A BACTERIOLOGICAL STUDY OF NORMAL THROATS, PATH-OLOGICAL THROATS, AND EXCISED TONSILS, MADE IN PUERTO RICO *

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Increasing interest concerning the etiology of influenza, the common cold, and allied conditions, has of late years evoked extensive and detailed studies in the definite establishment of the normal flora of the throat as found in different regions of the world.

We are, therefore, endeavoring to find out which are the predominant bacteria of the normal throat in the West Indian island of Puerto Rico, situated well within the tropic zone. Together with this we have included studies of pathological throats and of excised tonsils.

Two hundred normal throats were cultured and studied in two separate series of one hundred each. The method and media employed in each series differed in some respects, and the results obtained showed the expected corresponding variations. We have, therefore, reported the series separately.

The flora of the naso-pharynx of the one hundred apparently normal persons of the second series were investigated for purposes of comparison.

A morphological study of the bacteria found in several pathological throats was made.

One hundred pairs of tonsils removed in Puerto Rico were examined and reported on.

NORMAL THROATS

First Series: One hundred individuals whose throats were without clinical signs of disease, and with no history of sore

* This work is intended as an introduction to more complete studies of the same subject. Received for, publication April 6, 1935.

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throat for at least two years, were examined. Of these, about ninety were patients coming to the Clinic of the Presbyterian Hospital at San Juan. The remainder included several nurses and two members of the medical staff.

The cases ranged from 5 to 60 years as follows: From 5 to 10 years, four cases; from 10 to 20, twenty; from 20 to 30, forty; from 30 to 40, nineteen; from 40 to 60, seventeen.

Cultures were taken during the months of February, March, April and May. About 50 per cent were taken during the month of April.

Four swabs were taken from each case; one from each tonsil, one from the pharynx (avoiding the region of the tonsils), and one from tonsils and pharynx.

Swabs were placed in sterile test tubes, and labelled. Seedings were made in each case from five to ten minutes after the materials had been collected.

The media used consisted of plain blood agar (1 c.c. of blood to 10 c.c. of agar), plain agar, glucose broth, and Loeffler's blood serum.

We used human blood for the first thirty cases, but in the rest of the series we employed defibrinated rabbit blood. In the first ten cases we used plain broth, but due to poor chain formation in this medium we changed to glucose broth, which proved to be much more efficacious.

Plain and blood agar plates were divided with a wax pencil into three equal sections. These were labelled, "Left tonsil", "Right tonsil", and "Pharynx". Streaks were carefully made on the surface of the medium with swabs taken from each of the three respective places. Cultures on blood agar were made first, and then, using the same swab, streaks were made on plain agar. The material (that from tonsils and pharynx) on the fourth swab was inoculated first into Loeffler's blood serum, and then into glucose broth.

Only aërobic cultures were made. They were incubated at 37°C and examined at the end of twenty-four hours, chiefly for the production of hemolysis. In the majority of cases a Gram stain and a methylene blue stain were then made from the growth on Loeffler's serum. At the end of forty-eight hours, a Gram stain was made of all the different types of colonies on plain agar, blood agar, Loeffler's blood serum and of the growth in broth. In several cases in which

blood agar plates exhibited hemolysis, we could not ascertain which was the hemolytic organism by direct examination of the cultures. The following method was employed to determine if a hemolytic streptococcus was present: The growth on glucose broth was stained by Gram's method. If chains were present, a small drop of the culture was placed in the center of a blood agar plate and spread over the entire surface of the medium with a sterile glass rod. The same was done using an emulsion in saline of the growth on the plate. In this way we could easily get isolated colonies. We thus detected in four instances the presence of hemolytic streptococci, which otherwise might have been overlooked. One of the great objections to the streak method is that when the growth is abundant, isolated colonies are often not obtainable. Pour plates are much better. In several cases identification of organisms was attempted, based upon their sugar reactions, motility, etc.

As to the classification of the different organisms encountered, it may be remarked that all the Gram-negative cocci were considered as a group; streptococci were classified as *S. viridans* (definite greenish zone around colonies on blood agar), hemolytic and non-hemolytic; staphylococci were classified as hemolytic or non-hemolytic and according to their pigment formation. All the different strains of diphtheroids were also considered as a group. All non-hemolytic hemoglobinophilic Gram-negative bacilli were designated as *H. influenzæ*; hemoglobinophilic Gran-negative bacilli producing hemolysis were grouped as "Bacillus X", according to the description of Pritchett and Stillman¹.

The following table shows the organisms encountered in the order of predomination in our series, the number of cases in which they were found, and the number of cases in which they predominated.

Gram-negative cocci and non-hemolytic streptococci were found in practically every case. Gram-negative cocci were the predominating organisms in more than 50 per cent of the cases, but were absent in two cases in which pure cultures of *B. proteus* were obtained, and one case from which a pure culture of *B. friedländeri* was obtained. The non-hemolytic streptococcus was the second predominating organism.

