Notes on the Biology of Pneumococci Isolated in Puerto Rico*

By C. BENÍTEZ *and* P. MORALES OTERO

From the Health Division of the Puerto Rico Reconstruction Administration and the School of Tropical Medicine, San Juan, P.R.

THERE ARE FEW problems in the field of Public Health that have been the subject of more study than the pneumonia problem, and few movements in public health have enjoyed a more rapid growth than has that of pneumonia control. In Puerto Rico, however, very little attention has been given to the incidence of pneumococcic infections. With the possible exceptions of the mention of the presence of pneumococcus in the flora of normal throats in San Juan, Puerto Rico, by Morales-Otero in 1932,[1] and by Pomales Lebrón in 1936,[2] and the work on the "Study of Lobar Pneumonia in Puerto Rico" by Suárez, in 1930,[3] very few reports of the incidence of this organism on the Island have appeared in the literature.

A close survey of the demographic statistics of our Department of Health for the last years reveals the fact that the mortality from pneumonia** in Puerto Rico ranks high in order of magnitude among the most important causes of death for those years. In the year 1933 it was the fifth principal cause of death, and in the year 1934, it jumped from fifth to fourth place, being responsible for 7.2% of the total deaths. In 1935 it moved to third place, being responsible for 8.5% of all deaths and in 1936 there was a slight decrease in the rate, causing only 7.9% of deaths in that year. In the last two years (1937 and 1938) it has been reported as the third most important cause of death on the Island.

In this study we were primarily interested in those cases in which death was caused by a true pneumococcic infection.

The records of the Department of Pathology of the School of Tropical Medicine reveal perhaps a true picture of the rate of deaths in Puerto Rico due to pneumococcic infections. According to these records, pneumococci have been responsible for 4.6% of deaths in 1000 autopsies performed in the Department of Pathology. We are including here only those cases in which the pneumococcus has been reported as the causative organism, or in which the infection has been described

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** The term "pneumonia" in these records includes both "lobar" and "broncho-pneumonias," as well as cases in which the type of pneumonia was not specified.

as a typical pneumococcic infection. This comprises most of the cases of lobar pneumonia, a few cases of primary bronchopneumonia, and a limited number of cases of leptomeningitis. There is a large number of cases of bronchopneumonia in which a bacteriological report is missing, so our estimate is very conservative.

For the past two years we have endeavored, at the School of Tropical Medicine, to study the incidence of pneumococci among Puerto Ricans. Recent bacteriological studies of the flora of the respiratory tract in healthy Puerto Ricans reveal that a rather high percentage of the population harbors the organism in its throats, and studies of pathologic conditions have come to verify the belief that pneumococci are rather widespread in Puerto Rico.

We have divided our present work in two parts:

Part I.—Isolation and study of strains from normal throats.

Part II.—Isolation and study of strains from pathologic conditions.

PART I

The method used in the isolation and study of strains from normal throats was briefly as follows:

Swabs were taken from the normal throats and were immediately plated in streak and pour plates of rabbit's blood agar. Both deep and surface colonies were carefully studied after 24 hours' incubation, and those suspected of being pneumococcus were transplanted to beef heart phosphate broth.

The criteria adapted for the identification of an organism as a pneumococcus were typical morphology and bile solubility. All the strains fermented inulin with coagulation of beef serum, but a strain would not have been discarded had the ability to ferment this carbohydrate been lacking.

One hundred and twenty-nine (129) strains of pneumococci were isolated during the months of September to April (1937-38) from 366 samples taken from apparently normal throats; that is, 35.24% of the total number of throats studied harbored pneumococci.

Only 83 of the strains were pathogenic when injected intraperitoneally into white mice. The remaining 46 were not pathogenic for mice at the time of their isolation, and all efforts to enhance their virulence have so far failed. The standard test for pathogenicity was an intraperitoneal inoculation into white mice of 0.1 cc. to 0.5 cc. of a 1:10 dilution of an 18-hour broth culture. Milam and Smillie,[4] who performed similar studies and used more or less the same standards of

virulence, found that most of the strains isolated by them had little or no virulence for mice.

The morphology of the different strains isolated show very little. A description of their morphology is a repetition of the classical description of the pneumococcus; that is, that it occurs as an oval or oat-shaped small coccus, usually in pairs, but frequently also in the form of short chains. The coccus has a characteristically pointed or elongated end. It can easily be stained with the common aniline dyes, and retains the initial stain in Gram's method. Loss of the characteristic staining properties is associated with the beginning of autolysis.

