ENUMERATION OF β-HAEMOLYTIC STREPTOCOCCI ON NORMAL SKIN BY DIRECT AGAR CONTACT

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Several studies have indicated that group-A β-haemolytic streptococci may be found on the normal skin of members of populations in which streptococcal pyoderma is prevalent (Markham and Stenhouse, 1959; Dudding et al., 1970; Ferrieri et al., 1972). In epidemiological investigations of streptococcal impetigo in this laboratory (Dudding et al., 1970; Ferrieri et al., 1972) a moist-swab technique has been used for obtaining specimens for culture from a defined area of normal skin. These cultures usually yielded less than 10 colonies of streptococci (a term used in this paper as a synonym for β-haemolytic streptococci), but the isolation of group-A streptococci from the skin of children without cutaneous lesions was associated with a high probability that the same children would develop impetiginous lesions containing group-A streptococci of the same serological type within the ensuing few weeks.

In this report we describe the isolation of β-haemolytic streptococci from normal skin by direct agar contact, by means of a special plate for microbiological sampling of surfaces (Hall and Hartnett, 1964), and compare the frequency of finding streptococci by direct agar contact and by the moist-swab method. When samples obtained by both methods were positive for β-haemolytic streptococci, the quantitative results are analysed and compared.

MATERIALS AND METHODS

Methods of sampling and culture

The Hall and Hartnett plate is now marketed under the trade name "Rodac" (Falcon Plastics, Division of B-D Laboratories, Oxnard, Calif. 93030, USA). Two features of this plate facilitated its use in the present study: the meniscus of the medium is above the side wall of the plate, and the back of the plate has a 16-cm² grid, so that colony-forming units imprinted on the agar can be enumerated with respect to the exact surface area sampled.

Tryptose Blood Agar Base (Difco) containing Todd-Hewitt Broth (Difco) 1% (w/v) and sheep blood 6% (v/v) was prepared and poured by hand into "Rodac" plates until the meniscus was above the side wall of the plate. After the medium had solidified, the covers were set in place and the plates inverted, stacked, and kept at 4°C for 1 to 2 weeks before use. This interval allowed for partial drying of the agar. Fresh agar proved too moist, causing "smudging" of recovered bacterial colonies.

When the agar was applied to skin-surface sites, sufficient pressure was exerted so that skin-texture features (hair, pores) were imprinted on the agar. A rolling action was necessary to follow the contour of the surface sampled and to avoid air entrapment. Though time was not carefully controlled, the period of contact was approximately 1 sec. Care was taken to

Received 11 Mar. 1975; accepted 25 Mar. 1975.
avoid sliding between the agar and skin surface which would have smudged the exact replication of organisms. After sampling of selected skin sites (see below), inverted plates were incubated at ambient summer temperatures (20°C to 35°C), necessitated by field conditions, for 24 h, and the resulting colonies enumerated as β-haemolytic streptococci and "other aerobic flora". Plates negative for streptococci at 24 h were incubated another 24 h at 35°C and re-examined. Selected streptococcal colonies were grouped serologically (Lancefield, 1933).

Swab specimens of normal skin areas were obtained by scrubbing an approximate 4x4-cm area of skin with a sterile cotton-tip applicator moistened with Todd-Hewitt Broth. These were immediately inoculated on to blood-agar plates and within several hours the plates were streaked with a wire loop. After incubation under conditions similar to those used for contact plates, the plates were examined and the number of colonies of β-haemolytic streptococci recorded (Dudding et al., 1970; Ferrieri et al., 1972).

Lesions were cultured, after removal of crusts, with a cotton-tip applicator moistened with Todd-Hewitt Broth; subsequent bacteriological procedures were identical with those used for swab cultures of normal skin.

Study population

From 24 June to 12 Sept. 1972, children between the ages of 1 year and 14 years in the Red Lake Indian Reservation in northern Minnesota had normal skin cultures taken by the direct agar-contact technique at weekly intervals. The children were participants in studies for which the informed consent of parents had been obtained. The number of children examined each week ranged from 21 to 85, with an average of 56. During the first three weekly visits, only children under 5 years of age were examined. After the first three weekly visits, children were examined weekly regardless of age. One agar-contact culture of the volar surface of the mid right forearm was performed at each examination. Simultaneously, a moist-swab sample of an approximate 4x4-cm area of the adjacent volar surface of the right wrist was obtained. If pyoderma was present on the right forearm, the left forearm was sampled. A similar moist-swab culture of the medial aspect of the right ankle was also obtained but the results are not included in this analysis.