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| Organisms* | Cases in which they were found | Cases in which they predominated |
|---|--|---|
| Gram-negative cocci Non-hemolytic streptococci Gram-positive cocci Staphylococcus aureus Alpha prime hemolytic streptococci Diphtheroids Gram-negative bacilli (unidentified) Streptococcus albus H. influenzae B. friedlanderi Pneumococci Staphylococcus citreus Micrococcus citreus Micrococcus tetragenus Sarcinae Bacillus "X" Gram-positive bacilli (unidentified) B. proteus. Beta hem. strep. | $97 \\ 92 \\ 58 \\ 43 \\ 25 \\ 14 \\ 13 \\ 10 \\ 10 \\ 10 \\ 7 \\ 6 \\ 6 \\ 5 \\ 4 \\ 4 \\ 4 \\ 3 \\ 2 \\ 1$ | $ \begin{array}{c} 52\\ 24\\ 6\\ 7\\ 3\\ 2\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\$ |

TABLE I

*Findings in 100 individuals (First Series).

These findings are in accordance with those of Bloomfield² and Shibbley, Hanger and Dochez³ in the United States. Noble *et al.*⁴ found the average flora to consist of green streptococci and Gram-negative cocci. He states that green streptococci only were basic for all subjects.

Staph. aureus and Gram-positive cocci are common transients. We found Gram-positive cocci in 58 per cent of cases. Some strains produced slight hemolysis on blood agar. Staph. aureus was present in 43 per cent of cases. In nineteen it was hemolytic and in twenty-four non-hemolytic. This organism predominated in seven cases. In three it was hemolytic and in four, non-hemolytic. The various hemolytic strains encountered differed in the amount of hemolysis produced, and from our findings we can designate them as slightly hemolytic, hemolytic, and markedly hemolytic. Some variation was also observed in the size of the colonies and in the intensity of colors. In the majority of cases hemolytic Staph. aureus was limited to the region of the tonsils. Bloomfield ⁵ does not consider this organism as a normal inhabitant of the upper air passages. He encountered it under two conditions:

First, as a transient in nose and throat; and secondly, associated with chronic focal infections.

Hudson ⁶ in an attempt to classify the staphylococci in the throat of normal persons and of persons with common cold, found *Staph. aureus* to be the most active in producing biochemical changes.

We would emphasize the fact that hemolytic *Staph. aureus* is very common in this country. We have isolated it repeatedly from normal and pathological throats, furuncles, the blood stream, sputum, nose, etc. Out of the sixteen plates exposed in different parts of the hospital, we obtained this organism in one, thus showing that it is also present in the air.

Alpha prime hemolytic streptococci were found in twentyfive cases, and in the majority were limited to the region of the tonsils; they predominated in three cases. Real beta hemolytic streptococci were encountered but once.

From the Journal of Laboratory and Clinical Medicine (Vol. V:135) we take the following table showing the incidence of hemolytic streptococci in apparently normal throats according to different investigators:

TABLE II

| Author | Per cent of cases harboring |
|-----------------------------|-----------------------------|
| Spooner | 61. 71 |
| Cumming, Sprunt and Lynch | 6 |
| Nichols | 75 |
| Opie, Freeman and Blake | |
| Blandon, Burhaus and Hunter | |
| Smillie | 50 |
| Ruediger | 59 |

Smillie⁷ studying the flora of the normal throat and nasal pharynx of the inhabitants of an isolated region in Alabama, did not find hemolytic streptococci. In similar investigations by the same worker carried on in Labrador, hemolytic streptococci were found to be exceedingly rare. The same investigator, in studies conducted elsewhere ⁸, found hemolytic streptococci in 50 per cent of the normal throats examined, but beta hemolytic streptococci were found only once. Bloomfield declared them non-existent in a detailed study of the throat flora of six healthy persons from whom serial cultures were made.

Bloomfield and Felty⁹ studying the hemolytic streptococcous parasitism in the upper air passages, find that

the hemolytic streptococcus is less frequently found in the throats of those whose tonsils have been removed. They conclude that:

the hemolytic streptococcus under average conditions is strictly adapted to a local growth in foci of lymphadenoid tissue in the upper air passages, especially the tonsils.

Pilot and Davis reached similar conclusions. Davis ¹⁰ finds that:

cultures taken at short intervals sooner or later reveal the presence of hemolytic streptococci in the throat of practically all normal persons.

Diphtheroids were found in 14 per cent of the throats examined by us. Although they predominated only twice, they occurred in comparatively large numbers in most of the cases.

Among the unidentified Gram-negative bacilli, there was one which showed all the characteristics of B. coli, except that it gave a negative indol test. It was the second predominating organism in the cultures.

Staph. albus was not common. It was hemolytic in one case. In several cases we suspected staphylococcous colonies on blood agar to be of the albus type, but when transplanted to plain agar slants and growth exposed to light at room temperature during twenty-four hours or more, a definite pigment formation resulted. Pneumococci were rarely encountered. Longcope and Fox 11, and Park and Williams 12, report finding typical pneumococci in a large percentage of the throats of normal individuals, especially during the winter months. Many investigators have obtained similar results. H. influenzae were reported in seven cases, and predominated in none. It must be taken into consideration that the method and media employed were not ideal for the detection of this organism, and we might have overlooked it in some instances. Bacillus "X" was present in four cases. Although it never predominated it always occurred in relatively large numbers, especially in the region of the pharynx.

