Absence of Wide-Zone, Alpha-Hemolytic Streptococci in Children with Pharyngitis

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Wide-zone, alpha-hemolytic streptococci are potential sources of false-positive throat cultures since they can be misinterpreted as beta-hemolytic streptococci. However, quantitative information regarding their occurrence in sick children was not available. Throat swabs from 312 children with pharyngitis were processed conventionally and also with pour-plate methods and examination of the positive cultures under magnification. One-third of all cultures were positive for beta-hemolytic streptococci. There were no significant differences in the number of positive cultures among or between the different methods. Wide-zone, alpha-streptococci were found in none of the cultures. Therefore, they are unlikely to be a common cause of false-positive cultures in children with pharyngitis.

Although wide-zone, alpha-hemolytic streptococci are potential sources of false-positive throat cultures, the magnitude of this problem was unknown (4). The relationship of group A beta-hemolytic streptococcal pharyngitis to the later occurrence of rheumatic fever is well established, but infections with alpha-hemolytic streptococci and beta-hemolytic streptococci other than group A do not cause this complication. Therefore, antibiotic treatment could be withheld for "positive" throat cultures due to wide-zone, alpha-streptococci.

Most hospital laboratories use surface inoculation for throat cultures and do not examine the type of hemolytic reaction under magnification. Taranta and Moody (4) state that pour-plate culture methods permit a better definition of the hemolytic reaction when the area adjacent to colonies growing deep in the agar is examined under magnification. On gross examination, wide-zone, alpha-streptococci have a zone of clear hemolysis near the colony which becomes larger with continued incubation and can be confused with beta-hemolysis. However, under magnification there are intact or discolored erythrocytes adjacent to the colonies. With beta-hemolysis, few or no intact erythrocytes are seen adjacent to the colonies.

In this study, we prepared both surface-inoculated and pour-plate cultures from a group of children with pharyngitis to determine the prevalence of wide-zone, alpha-streptococci.

MATERIALS AND METHODS

Throat cultures were available from 312 children with signs and/or symptoms of pharyngitis who were seen in the Medical Clinic, Evening Clinic or Emergency Room at Children's Hospital of Pittsburgh from 15 January 1974 through 5 May 1974.

A sterile, dry cotton swab was used to culture the tonsillar areas and posterior pharynx and was then stored in a cotton-stopped glass tube. Streptococci can be recovered, without change in quantitation, from dry swabs that have been stored for weeks or even months (1). This single swab was used to inoculate three different culture plates.

In culture A, the surface of a previously prepared sheep blood agar plate was inoculated, streaked, and stabbed as described by Wannamaker (5). The plate was prepared by pouring 15 ml of Trypticase soy agar (BBL) with 5% defibrinated sheep erythrocytes in a 9-cm plastic petri dish.

To prepare culture B, the dry swab was first vigorously scrubbed up and down in a tube containing 1 ml of Trypticase soy broth (BBL). One loopful (0.3 mm, internal diameter) of the broth suspension was mixed with 15 ml of melted Trypticase soy agar to which 0.8 ml of defibrinated sheep erythrocytes had been added. This was poured into a 9-cm plastic petri dish and allowed to solidify.

Culture C was prepared to check on any change in quantitation that might occur in the broth suspension. One loopful was inoculated onto the surface of a sheep blood agar plate and was streaked and stabbed as for culture A. The broth suspension for the B and C cultures was used as soon as prepared and was not preincubated.

For throat swabs obtained during the day, all three cultures were processed simultaneously. When swabs were obtained at night, the house staff inoculated culture A and the same dry swab was used the following day to prepare cultures B and C.

All cultures were incubated at 37°C in atmospheric air and inspected at 24 and 48 h for beta-hemolysis. The colony morphology of beta-hemolytic colonies was studied to determine if they were strep-
toccoci. Questionable colonies were Gram stained and/or catalase tested for confirmation. Cultures positive for streptococci were quantified according to the scale described by Breese et al. (2). Zero to 10 colonies were graded rare, 11 colonies through 25% of the visible growth appearing to be streptococci were graded 1+, 26% to 50% graded 2+, 51% to 75% graded 3+, and over 75% were graded 4+.

Colonies showing apparent beta-hemolysis in culture B (pour plate) were studied under ×15 magnification. The edges of the colonies were examined for the presence or absence of intact erythrocytes as shown in the figures in Taranta and Moody’s article (4).

Cultures positive for beta-hemolytic streptococci were subcultured for bacitracin testing. Streptococci with any degree of inhibition of growth around the bacitracin differential disks (Taxo A, BBL) were described as presumptively group A (3). Known strains of group A and non-group A streptococci served as controls for the bacitracin disks during the study.

RESULTS

Of the 312 throat swabs, 103 (33%) were positive for streptococci of any grade (rare through 4+) in one or more of the three cultures at 24 or 48 h. Eight-five of the positive cultures (83%) were presumptively group A by bacitracin sensitivity.

A satisfactory pour plate (culture B) was available for examination in 91 of the 103 cultures. In three cases, no beta-hemolytic streptococci were isolated from culture B. An additional four cultures were not usable because the growth was too light or too heavy. Five other cultures were accidentally discarded before being examined. In none of the 91 satisfactory cultures were intact erythrocytes seen adjacent to the edges of the colonies when examined under magnification.