RESULTS

During the study 600 agar-contact cultures were obtained. Among the 554 plates that were technically acceptable for inclusion, β-haemolytic streptococci were present on 98 (18%). Serological grouping was performed on a single-colony isolate from 91 of the 98 plates positive for β-haemolytic streptococci. Eighty-five belonged to group A, two to group C and four to group G.

Frequency of detection and enumeration of streptococci by the agar-contact and moist-swab methods

Cultures were obtained simultaneously by direct agar-contact and by moist-swab sampling of an adjacent area of the same extremity in 480 instances. Isolation of β-haemolytic streptococci was significantly more frequent (P = <0.05) by the agar-contact method (83 instances; 17.3%) than by the moist-swab method (59 instances; 12.3%). Table I presents a more detailed analysis of these results. There was concordance for presence (+) or absence (−) of β-haemolytic streptococci in 84% of pairs of samples examined by the two methods. However, the employment of both methods appreciably increased
TABLE I
Isolation of β-haemolytic streptococci from normal skin by the agar-contact and the moist-swab method; concordance and non-concordance between results obtained from 480 simultaneously collected pairs of samples

<table>
<thead>
<tr>
<th>Agar-contact method</th>
<th>Moist-swab method</th>
<th>Number (and percentage) of pairs of samples giving the indicated result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>32 (6.7)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>370 (77.1)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>51 (10.6)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>27 (5.6)</td>
</tr>
</tbody>
</table>

β-Haemolytic streptococci: + = isolated; - = not isolated.

TABLE II
Comparison of counts of β-haemolytic streptococci obtained by the agar-contact and the moist-swab method on 475 simultaneously collected pairs of samples*

<table>
<thead>
<tr>
<th>Agar-contact method; colony count</th>
<th>Moist-swab method; number of pairs giving a colony count of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>nil 1-9 10-50 &gt;50 any number</td>
</tr>
<tr>
<td>Nil</td>
<td>370 22 4 1 397</td>
</tr>
<tr>
<td>1-9</td>
<td>43 18 3 0 64</td>
</tr>
<tr>
<td>10-50</td>
<td>5 3 3 0 11</td>
</tr>
<tr>
<td>&gt;50</td>
<td>0 1 1 1 3</td>
</tr>
<tr>
<td>Any number</td>
<td>418 44 11 2 475</td>
</tr>
</tbody>
</table>

* Five pairs of samples in which contact plates were positive for streptococci (included in table I) were not included here because accurate counts of streptococcal colonies could not be made.

the total yield of positive cultures. In the non-concordant pairs, the frequency of isolation of streptococci by direct agar contact was almost double that by the moist-swab method.

Quantitative results, on 475 pairs of samples in which countable cultures were obtained, are presented in table II as the distribution of pairs according to the range of colony counts obtained by the two methods. When grouped in this way the results were fairly well correlated. Among the 30 pairs positive for β-haemolytic streptococci by both methods, members of 22 pairs fell into the same quantitative group; there was a difference of one group between members of seven pairs, and a difference of two groups between members of only one pair. Among the eight pairs positive by both methods but falling into different quantitative groups, five showed higher counts by the agar-contact method and three showed higher counts by the moist-swab method. Among the 75 sample pairs positive for β-haemolytic streptococci by one method and negative by the other, 48 were positive by the agar-contact method and 27 by the moist-swab
method. In these 75 pairs, the positive samples usually exhibited low counts. The greater frequency of positive results obtained by the agar-contact method (also shown in table I) appeared to be due to an increased sensitivity in detecting small numbers of colony-forming units (c.f.u.) of \( \beta \)-haemolytic streptococci.

**Quantitative comparison of streptococci and other aerobic flora by the agar-contact method**

Table III summarises the quantitative data from 80 agar-contact plates positive for streptococci, where both streptococci and other aerobic flora could be adequately counted. The range of counts was rather wide, covering an approximately 3-log span both for streptococci and for other aerobic flora. There was a considerable difference between the mean and the median values for the streptococcal counts. Because the means were skewed upward by a relatively small number of samples with very high counts, the streptococcal data are perhaps better represented by the median of 3 rather than the average of 14 c.f.u. per 16 cm\(^2\).