Dible ¹³, in a study of the hemophilic bacteria of one hundred individuals, found bacilli morphologically identical with *H. influenzae* in forty-seven cases. Thirty of these he considered true *influenzae* bacilli. Some of these hemophilic,

hemolytic Gram-negative bacilli ("Bacillus X") were also encountered. Other investigators have arrived at more or less the same results.

Wollstein¹⁴ and Bloomfield¹⁵ did not find *H. influenzae* in the throats of healthy children. Bloomfield reports that neither was he able to find either pneumococci or hemolytic streptococci—organisms which are relatively frequent in adults. He calls attention to the fact that non-hemolytic streptococci are found constantly in large numbers from a few hours after birth.

We obtained *Strep. viridans* in ten cases. It predominated in none, but occurred in comparatively large numbers in all.

B. friedländeri was isolated in six cases and predominated in three. It was always found in large numbers, and in four out of six cases the organism was limited to the region of the tonsils.

Staph. citreus, although frequently found as a contaminating organism, was encountered in only five cases and in sparse numbers.

B. proteus was found twice, and in both cases in pure culture.

Second Series: One hundred cases with apparently normal throats were selected. Sixty were nurses and servants of the Presbyterian Hospital at San Juan; twenty were inmates of the Boys' Charity School at Santurce, and twenty included some other volunteers. Cultures were taken during the months from June 1929 to February 1930.

Three swabs were taken from each case, one from each tonsil and one from the pharynx (avoiding the region of the tonsils). Cultures were taken also from the nasal pharynx, but the results obtained are discussed separately.

In the first series, swabs were kept in sterile test tubes until seedings were made. In the present series, seedings were made immediately after collecting the material.

Plain blood agar and Loeffler's blood serum were the media used. Plain agar and glucose infusion were omitted in this series.

Blood agar plates* were prepared as follows: carefully

^{*} This medium was recommended to us by Dr. Morales Otero of the School of Tropical Medicine at San Juan.

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clarified plain agar (pH 7.6–7.8) was distributed into Erlenmeyer flasks in amounts of 250 c.c. and sterilized. The agar was melted and subsequently cooled to 45°C, when 7 c.c. of fresh, defibrinated rabbit-blood were added to each 250 c.c. of agar. Agar and blood were mixed thoroughly by gentle shaking, avoiding the formation of air bubbles. It was then distributed into sterile Petri dishes so that each plate contained approximately 25 c.c. of the medium. As soon as it hardened the plates were inverted to prevent the water of condensation from falling over the surface of the agar. This culture media was always used while fresh, and never, in this series, more than three hours after the plates had been poured. This proved better than the 10 per cent blood agar employed in the first one hundred cases.

With this medium hemolysis can be easily detected, and the greenish color of the colonies in the case of *Strep. viridans* comes out readily and prominently.

Plates were divided in the same manner as for the first one hundred cases. The way in which material was cultured varied a little: as soon as the material was obtained, the swab was rubbed on the surface of the medium over an area about the size of a nickel. Then, using an inoculating loop about 4 mms. in diameter, some of the material adhering to the surface of the agar was recovered, and tubes of Loeffler's blood serum inoculated. Using the same loop, and starting from the area previously inoculated with the swab, parallel streaks were carefully made on the surface of the medium.

This technique enabled us to make perfectly isolated colonies in both blood agar plates and slants of Loeffler's blood serum. In all other respects we proceeded as in the first series.

Table III shows the results obtained in our second series of one hundred cases. The findings from the posterior and naso-pharynx are also included so that they may be compared with those from the region of the tonsils. The organisms cultured from the right and left tonsils are recorded separately.

The following discussion will refer only to those organisms cultured from the surface of the tonsils unless otherwise noted.

As in the first series, Gram-negative cocci were the predominating organisms. They were encountered in 92 per cent of the cases. Some strains produced slight hemolysis on blood agar.

Strep. viridans ranked second. This organism was cultured in 75 per cent of the cases. Most of the streptococci reported as non-hemolytic streptococci in our first series were probably Strep. viridans. The differentiation we attribute to the composition of the culture medium (blood agar) used in both cases.

Hemolytic Staph. aureus were isolated in 18 per cent of the throats examined, non-hemolytic Staph. aureus in 9 per cent. The former was the predominating organism in one case, the latter predominated in none. Hemolytic streptococci were encountered in 17 per cent. Of these, three were of the beta type. They predominated in five cases and in two of them they were beta hemolytic streptococci.

No systematic study has been made of the virulence of the hemolytic streptococci isolated from apparently normal throats in Puerto Rico.

Diphtheroids were cultured from twelve cases and predominated in six.

H. influenzae appeared in eight cases and predominated in one.

Both diphtheroids and influenzae bacilli, as we shall see later, seem to be found in greater numbers and in a larger number of cases, in the posterior and nasal pharynx.

Pneumococci were cultured only once.

Bacilli of the Friedländer's group were obtained in four cases, but predominated in none.

Bacillus "X" and sarcina were cultured once each; Grampositive bacilli and *B. proteus*, twice.

We found *Staph. albus* three times; twice it was hemolytic and sparse in numbers. This organism is comparatively rare in normal throats in Puerto Rico.