From this same group of 91 cultures, all cultures with beta-hemolytic streptococci in culture A on gross examination were confirmed as beta-hemolytic when examined under magnification in culture B. Where no beta-hemolytic streptococci were isolated in the three additional B cultures referred to above, the corresponding A cultures had only rare growth in two cases. In the third, the original culture A had 4+ growth, but a repeat pour plate made from that same culture grew heavy streptococci that were beta-hemolytic when examined under magnification. Conceivably, there was a laboratory error in the original culture B.

We also analyzed the overall recovery rates of beta-hemolytic streptococci, as determined grossly, by the three different culture methods. Table 1 shows the 48-h results for the sets of cultures where one or more was positive for beta-hemolytic streptococci and the results from all three cultures were available. Cultures with any degree of positivity from rare through 4+, whether group A or not, were assigned to the positive category. The differences in recovery rates are not significant by chi-square analysis.

A more specific paired comparison of the recovery of beta-hemolytic streptococci from the conventional surface-inoculated plate (culture A) to the pour plate (culture B) was made for 93 pairs where 48-h results for both were available and at least one was positive. Using the same assignment to the positive category as above, both cultures A and B were positive in 85 pairs. In three pairs, culture A was positive where culture B was negative. In five other pairs, culture B was positive where culture A was negative. These differences are not significant by the binomial test.

DISCUSSION

How often wide-zone, alpha-hemolytic streptococci masquerade as beta-hemolytic streptococci in children with pharyngitis had not been established. R. R. Facklam estimated that as many as 5% of the presumed beta-hemolytic streptococci sent to the Center for Disease Control may be wide-zone alpha, but these were from a variety of highly selected clinical specimens and usually not throat cultures (6). Facklam also refers to a high incidence of wide-zone, alpha-streptococci in school children (6). However, additional information obtained from him indicates that the children were not sick when they were cultured and also that the circum-

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**Table 1. Recovery of beta-hemolytic streptococci by three different culture methods at 48 h**

<table>
<thead>
<tr>
<th>Cultures</th>
<th>No. positive for beta-hemolytic streptococci</th>
<th>No. negative for beta-hemolytic streptococci</th>
<th>Positive cultures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Surface inoculation—dry swab</td>
<td>86</td>
<td>5</td>
<td>94.5%</td>
</tr>
<tr>
<td>B Pour plate—broth suspension</td>
<td>88</td>
<td>3</td>
<td>96.7%</td>
</tr>
<tr>
<td>C Surface inoculation—broth suspension</td>
<td>83</td>
<td>8</td>
<td>91.2%</td>
</tr>
</tbody>
</table>

* N = 91. Sets where results of all three cultures were available and one or more was positive for beta-hemolytic streptococci.
stances and the culture methods were somewhat unusual.

An apparent outbreak of streptococcal pharyngitis occurred in a small isolated community. The local laboratory was using human blood agar and reading the hemolysis from poorly streaked plates. Three hundred and twenty children were recultured several days later when they were asymptomatic. These cultures were incubated anaerobically overnight at 37°C and then set out at room temperature in atmospheric air for an additional 24 h. Interestingly, all cultures appeared negative for beta-hemolysis at 18 h, but after 24 h at room temperature, approximately 35% of the cultures showed hemolysis. Replating the cultures showed only alpha or wide-zone, alpha-streptococci. All cultures were grown on rabbit blood agar since the Center for Disease Control routinely works with subcultures and not primary isolations. (Letter dated 25 September 1975 from Richard R. Facklam, Center for Disease Control, Atlanta, Ga.)

Therefore, the relevance of these findings to sick children with pharyngitis whose cultures would be processed by the usual clinical laboratory using sheep blood agar is unknown. There may be different recovery rates of wide-zone, alpha-streptococci with different blood and incubating conditions. Also, the outbreak described above occurred in a closed population.

We studied 312 children with pharyngitis. Several culture methods, all using sheep blood agar, were used for each swab processed. Pour plates of cultures with apparent beta-hemolysis were examined under ×15 magnification for the presence or absence of intact erythrocytes around the individual colonies. We did not find wide-zone, alpha-streptococci in any of the cultures examined. Although the population size was not large, the cultures were obtained during a season when streptococcal pharyngitis is frequent in Pittsburgh. Whether these findings can be generalized to other geographic areas and seasons is not known.

Except for three cases, all cultures with beta-hemolysis in the conventional surface-inoculated culture A were confirmed as beta-hemolytic by examination of the corresponding pour-plate culture B under magnification.

Although pour-plate culture methods are regarded to be more sensitive, analysis of recovery of beta-hemolytic streptococci by the different culture methods did not show a significant difference. This was true whether overall recovery rates were compared or a more specific paired comparison of the conventional surface-inoculated plate to the pour plate was made. Therefore, we do not feel that the added time and expense of using special pour-plate techniques is justified for increased recovery of beta-hemolytic streptococci or to detect wide-zone, alpha-streptococci in usual clinical practice.

LITERATURE CITED