On most agar-contact plates the numbers of other aerobic flora were considerably higher than the numbers of \( \beta \)-haemolytic streptococci. In these 80 samples, the ratios of other aerobic flora to streptococci on individual plates varied over a wide range (low 0.3:1; high 1212:1). The mean ratio was 40:1 and the median ratio 60:1. Contact plates positive for streptococci but with uncountable colonies of other aerobic flora, and contact plates negative for streptococci were not included in this analysis.

**Distribution of streptococcal counts by time**

The figure represents the distribution of counts on the 91 agar-contact plates containing countable colonies of streptococci according to the specific dates on which the samples had been obtained. It demonstrates the consistently low counts and low means in most sample periods, especially in early summer. Towards the end of the summer a small number of samples had high counts.

**Table III**

*Comparison of colony counts of \( \beta \)-haemolytic streptococci and other aerobic flora on 80 samples from normal skin by the agar-contact method*

<table>
<thead>
<tr>
<th>Range</th>
<th>( \beta )-haemolytic streptococci</th>
<th>other aerobic flora</th>
<th>total aerobic flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arithmetic mean</td>
<td>14.3</td>
<td>269</td>
<td>284</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>187</td>
<td>214</td>
</tr>
</tbody>
</table>

* Area sampled = 16 cm\(^2\).*
In all, only 20 (22%) of the 91 countable samples positive for β-haemolytic streptococci yielded 10 or more colonies per 16 cm².

**Relationship between the number of streptococci isolated from normal skin and the presence of lesions**

In table IV the number of streptococci (c.f.u.) isolated from the normal skin by agar-contact plates is related to the presence or absence of streptococcal skin lesions at the time the sample was obtained. The presence of skin lesions containing streptococci was not regularly associated with the isolation of streptococci from the area of normal skin sampled. In 37 of the 76 samples (49%) taken from children who had streptococcal impetigo at the time of examination, streptococci were not isolated from normal skin by agar contact of the forearm. When positive streptococcal cultures of the normal skin were obtained from children with streptococcal impetigo, the counts were generally low; only 12 (32%) of the 37 countable positive samples obtained from children with lesions gave counts of 10 or more streptococcal colonies per 16 cm². Most agar-contact cultures obtained on children without lesions were negative for streptococci (419 of 478; 88%). Of the 54 positive countable samples obtained from children without lesions, only eight (15%) exhibited counts of streptococci of 10 or more c.f.u. per 16 cm².

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**Ficim.-Distribution of counts of β-haemolytic streptococci among positive agar-contact samples according to date of collection. (Seven samples on which it was impossible to obtain exact counts are omitted.) Each solid circle represents the number of streptococcal colonies found by direct agar contact (16-cm² surface) on one positive examination of one child. The horizontal lines represent the mean counts for the recorded date.**
TABLE IV

Relationship to presence or absence of streptococcal skin lesions of counts of β-haemolytic streptococci on agar-contact samples from normal skin

<table>
<thead>
<tr>
<th>Streptococcal count*</th>
<th>Number of samples† obtained from children with lesion or lesions</th>
<th>without lesions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37</td>
<td>419</td>
<td>456</td>
</tr>
<tr>
<td>&lt;10</td>
<td>25</td>
<td>46</td>
<td>71</td>
</tr>
<tr>
<td>≥10</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Not countable‡</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Any number</td>
<td>76</td>
<td>478</td>
<td>554</td>
</tr>
</tbody>
</table>

* Per plate (area sampled = 16 cm²).
† Analysis includes samples obtained from the same or different children at weekly intervals.
‡ Positive for β-haemolytic streptococci but not countable.

Effect of penicillin on the isolation of streptococci from the normal skin

Some of the data included in this report were obtained while the children were enrolled in a study of benzathine-penicillin prophylaxis of double-blind cross-over design. The possibility that penicillin might have an effect on the isolation of streptococci from the normal skin was considered. Table V summarises the results of 306 contact samples obtained during a period when penicillin might have affected the results (arbitrarily defined as the 4-week period after injection). There was a significant decrease (P = <0.01) in the number of normal skin samples positive for streptococci after the administration of penicillin. Penicillin appeared to be most effective in reducing the isolation rate for streptococci soon after injection. After 4 weeks, the frequency of isolation of streptococci appeared to be unrelated to the type of injection given at the beginning of that period.