Staph. citreus was not found in any of this series.

The assertion may be made that it is a well-established fact that *Strep. viridans* and Gram-negative cocci constitute the normal basic throat flora in Puerto Rico.

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| TABLE III* | | | | | | | | |
|--|--------------|-------------|--|---|---|---|---|---|
| Organism | Right tonsil | Left tonsil | Times encoun- tered in region of tonsils | Times predom- inating in region of ton- sils | Times encountered posterior pharynx. | Times predom- inating in posterior pharynx | Times encoun- tered naso- pharynx | Times predom- inating in naso-pharynx |
| Gram-negative cocci | 88 | 86 | 92 | 41 | 78 | 50 | 82 | 45 |
| Strep. viridans | 68 | 61 | 10 | 40 | 30 | 19 | 32 | 10 |
| Hemolytic Staph. aureus | 13 | 12 | 18 | 1.1.1 | 12 | 4 | 14 | 3 |
| Anhemolytic streptococci | 15 | 16 | 18 | 5 | 24 | : | 14 | 8 |
| Hemolytic streptococci | 16 | 14 | 17 | 0 | 9 | 1 | 14 | 3 |
| Diphtheroids | 11 | 12 | 12 | 0 | 17 | 1 | 17 | 4 |
| H. influenzae | 6 | 8 | 8 | 1 | 8 | 3 | 26 | 9 |
| Non-hemolytic Staph. aureus | 8 | 5 | 9 | 0 | 6 | 3 | 6 | 3 |
| Gram-positive cocci Gram-negative bacilli (uni- | 7 | 8 | 8 | 0 | 2 | 1 | 5 | 1 |
| dentified) | 8 | 10 | 10 | 1 | 12 | 1 | 14 | 4 |
| B. friedlanderi | 4 | 3 | 4 | 0 | 3 | 0 | 3 | 0 |
| Non-hemolytic Staph. albus. | 1 | 1 | 1 | 0 | 3 | 0 | 4 | 0 |
| Hemolytic Staph. albus | 1 | 2 | 2 | 0 | 2 | 0 | 3 | 0 |
| B. proteus | 1 | 1 | 2 | 0 | 3 | 3 | 4 | 3 |
| Pneumococci | .1 | 1 | 1 | 0 | 3 | 1 | 5 | 1 |
| Bacillus "X" | 1 | 1 | 1 | 0 | 0 | . 0 | 2 | 0 |
| Gram-positive bacilli | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| Sarcinae | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 |

* Findings in one hundred apparently normal individuals (second series)

NASO-PHARYNX

Cultures of the naso-pharynx were taken at the same time from the same individuals whose throats were cultured in the second series.

Material was obtained using curved cotton swabs mounted on wire.

One blood agar plate and one tube of Loeffler's blood serum were used for each case. We proceeded in all respects as described in the case of normal throats, second series.

The bacteriological findings have already been tabulated together with those of normal throats, second series. Table IV gives the list of bacteria encountered in the order in which they predominated in the series. As shown in this table, Gram-negative cocci prevailed.

Shibbley, Hanger and Dochez⁸ and Bloomfield⁵ found Staph. albus and diphtheroids to be the predominating organisms in the naso-pharynx of normal individuals.

From Ford's Bacteriology (1927, p. 791) we take Table V that gives a synopsis of Neumann's findings in a study of two hundred individuals, of whom one hundred and eleven were normal.

| Organism | Number of cases in which it was found | Number of cases in which it predominated | |
|-------------------------------------|--|---|--|
| Gram-negative cocci | 82 | 45 | |
| Strep. viridans | 32 | 16 | |
| H. influenzae | 26 | 9 | |
| Diphtheroids | 17 | 4 | |
| Hemolytic Staph, aureus. | 14 | 3 | |
| Non-hemolytic streptococci | 14 | 8 | |
| Alpha prime hemolytic streptococci | 10 | 1 | |
| Gram-negative bacilli | 14 | Â | |
| Non-homolytic Stank courses | 6 | 2 | |
| Crem positive coosi | 5 | 0 | |
| Gram-positive cocci | 5 | | |
| Pheumococci | 0 | 1 | |
| Non-hemolytic Staph. albus | 4 | 0 | |
| Beta hem. Strep | 4 | 2 | |
| B. proteus | 4 | 3 | |
| Bacilli of the friedlanderi's group | 3 | 0 | |
| Hemolytic Staph. albus | 3 | 0 | |
| Bacillus "X" | 2 | . 0 | |
| Sarcinae | . 1 | 0 | |
| | CITES A MARCIN | | |

TABLE IV*

*Findings in one hundred individuals (naso.pharynx).

TABLE No. V

| Organism | Per cent |
|----------------------|----------|
| Diphtheroids | 98-100 |
| Staph. albus | 98 |
| Staph. aureus | 30 |
| Staph. citreus | 12 |
| Sarcinae | 8 |
| Pigmented micrococci | 8 |
| Micrococcus roseus | 4 |
| Pneumococci | 4 |
| Friedländer bacilli | 6 |
| Lactis aerogenes | 4 |
| Ozoena bacillus | 3 |
| B. coli | 12 |
| Molds | 20 |
| Yeasts | 2 |
| B. mesentericus | 3 |
| | |

We isolated diphtheroids in 17 per cent of the cases examined. They predominated in 4 per cent. *Staph. albus* was cultured only in four cases, and always in small numbers. In this respect, there is almost absolute discrepancy between the numbers cited above, and those obtained by us.

