitracin disks, is less than has been reported elsewhere (3, 9) and agrees with others (1, 8). Sensitivity tests are subject to many variables, particularly with low concentrations of antibiotic. Variations in inoculum, concentrations of antibiotic, length of time which plates are left at room temperature prior to incubation, and many other factors are likely to have a profound influence on the results of

different workers. Groups C and G gave the highest rate of false-positive readings, similar to the experience of Chitwood et al. (2) and Moody (9). Unlike these studies, Ederer et al. found that group B organisms had a greater percent of sensitive strains than did group C. These variations in results may reflect regional differences in strains.

More false-positive bacitracin reactions occurred in those groups which were commonly found in wounds and the upper respiratory tract; thus, if bacitracin sensitivity is the major criterion used for classification, the likelihood of misidentification of an isolate as group A may be significant. In recent years, increasing emphasis is being placed on the infections due to groups of streptococci other than A and D and their involvement in human infections (4, 9). The predominance of group B organisms in lower respiratory and genitourinary tract specimens indicates that more effort should be made to identify these organisms and determine their role in human disease.

Although the bacitracin test is a relatively good screening tool, the involvement of non-group A streptococci in human infection suggests that more consideration be given to more routine precipitin grouping, as suggested by Ederer et al. (3), and to the development of simple biochemical methods for identifying group B streptococci.

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