Perhaps the only significant fact revealed by the morphological study of strains isolated in the laboratory was that the more virulent strains have a greater tendency to appear in pairs, or as diplococci, while the more avirulent ones frequently appear in the form of more or less short chains. Most of the strains isolated showed the presence of a capsule at least at the time of their isolation, the great majority of them losing their capsule after a few weeks of artificial cultivation. As to characteristics of the capsule, it was hard to notice any marked difference with the available microscopes, beyond the fact that in those organisms which later turned out to belong to Type III, the capsule was slightly more prominent than in other strains.

The surface colonies in the blood agar plates appear as slightly raised convex discs of variable size, with a smooth glistening surface. There is usually a green discoloration of the red cells under the colony and immediately adjacent to it, which is due to metahemoglobin production.

The deep colonies are very hard to differentiate from the colonies of Streptococcus viridans, or "Alpha type." The colonies are surrounded by a zone (1-2 mm.) of greenish corpuscles which show little lysis or decolorization. A surrounding and narrower translucent zone of destroyed corpuscles is also present, giving the impression of a distinct halo.

With subsequent cultivation the colonies lose their smooth, glistening surface, which turns granular or uneven, the colonies becoming larger, exhibiting a fringed periphery and the individual components displaying no capsulation. They are now called the "rough strains," in contrast with the "smooth strains" which were described before.

The difference in characteristics between the "smooth" and "rough" strains is more easily brought out if dog's blood, instead of rabbit's blood, is used in the culture media.

Chung and Sia,[5] working at Peiping, have described a very simple and satisfactory medium for the differentiation of "smooth" and "rough" pneumococci. Their medium consists of ordinary beef infusion agar (pH 7.8) to which 5 to 10% of defibrinated blood of the dog is added. When the "smooth" and "rough" forms of pneumococci are grown in such a medium, the "R" colonies show a markedly rough surface and can easily be distinguished from the "S" colonies.

The "R" colonies have a comparatively dry surface, firm consistency, and are quite adherent to the medium in dog's agar. The "S" colonies are smooth, shiny, moist and soft, and can easily be scraped out.

According to Chung and Sia,[5] the differential quality of the medium depends upon the presence of free hemoglobin. The free hemoglobin in the medium is readily made available because the dog erythrocytes are highly suceptible to hemolysis, both by physical factors and the hemolytic action of pneumococci.

The mechanism whereby the "R" strains assume a rough appearance when grown in hemoglobin agar has never been well explained.

The importance of differentiating between the "S" and the "R" forms is more readily appreciated if one bears in mind that the "S" pneumococci are virulent, possess capsules, are type-specific and elaborate the specific soluble substance, while the "R" forms are avirulent, devoid of capsule, not type-specific and do not elaborate the specific soluble substance.

We have given most of our attention to the typing of the different strains isolated. Unfortunately, at the time that these organisms from normal throats were isolated, the only available anti-pneumococci sera in this laboratory were those belonging to the specific Types I, II, and III, and typing could be made with these three sera only.

Of the 129 strains isolated, only 4 gave positive Quellung reaction, and agglutinated with Type III antisera, one agglutinated with Type I, and the remaining 124 strains fell into Group IV.

The following table shows the incidence of the different types in apparently normal throats, according to different investigators. (See table I)

The results obtained by the first four investigators differ somewhat from those obtained by Milam and Smillie, and by us. It is not surprising that it should be so, because the first four investigators did their work under very different climatic and environmental conditions, while Milam and Smillie did their investigation in St. John's, one of the Virgin Islands, where climatic and other conditions are very similar to ours.

TABLE I

Type Incidence of Pneumococcus in Normal Persons for Different Localities

Locality	*Author*	*No. of persons*	*No. of exams.*	*Pneumococcus present in %*	*Type I*	*Type II*	*Type III*	*Group IV*
New York	Stillman, E.	297	297	39.0	0.8	0	28.1	74.1
Boston	Rosenau, Felton and Atwater	180	180	47.3	2.2	4.4	9.9	83.5
Boston	Powell, Atwater and Felton	93	418	63.6	4.3	6.7	0.7	78.3
Germany	Gundel and Linder	99	1,113	55.9	3.9	4.5	16.4	75.9
Virgin Is.	Milam and Smillie	385	813	31.16	0	0	3.08	96.92
Puerto Rico	Benítez and Morales	366	366	35.24	0.9	0	3.66	95.33

There are probably a number of factors which determine the distribution of pneumococci in healthy individuals. Longcope and Fox[6] have suggested a seasonal effect in the distribution of pneumococci. These investigators observed a greater incidence of the organisms in normal beings during the winter months than during the milder seasons. Brown and Anderson[7] noted a correlation between the incidence of pneumococci in the throats of normal persons and periods of inclement weather. Other investigators have concluded that practically every person acts as a host, at some time or other, and probably at repeated intervals for pneumococci.