Strep. viridans was the second predominating organism. It was cultured in 32 per cent of cases and predominated in 16 per cent.

H. influenzae was cultured twenty-six times, and predominated nine times.

Hemolytic streptococci were isolated from fourteen of the individuals examined. They were of the beta type in four. They predominated in three instances and were of the beta type in two. It seems that under certain conditions these organisms can grow freely on the mucous membranes of the throat.

We got hemolytic *Staph. aureus* in fourteen cases, and it was found to predominate in three. In one case this organism was isolated in pure culture without having created any obvious symptoms.

Out of the fourteen times in which Gram-negative bacilli were cultured, they were of the hemolytic type (already described in the case of normal throats, second series) in ten cases. Out of these ten cases, the colonies were of type No. 2 in four, and of type No. 1 in six. The organism predominated twice, and in both cases the colonies were of type No. 2.

Pneumococci were obtained from five cases.

B. proteus was encountered four times, and predominated in three.

PATHOLOGICAL THROATS

Ninety-nine pathological cases were examined. Fifty were cases of tonsillitis. Of the remaining forty-nine, twentythree were more or less typical cases of diphtheria in which the bacteriological findings corroborated the clinical findings.

Nine were cases of sore throat. In six of these the predominating organism was hemolytic streptococcus. In the other three, hemolytic *Staph. aureus* predominated.

The findings in the other seventeen cases are copied here as they appeared in our original records, because they illustrate certain important points (See Table VII).

In the case of individuals with diseased tonsils, we proceeded to collect material as in the case of normal throats. In the other forty-nine cases, cultures were taken from the part affected, only. Direct smears were made in each case, and stained with Giemsa's and by Gram's method for the detection of spirochetes and the organism of Vincent's angina.

In the following table we group the bacteria found in the throats of the fifty cases of tonsillitis. The organisms are listed in the order in which they predominated in the series.

| Organisn: | Cases in which found | Cases in which predominated | | |
|---|----------------------------|--|--|--|
| Gram-negative cocci Non-hemolytic streptococci Staph. aureus. Gram-positive cocci Hemolytic streptococci | 40 30 30 25 16 | $ \begin{array}{r} 15 \\ 4 \\ 12 \\ 5 \\ 6 \end{array} $ | | |
| Strep. viridans Staph. albus Diphtheroids Pneumococci | 13 7 5 5 | 5 0 2 0 | | |
| Gram-positive bacilli (unidentified) Sarcinae B. friedlanderi. Gram-negative bacilli (unidentified) B. proteus. | | 0 0 1 0 | | |

TABLE VI

The findings in the seventeen cases referred to above are listed as follows:

TABLE 7

| Description of Condition | Bacteriological findings. (Organisms listed in order in which they predominate.) | Remarks |
|---|--|--|
| 1—Extensive ulceration of pharynx and soft palate. Five months duration. | 1—-Slightly hemolytic Gram-pos- itive cocci in practically pure culture. | Smears: spirochetes morpho- logically like Vincent's. Few isolated fusiform bacilli seen. |
| 2-Congestion of anterior pil- lars. Chronic granular pharyngitis. | 1—Non-hemolytic strep. B. proteus. 3—Diphtheroids. | B. proteus, although not predom- inating, was present in rela- tively large numbers. |
| 3—Congestion of anterior pil- lars. Sore throat. | 1-Gram-positive cocci. 2-Gram-negative cocci. 3-Non-hem. strep. 4-Non-hem. Staph. aureus. | Direct smears: negative. |
| 4—Mucous patches of both tonsils and pillars, Bilateral cervical adenitis. 2 weeks' duration. Swabs sent to laboratory for culture. | 1—Non-hem. strep. 1—Non-hem. Staph. aureus. 3—Gram-positive cocci. 4—Gram-negative cocci. | Dark field examination and di- rect smears (Giemsa's) showed spirochetes very suspicious for <i>T. pallidum</i> . Kahn test-4.3.2. |
| 5—Pseudo-membranes grayish, bleeding easily, extending from both tonsillar pillars to posterior nares. Child very toxic. Twelve days duration. | Beta hemolytic streptococci and <i>B. diphtheriae</i> in about equal numbers. | Report from another laboratory read: "B. <i>diphtheriae</i> in pure culture". Diphtheria antitoxin given. No improvement. Child died two days later. |
| 6—Mucous patches in poste- rior pharynx. Ulceration of surface of left tonsil. Bilateral cervical adenitis. | 1—Gram negative cocci. 2—Slightly hem. Staph. aureus. | Direct smears and dark field negative for spirochetes. No fusiform bacilli found. Kahn test 4.3.2. |