When streptococci were isolated after penicillin injection, the colony counts obtained on contact plates (range 1–80, mean 11-6, median 3) were essentially the same as those found after the placebo (range 1–122, mean 11-5, median 3). Penicillin did not influence the mean and median number of other aerobic flora on the normal skin. Five children studied by agar contact developed lesions within 28 days after penicillin injection. At the time lesions were present, samples from three of these children yielded no streptococci; those from one child yielded one colony and from one other child yielded two colonies of streptococci. Two of these five children had streptococci present in very low numbers on normal skin the week before the discovery of the skin lesions. Another five positive contact samples were obtained from children who were excluded from the analysis presented in table V because they were receiving antibiotics extraneous to the study. Two of these yielded 10 or more streptococcal colonies per 16 cm². A more detailed analysis of the effects of
**TABLE V**

**Effect of penicillin on the isolation of β-haemolytic streptococci from normal skin by the agar-contact method**

<table>
<thead>
<tr>
<th>Time interval after injection (weeks)</th>
<th>Benzathine penicillin*</th>
<th>Saline placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of samples examined</td>
<td>Number positive</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>Any†</td>
<td>151</td>
<td>14</td>
</tr>
</tbody>
</table>

* All children (except those allergic to penicillin) received benzathine penicillin (600,000 units for children 6 years of age or younger; 1,200,000 units for children 7 years of age or older) intramuscularly during either the first or the second period of the study. For the purposes of this analysis, data from the two periods were combined according to the type of injection received at the beginning of the period.
† Up to 4 weeks.

Penicillin prophylaxis on the detection and enumeration of streptococci on the normal skin will be given elsewhere (Ferrieri et al., unpublished).

**DISCUSSION**

All semi-quantitative methods of sampling surfaces for bacteria have advantages and limitations, some of which are specific to the method employed (Rubbo and Dixson, 1960; Armbruster, 1962; Green et al., 1962; Favero et al., 1968). The cotton swab has proved to be useful for field investigations of streptococcal infections. It permits vigorous scrubbing of surfaces of varying contours and may be used to obtain samples from small or less readily accessible areas, such as the nose, throat and skin lesions.

In this study, agar-contact plates were also easily managed in the field. The procedure of skin contact is rapid, and after contact the culture plate is ready for incubation without further manipulation. This may be a special advantage when field conditions do not permit streaking of plates before growth of the inoculum begins. Our experience with the agar-contact plate also substantiated its obvious theoretical disadvantages. It may not be used on grossly irregular or out-of-the-way surfaces. When dense bacterial populations are present on sampled surfaces, counting their replicates on agar is difficult if not impossible.

In swab methods, one cannot determine where a particular organism resided within the sampled area. Moreover, factors influencing transfer of the organisms from the swab to the agar surface are uncontrolled. In direct agar-contact techniques, the organisms isolated are related to specific locations in the area sampled rather than to the general area. Indeed, the colonies formed on agar reflect the precise in-situ surface position of their ancestors.

In addition to the advantage of increased anatomical specificity, the direct
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The agar-contact technique compares favourably with the moist-swab method in frequency of isolation of β-haemolytic streptococci from normal skin sites. Our data suggest that the agar-contact method may be more sensitive for streptococcal counts in the lower range.

In neither method is there control of factors governing adherence of the organisms to the surface on which they reside or in which they are embedded. Isolation of streptococci by the relatively brief and unperturbing skin-agar contact implies that the streptococci were located superficially on the skin.

Quantitative information obtained by both of the methods used in this study suggest that when streptococci are present on normal skin they are usually there in low numbers. Streptococci also comprise only a small proportion of the total aerobic flora of the skin as indicated by the high ratio of other aerobic flora to streptococci on the agar contact plates.

The comparison of the two methods presented here must be interpreted with some caution, because separate though adjacent skin sites were sampled. However, analysis of information obtained previously in this laboratory indicated that, when multiple sites on the same forearm are sampled by the moist-swab method, there were no appreciable differences between adjacent sites in the frequency of detection of streptococci or in the number isolated (Ferrieri, Dajani and Wannamaker, 1970, unpublished). Indeed, in earlier studies (Dudding et al., 1970; Ferrieri et al., 1972) normal skin cultures taken from as remote a site as the mid-back were only slightly less frequently positive for streptococci than were cultures taken from the wrist.