In our studies we were able to observe that the incidence was higher during the months of February, March and April.

PART II

The material studied in this section of the work consisted of:

1. Samples obtained by swabbing the throats of children under 12 years of age having a condition diagnosed either as lobar pneumonia or bronchopneumonia.

2. Specimens of sputum from adult patients suffering from lobar pneumonia, one case of bronchopneumonia and one of bronchitis.

3. Material from other pathologic conditions as otitis media, pleurisy and sinusitis.

In these cases we used, for typing, two different lots of sera, one from the Department of Health of New York City, the other from the Lederle Laboratories. The method used by us was the Neufeld "Quellung" method. This is based on the original observations of Neufeld, that swelling of the capsule of the pneumococcus occurs when the organism is brought into contact with its homologous antiserum produced in rabbits. When the reaction is positive, swelling and a definite outline of the capsule are visible under the oil immersion. The peculiar ground-glass appearance of the capsule and the sharpness of its outline are more helpful in the determination of the positive reaction than is the actual size of the capsule. In pneumococci showing a negative reaction, the capsule is scarcely distinguishable and very translucent. It appears as a narrow halo without definite outline.

A positive reaction usually occurs within a few minutes, though sometimes it may require 20 to 30 minutes or even longer to appear. Failure in immediate typing may be due to the use of too much sputum and too little typing sera, or to the scantiness of the organism. There is no doubt that a certain amount of experience and often a considerable

degree of patience are required for maximum success, particularly if there are few organisms present in the sample submitted.

Previously, only monovalent serum was used for typing, but it was later found that for preliminary typing, mixtures or combinations of different antisera could be used, and the results were satisfactory, thus decreasing the labor involved in the use of so many monovalent sera in separate tests.

We have found that the following technique offers a simple and efficient means of obtaining satisfactory results.

Using the six mixtures—A, B, C, D, E, F—offered by the Lederle Laboratories, Inc., we proceed as follows:

A tiny speck of sputum is mixed in each of six cover glasses with the amount of serum supplied in a capillary tube, and a standard loopful of Loeffler's methylene blue. The cover glasses are inverted on flat slides, and a little pressure is exerted so as to secure a thin layer of sputum between the cover glass and the slide. Each mixture is allowed to stand for five minutes at least, and then examined under the oil immersion lens with a powerful microscope light.

If there is a reaction on one of the combinations, tests are carried out with the monovalent antisera included in the combination with which the reaction was obtained.

In samples where the number of organisms is too few, we use Taplin et al's[8] concentration method as described by them.

The results obtained by us with this procedure have been consistently satisfactory, and we have thus been able to identify the type of pneumococcus, or, if more than one type is present, the type of pneumococci in a large percentage of the samples submitted to us.

From young children, we nearly always fail to obtain sputum in the usual way, so we obtain our material by gently irritating with an applicator the pharyngeal wall, thus trying to induce coughing, and collecting the forthcoming secretions in a swab held as close to the larynx as possible. The swabs are then brought to the laboratory and plated. Only in a very few instances were we able to type the pneumococcus directly from the material on the swab. In most cases we had to type them from the culture, or animal exudate, after inoculation with the broth culture.

Even though we tried different methods for typing, pneumococci of undeterminable types were encountered now and then.

Table II shows the distribution of different types among Puerto Rican children suffering from various pneumonic infections.

From this table we see that the six types of pneumococci occurring

in a series of 53 cases of pneumococcic infection in children, in their order of frequency, are: Types XIV, I, XVIII, VI, III and XVII.

The number of cases examined is too small to allow accurate statistical deduction, but the trend is significant and points to the fact that the frequency of organisms found in pneumonic infection in children in Puerto Rico, is not very different from those reported in other geographical areas.