| Description of Condition | Bacteriological findings. (Organisms listed in order in which they predominated) | Remarks |
|--|---|---|
| 7—Extensive membranous pat- ches scattered all over the mouth and pharynx with areas of inflammation. Lesions have a spongy ap- pearance. | 1—B. proteus. 2—Gram-positive cocci. | Direct smears negative. |
| 8—Diseased hypertrophic ton- sils. Mild sore throat. | 1—Diphtheria–like bacilli. 2—Non-hemolytic streptococci. | Virulence test negative. |
| 9—Tonsils enlarged and cov- ered with grayish mem- branes which did not ex- tend to the pillars or to the soft or hard palate. Some difficulty in swallow- ing. Two days' duration. | 1—Beta hemolytic streptococci. 2—B. friedlanderi. | Swabs were sent to laboratory for <i>B. diphtheriae</i> . |
| 10—General inflammation of the mucous membranes of entire mouth. White patches all over. Gums bleeding easily. | 1—Strep. viridans. 2—Hemolytic Staph. aureus. No fungi in Sabouraud's maltose agar after ten days' incuba- tion. | Sent to laboratory for culture Direct smears showed organism of Vincent's angina in large numbers. |
| 11—Dry necrotic ulcer of right tonsil. | 1—Gram-negative cocci. 2—Hemolytic streptococci. | Direct smears show organism of Vincent's angina. |
| 12—Dry necrotic ulcer on cheek. | Cultures not made. | Direct smears showed organism of Vincent's angina. |
| 13—Acute attack of follicular tonsillitis, Four days' du- ration. Tonsils greatly enlarged. Deposits in crypt. Great difficulty in swallowing. | 1—Beta hemolytic streptococci in practically pure culture. | Direct smears negative. |
| 14—Acute attack of follicular tonsillitis. Tonsils very large. Deposits in crypts | Decidedly hemolytic Staph. au- reus in pure culture. | Direct smears. Negative. |
| 15—White patches all over tongue and soft palate. Tonsils not affected. | 1—Gram-neg. cocci. 2—Strep. viridans. Sabouraud's agar showed no fungi after ten days' incubation. | Direct smears showed organism of Vincent's angina in large numbers. |
| 16—Whitish patches all over tongue. Congestion of pillars and pharynx. | 1—Gram-neg. cocci. Sabouraud's agar: Monilia psilosis (Ash- ford) in large numbers and in pure culture. | Direct smears revealed spiro- chetes occasionally. Fusiform bacilli absent. |
| 17—Extensive ulceration of the mucous membranes of the upper and lower lips and cheeks. Tonsils and gums unaffected. The ulcers are covered with a dirty gray- ish exudate which is abundant; mucous mem- branes bleed easily. | 1—Gram-neg. cocci. 2—Strep. viridans. 3—Large Gram-positive cocci. Sabouraud's: no fungi. | Direct smears. Organism of Vincent's angina in very large numbers. |

TABLE 7-Continued

Gram-negative cocci and non-hemolytic streptococci were the organisms most commonly encountered, as in the case of normal throats.

Staph. aureus was the third predominating organism. Out of the thirty cases in which it was cultured, in fourteen it was hemolytic. It was found to predominate in twelve, and out of these twelve it was hemolytic in nine.

In normal throats *Staph. aureus* was found to predominate in 7 per cent, while in the case of pathological throats it predominated in 24 per cent of cases, in the vast majority of which the organism was hemolytic.

We do not attach any etiological significance in the case of diseased tonsils to the non-hemolytic *Staph. aureus*, or even to the slightly hemolytic strains; but we believe that when by careful swabbing of the surface of the tonsils a pure, practically pure, or even a large number, of decidedly hemolytic *Staph. aureus* is obtained, the organism, as in the case of beta type of hemolytic streptococci, has a diagnostic significance.

In the case of normal throats we got alpha prime hemolytic streptococci in 26 per cent of the cases, and they predominated in 3 per cent. Beta type hemolytic streptococci were encountered only once. Out of the fifty cases of diseased tonsils cultured, we obtained hemolytic streptococci in 32 per cent; they predominated in 12 per cent, and the beta type was isolated in 14 per cent.

Pilot and Davies¹⁷ recovered hemolytic streptococci by swab cultures in sparse numbers from the pharynx and tonsillar surfaces in 61 per cent of throats. The individuals examined were children with hypertrophic tonsils.

From the crypts of the excised tonsils of the same cases, they got the organism in 97 per cent, usually in predominating numbers. They conclude that swab cultures are unreliable in determining the incidence of this organism in the respiratory tract.

Valentine and Mishulow 18, studying:

the cultural and serological relationship of hemolytic streptococci isolated from inflammatory conditions of the respiratory tract

conclude that the different strains fall into many cultural groups and subgroups, and they found so few similarities among them that:

the probability of a dominant strain seemed remote.

They did not consider any of the strains isolated by them to be of primary etiological importance.

Otteraaen¹⁹, after similar studies, concludes that hemolytic streptococci are frequent inhabitants of the throat of normal subjects and of persons suffering from acute infectious diseases. His findings that these streptococci are not virulent, are based on results obtained by animal inoculations and phagocytoses experiments.

Fox and Stone²⁰ come to the conclusion that:

the streptococcal contents of the human throat maintain a constant standard as regards both quantity and type; the only important change associated with local pathological conditions is an invasion by hemolytic streptococci.