Both the frequency of isolation of streptococci from normal skin and the number of streptococcal colonies obtained were higher in earlier studies reported from this laboratory than in the present studies. Culture of the wrist by the moist-swab method in previous studies (Dudding et al., 1970; Ferrieri et al., 1972) was positive for β-haemolytic streptococci in c. 33% of samples, in comparison with 17 and 12% of samples in the present study by the agar-contact and swab methods respectively. Dudding et al. (1970) estimated that one-third of their wrist samples positive for streptococci yielded more than 10 colonies, and Ferrieri et al. (1972) found that 43% of their normal skin samples positive for streptococci yielded 10 or more streptococcal colonies per plate; the moist-swab technique was the only method used in these earlier studies.

Among the paired samples of the present study 18% of positive samples obtained by the agar-contact method and 23% of positive moist-swab samples yielded 10 or more colonies of streptococci.

These differences are probably not attributable to penicillin prophylaxis. In the present study, the injection of benzathine penicillin seemed to influence the frequency of isolation of streptococci from normal skin but not the number of streptococcal colonies in the cultures that were positive. The effect of penicillin prophylaxis on the frequency of isolation of streptococci from the normal skin may have been indirect, i.e., a reduction in the amount of exposure to streptococci (Ferrieri et al., unpublished). As reported in an earlier pilot study (Ferrieri et al., 1974), streptococci were isolated as soon as 2 weeks after a prophylactic injection of benzathine penicillin.
As might be expected, β-haemolytic streptococci were more frequently obtained from samples taken from children with lesions (51%) than from those without lesions (12%). Although some children with lesions had streptococcal counts in the upper range (10 or more), our data indicate that higher counts from the skin were not consistently associated with the presence of lesions elsewhere. However, the counts in children with lesions might have been higher if samples obtained in close proximity to a lesion had been included in the study.

Although the information in this report mainly substantiates that already obtained by the moist-swab method, future studies could well make better use of the advantages of agar-contact methods. Fundamental issues such as the nature of deposition, survival and possible colony formation of streptococci on the skin could be studied by this technique (Wannamaker et al., 1974). Indeed, a method has been described for estimating the size of staphylococcal microcolonies on normal adult skin by means of replicate impression cultures in conjunction with a scrub-sampling technique (Holt, 1971). Thus, the agar-contact plate may also be applicable to a more incisive analysis of the interrelationships of streptococci and their cutaneous environment.

**SUMMARY**

Normal skin sites in children from a population in which streptococcal impetigo is endemic were examined for the presence of β-haemolytic streptococci by a direct agar-contact technique. Ninety-eight of 554 samples (18%) were positive for these organisms. Penicillin prophylaxis reduced the frequency of isolation of streptococci from the normal skin for a period of 3 weeks, perhaps accounting in part for the lower isolation rate in this than in earlier studies.

Numbers of streptococcal colony-forming units in positive samples were generally low, both in terms of absolute numbers isolated from the surface area sampled and in comparison with numbers of other aerobic flora recovered. The presence of streptococcal pyoderma at the time of agar contact was not necessarily associated with the presence of or with increased numbers of streptococci on samples obtained from normal skin sites. Low counts were consistently found in early summer and higher counts in some samples in late summer.

In a simultaneous comparison of paired samples taken from adjacent sites, the frequency of detection of streptococci by direct agar contact compared favourably with that obtained with a moist-swab method. The increased frequency of detection by the agar-contact method appeared to be related to an increased sensitivity for the detection of low numbers of streptococcal colony-forming units on the normal skin.

We thank the participants in the study, the members of the Red Lake Comprehensive Health Services and the physicians and administrative personnel of the US Public Health Service Hospital at Red Lake for their co-operation. The technical assistance of T. LeVin, D. Johnson and Judy Jaqua is gratefully acknowledged. We also wish to thank Drs H. Hill, E. Kaplan and P. Cleary for general assistance in gathering data in the larger study.
This work was supported by a research grant from the US Public Health Service (AI 09527) and conducted in part under the sponsorship of the Commission on Streptococcal and Staphylococcal Diseases, Armed Forces Epidemiological Board, and with the support of the US Army Medical Research and Development Command under research contract DADA17-70-C-0081. L. W. W. is a Career Investigator of the American Heart Association. P. F. was supported in part by a grant from the US Public Health Service (H.L. 06 314-14) during the period of this study.

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