In the study of cases from adult patients, sputum samples are either

TABLE II

Distribution of Prevalent Types of Pneumococci in the Cases Studied Among Puerto Rican Children

Type	*Patients with lobar pneumonia*	*Patients with broncho-pneumonia*	*Other infectious processes*
I	5	3	1
III	3	1	1
VI	2	3	1
XIV	6	8	2
XVII	—	1	—
XVIII	3	3	1
Undeterminable	2	7	—
TOTAL	21	26	6

submitted or collected. In every case the sputum used for immediate typing is injected into a mouse and 4 or 6 hours afterwards serological typing is carried out from the peritoneal exudate. No discrepancy has been found in typing performed either by the immediate method or by mouse inoculation.

There were a few failures of immediate typing, even though the concentration method was applied to these samples. We attribute the failure to identify by direct method the organisms in these cases to the scantiness of pneumococci.

Table III shows the types and frequency found in grown-up cases.

The data presented shows that 30% of the studied pneumonias in adults were due to organisms belonging to Type I, 22% to organisms belonging to Type III and 48% were listed under Group IV.

We recognize the fact that the number of cases studied is too limited to arrive at any definite conclusions and work should be continued for a longer period of time.

It has been suggested by Bullowa and Wilcox[9] that the specific types

of endemic pneumonia may have individual cycles, and they were able to notice a year by year variation in the percentage distribution of the various types of pneumococci. This might explain the complete absence of Type II organisms from our series, whereas a rather high percentage of Type II organisms occurring in cases of lobar pneumonia in Puerto Rico has been previously reported by Suárez[3].

TABLE III

Distribution of Prevalent Types of Pneumococci in the Cases Studies Among Puerto Rican Adults

Type	*Patients with lobar pneumonia*	*Patients with broncho-pneumonia*	*Other infectious processes*
I	12	—	—
III	6	—	3
IV	3	—	—
VI	1	1	—
VII	2	—	—
IX	—	1	—
XII	—	1	1
XIV	1	—	—
XVIII	2	—	—
XIX	—	—	2
XXI	—	—	1
XXVIII	—	—	1
XXIX	—	—	1
Undeterminable	—	—	2
TOTAL	27	3	11

All the strains isolated from the pathological cases, with very rare exceptions, were virulent when injected intraperitoneally into white mice. The avirulent strains belong to Type VI and XXIX. We have not noticed any remarkable morphological or biological difference between these strains isolated from pathological material and those recovered from apparently healthy individuals.

DISCUSSION

The presence of pneumococci either as normal habitant or infecting organisms among Puerto Ricans has only been admitted in the last few years. Suárez,[3] in 1929, made the following statement: "The prevalent idea among the medical profession of the Island is that lobar pneumonia is very rare with us. Some go so far as to deny its occurrence in Puerto Rico. Others, while admitting it, ascribe to it an atypical or

benign course." We feel sure that in the short time that has elapsed since this statement was made, the medical profession has become aware of the importance that the pneumonia problem has acquired in the Island.

The number of cases reported lately compared with those reported in former years shows a steady increase in the incidence of pneumonia. Also, the number of normal throats harboring pneumococci has shown a great increase. The higher incidence could not be accounted for wholly by the fact that the disease processes are better known now than they were in former years, and that the laboratory facilities have greatly improved in the last years, but we feel safe in asserting that the introduction of carriers, which has been going on continuously due to the increased trade relation with the mainland, may influence the increase of the pneumococcus incidence here in the Island, as one of the conditions believed to be necessary to acquire pneumonia is the coming in contact with someone carrying virulent pneumococci (either as a case, or as a carrier of the germ without suffering from the disease).

CONCLUSIONS

1. Pneumococci are found in a high percentage of the normal throats of apparently healthy Puerto Ricans, especially during the months of February, March and April.

2. The majority of the organisms isolated from normal cases belong to the heterogenous Group IV, and they vary in virulence, most of them having little or no virulence.

3. Studies of pathologic conditions have revealed the fact that pneumococci are rather widespread on the Island.

4. The frequency with which the different types of pneumococci are found in Puerto Rican children suffering from pneumococcic infections is very similar to that reported in other geographical areas.

5. Thirty per cent of the pneumonias in Puerto Rican adults in the series studied were due to Type I pneumococcus, 22% to Type III and 48% to Group IV.

6. Judging by the previous reports, there has been a steady increase in the incidence of pneumococci in Puerto Rico during the last years, both in normal and pathologic conditions.

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