The tendency of hemolytic streptococci to be confined to local lymphadenoid foci has already been pointed out, but investigations by different workers tend to show that:

when streptococcal disease is highly epidemic, a different type of parasitism may exist.

Bloomfield states that under those conditions, in the army camps, practically every man, sick and well, was found to be a streptococcus carrier. In such cases the streptococci were not confined to local lymphadenoid foci and they grew freely, generally on the mucous membranes of the throat.

Bloomfield and Felty²¹ consider that:

a culture showing no beta hemolytic streptococci, if properly made, would be strong evidence against acute follicular tonsillitis and in favor of some other infections such as diphteria or Vincent's angina.

Here again, the decidedly hemolytic *Staph. aureus* must be taken seriously into consideration.

They also agree that:

beta hemolytic streptococci may persist in the tonsil for an indefinite time after acute tonsillitis, but die out rapidly after tonsillectomy, on the free mucous membranes of the throat.

These investigators examined a group of individuals for beta hemolytic streptococci. They then observed this experimental group for the purpose of finding out whether the carriers or non-carriers developed tonsillitis. When cases of tonsillitis began to appear, it was observed that the infection occurred in those that had been previously found to be noncarriers of beta hemolytic streptococci. The carriers, with

one exception only, did not develop tonsillitis. The investigators conclude that:

tonsillitis is distinctly not an autogenous infection, but results from invasion of an outside strain.

The work of these and many other investigators shows that beta hemolytic streptococcus is etiologically related to septic sore throat and acute follicular tonsillitis. Undoubtedly, as Young and Crooks point out, when hemolytic streptococci are obtained in pure or practically pure, culture, they have a diagnostic significance.

The number of cases showing other pathological conditions is so small that they present no conclusive evidence. Nevertheless, we want to point out the great practical value of the direct smear and the blood agar plate in such cases.

Young and Crooks²² found the organism of Vincent's angina in many cases clinically confused with diphtheria.

Morales Otero²³ in a study of one hundred cases clinically diagnosed as diphtheria, encountered diphtheria bacilli only in fifty-two. Hemolytic streptococci were obtained in nineteen cases; hemolytic staphylococci in six, and the organism of Vincent's angina in two.

Undoubtedly, Vincent's angina is a common infection in Puerto Rico.

TONSILS

During the twelve months from January, 1929, to February, 1930, cultures were made from crypts and from the interior of one hundred pairs of tonsils removed at operation from patients with a history of repeated attacks of tonsillitis. In some instances cultures were taken before operation to find out if simple swabbing of the tonsils were a reliable index of the predominating organism in the crypts. Our main purpose in conducting these preliminary investigations, was to ascertain which are the predominating organisms in the crypts and the interior of diseased tonsils in Puerto Rico.

The tonsils were received in the laboratory in sterile specimen bottles. The pits of the tonsillar crypts were carefully seared with a chromel wire inoculating loop. The loop was introduced into the crypts and streak cultures were made on blood and agar plates with the material obtained. Loeffler's blood serum and glucose broth were also inoculated.

Then, the outer surface of the tonsil was seared, and an incision made with a sharp, sterile knife, the inoculating loop was introduced through the incision and the material obtained cultured, as in the case of that from the crypts. Many of the tonsils received were badly macerated. We cultured only those that were intact. Table No. VIII shows the findings in the one hundred tonsils examined.

| elle di la contrata di sentente elle di sentente elle di la contrata di la contrata di sentente di sententente di sentente di sentente di sentente di sentente di | Numl tin encour | ber of nes ntered | Number of times it predominated | | | Number of times obtained in pure culture | | | |
|---|-----------------------|-------------------------|------------------------------------|----------------|---------------------------------|---|----------------|---------------------------------|--|
| Organism | Crypts | Inside | Crypts only | Inside only | Both crypts and inside | Crypts only | Inside only | Both crypts and inside | |
| Hemolytic Staph. aureus | 42 | 42 | 8 | 7 | . 19 | 7 | 6 | 17 | |
| Beta hemolytic streptococcus | 24 | 33 | 3 | 7 | 11 . | | 11 | 10 | |
| Gram-negative cocci | 12 | 12 | | 4 | 3 | | 1 | | |
| Alpha prime hemolytic | (TRUE) | 10301 | 0.903 | N.O.L | D I I R R | 191181 | 80.5 | III, POL | |
| streptococcus | 8 | 8 | 1 | 2 | 1 | 1 | 3 | 1 | |
| Non-hemolytic Staph. aureus | 8 | 6 | 1 | 3 | | 2 | 1 | - Ic | |
| Gram-positive cocci | . 8 | 6 | | | 1 | 1 | | | |
| Strep. viridans | 7 | 9 | 1 | 1 | 2 | | 3 | 1 | |
| H. influenzae | - 7 | 10 | 2 | 1 | 3 | 1 | 1 | 1 | |
| Diphtheroids | 3 | 3 | 1 | | 1 | 1 | 1 | 1 | |
| Hemolytic Staph. albus | 2 | 2 | | - 1 | | 1 | | | |
| Gram-negative bacilli (uni- | Section 1 | | | and the | | | 1. 201 | | |
| dentified) | 3 | 4 | 1 | | | | | | |
| Pneumococci | . 2 | 2 . | | | | 1 | | | |
| B. friedlanderi | . 1 | 1 | | | | | | | |
| Bacillus "X" | 2 | 2 | | | | | | 1 | |
| | 247 | RAR T | 100 | 11.11 | Sant | | 111 | Pida | |

TABLE VIII

Staph. aureus was the predominating organism; hemolytic streptococci ranked second.

Among the streptococci producing decided hemolysis, two different types of colonies were observed: 1. Punctiform, white, slightly transparent. 2. Opaque, sized and shaped like the head of a pin, dirty and greasy in appearance. The latter was much more common than the former.

Polvogt and Crowe²⁴ found hemolytic streptococci to be the predominating organism in a series of one hundred excised tonsils examined. Staphylococci were encountered in 8 per cent.

Pilot and Pearlman²⁵ isolated hemolytic streptococci in 95 per cent of the tonsils cultured.

Kilduffe and Hersohn²⁶ found staphylococci in 33 per cent

of four hundred pairs of tonsils examined.

None of these authors mention hemolytic staphylococci.

Streptococcus viridans was found to predominate once in the crypts, once in the interior of the tonsil and twice in both crypts and inside. It was obtained in pure culture three times from inside and once from both crypts and inside.

Pneumococci were cultured twice from the crypts, twice from inside, and they predominated once in both crypts and inside.

Diphtheroids were encountered three times in the crypts and three times in the interior. They were obtaind in pure culture from the three crypts in one case, and in another case they were found in pure culture in both crypts and inside.

H. influenzae was found seven times in the crypts, ten times in the interior, and was obtained in pure culture once from inside, once from the crypts and once from both crypts and inside.

In a detailed study of the bacteriology of tonsils and adenoids, Pilot *et al*²⁷⁻³⁰ found *Strep. viridans*, pneumococci, *H. influenzae*, and diphtheroids in relatively larger numbers. They conclude that the tonsils and adenoids are foci in which such organisms usually flourish.

They found diphtheroids to be:

decidedly more numerous in the pharyngeal vegetations than in the tonsillar crypts.

This is in accordance with our findings.

It must be noted that the hemolytic *Staph. aureus* was much more commonly encountered in those tonsils cultured during the months of June, July, August and September, than in those cultured during the four preceding months.

In sixteen cases cultures were taken from the surface of the tonsils before operation. In six of these the predominating organism on the surface was also the predominating organism in the crypts. Out of the remaining ten cases, in six the predominating organism in the crypts was the second predominating organism on the surface. In the remaining four, the predominating organism in the crypts was found only in sparse numbers on the surface cultures.

We believe that merely swabbing the tonsils, even if done with care, is not a very reliable index of the predominating organism in the crypts.

SUMMARY AND CONCLUSIONS

The bacteriological findings in two hundred normal throats, one hundred pathological throats and one hundred pairs of excised tonsils are reported. The results obtained from cultures taken from the naso-pharynx of one hundred apparently normal individuals are also included.

Gram-negative cocci and Strep. viridans constitute the normal basic flora in Puerto Rico.

Staph. albus and citreus, are rare in both throats and naso-pharynx of normal persons in Puerto Rico.

Staph. aureus is a relatively common transient in the case of normal throats.

Pneumococci were only occasionally encountered in the case of normal throats; in six cases in the first series, and in one case in the second series. This organism was obtained five times from the naso-pharynx.

Alpha prime hemolytic streptococci were isolated from 25 per cent of the normal throats in the first series, and from 14 per cent in the second. In the former, beta type hem. strep. were cultured in one case, and in the latter, in three cases. Alpha prime hem. strep. were cultured ten times, and the beta type four times from the naso-pharynx.

Hemolytic Staph.aureus was encountered fourteen times in the nasal pharynx. Although it is true the hemolytic streptococci and hemolytic Staph. aureus are in many cases limited to the region of the tonsils, it is no less true that under certain conditions they grow freely on the mucous membranes of the throat.

Hemolytic Staph. aureus was isolated from pathological throats (cases of tonsillitis) in fourteen out of fifty cases examined. Hemolytic streptococci were cultured in 32 per cent. In 14 per cent they were of the beta type.

Hemolytic Staph. aureus and hemolytic streptococci were the predominating organisms in the crypts and the interior of 100 pairs of tonsils examined.

Hemolytic Staph. aureus is a very common and important organism in this Island, and we think that when it is isolated in pure, or practically pure, culture from the surface of diseased tonsils, it has the same diagnostic significance as in the case of the beta hemolytic streptococcus.

Simple swabbing of the surface of the throat, even if carefully done, is not a very reliable index of the organism predominating in the crypts.

Vincent's angina is a common infection in Puerto Rico. The direct smear must never be omitted when swabs from pathological throats are sent to the laboratory for culture. The blood agar plate, Loeffler's blood serum and the direct smear must be employed routinely whenever possible in the bacteriological diagnosis of throat infections. Cultures on Sabouraud's maltose agar are also desirable.

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