The incidence of Diplococcus pneumonia Weichselbaum in a group of individuals at Montana State College
by Elizabeth M Carter

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Bacteriology at Montana State College
Montana State University

Abstract:
During the school years of 1958 to 1940,, a Survey to determine the carrier incidence of Diplocoacus pneumoniae in the throats of a group of individuals at Montana State College was carried out. Various methods of obtaining cultures were tried.

Using the results of this work and the suggestions received from other investigators as a guide, a more comprehensive survey was carried out from October 1940 through April 1941 on a group of 54 individuals.

The group was composed of some members of the college faculty, some members Of the clerical staff, but mostly it was made up of college students. Two methods, the gargle and the swab methods with modifications developed by the author, of obtaining cultures of Dlplocpccus pneumoniae were used in order that the relative merits of each might be determined.

Of the 54 individuals tested, 75.9 per cent (41) were carriers of Diplococcus pneumoniae at one time or another during the survey.

Experimental evidence is presented which indicates that neither the gargle nor the swab method can be depended on to secure cultures from 100 per cent of the carriers, and that both methods should be used simultaneously when determining the carrier incidence. Evidence is also presented which shows that beef heart infusion broth enriched with beef serum gives a much higher per cent of positive cultures than does the unenriched broth.

The individuals tested in this survey were grouped using the classification of the author.

Nineteen specific types of DiplococCus pneumoniae were found during this survey. Type 5 was the most common; types 1 and 2 were never found.

"...It is easy to make mistakes in this field of investigation; easier, perhaps, than to acknowledge them. And believing, as I do, in human fallibility, I have no hesitation in questioning the conclusions of the most illustrious workers in the field of microbiology, if they are in conflict with my own observations. On the other hand, if, upon fuller investigation, I am convinced that I have been mistaken in regard to this or any other question, I shall feel no hesitation in following the example of Pasteur in making a public announcement of my error."

--Sternberg (1885)

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ABSTRACT

During the school years of 1938 to 1940, a survey to determine the carrier incidence of Diplococcus pneumoniae in the throats of a group of individuals at Montana State College was carried out. Various methods of obtaining cultures were tried.

Using the results of this work and the suggestions received from other investigators as a guide, a more comprehensive survey was carried out from October 1940 through April 1941 on a group of 54 individuals. The group was composed of some members of the college faculty, some members of the clerical staff, but mostly it was made up of college students. Two methods, the gargle and the swab methods with modifications developed by the author, of obtaining cultures of Diplococcus pneumoniae were used in order that the relative merits of each might be determined.

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The individuals tested in this survey were grouped using the classification of the author.

Nineteen specific types of Diplococcus pneumoniae were found during this survey. Type 3 was the most common; types 1 and 2 were never found.

INTRODUCTION

This paper presents the results of a survey by the author of a group of individuals selected on the Montana State College campus to determine the incidence of the various types of Diplococcus pneumoniae Weickselbaum in the throats of this group. The results should throw some light on the incidence of Diplococcus pneumoniae among the individuals at that institution.

It is also the object of this paper to compare two different methods of obtaining cultures from the throats of individuals and to show the relative merits of the two methods.

HISTORICAL

Incidence of the carrier rate.--Although Klebs (1875) is given credit for first seeing pneumococci, it was Sternberg (1881) who first inoculated rabbits with normal saliva and isolated the characteristic lancet-shaped diplococci which we now recognize as Diplococcus pneumoniae. Sternberg, however, did not appreciate the significance of these organisms, and it remained for Friedlander (1884) to designate these diplococci as the causative agents of pneumonia.

Since the work of Dochez and Avery (1915), it has been known that pneumococci are present in the throats of a large number of healthy individuals. These authors found that, while pneumococci of types I and II caused over 60 per cent of the cases of lobar pneumonia in adults, the incidence in the normal mouth was less than 1 per cent. They found type III and group IV to be responsible for 30 per cent of the pneumococcus

cases and to be found frequently in the mouth.

Powell, Atwater, and Felton (1926) made several surveys during the period from September 1923 to March 1924 in Boston and found the incidence of pneumococci to be as follows:

(a) Of 104 laboratory workers 70 (67 per cent) were carriers of pneumonia organisms. Type I occurred in 2 (1.9 per cent) of those examined during the survey; type II in 10 (9.6 per cent); type III in 14 (13.4 per cent); and type IV (now referred to as group IV) in 57 (54.8 per cent).

(b) Of 112 high school boys 74 (66 per cent) were carriers. Type I occurred in 7 (6.2 per cent) of those examined during the survey; type II in 6 (5.3 per cent); type III in 5 (4.4 per cent); and type IV in 66 (58.9 per cent).

(c) Of 112 medical school students 74 (66 per cent) were carriers. Type I occurred in 4 (3.5 per cent) of those examined during the survey; type II in 3 (2.6 per cent); type III in 5 (4.4 per cent); and type IV in 67 (59.8 per cent).

(d) Of 90 student nurses 48 (53 per cent) of those examined during the survey were carriers. Type I occurred in no cases; type II in 1 (1.1 per cent); type III in 8 (8.8 per cent); and type IV in 44 (48 per cent).

(e) Of the total group of 93 persons on whom 418 examinations were made, type 1 occurred in 3.1 per cent of the examinations; type II in 4.8 per cent; type III in 7.7 per cent; and type IV in 56 per cent.

Meyer (1920) found no strains of type I or II among 100 healthy individuals; only 3 strains (3 per cent) of type III; and 17 strains

(17 per cent) of group IV organisms.

Webster and Hughes (1931) reported that in an extended investigation embracing monthly examinations over a period of from two months to two and one-half years, of 105 children and adults living in New York City, pneumococci were obtained at one time or another from the nasal passages or the throat of 80 per cent of the persons studied. They also noted that the incidence of pneumococci in all of the individuals included in the study underwent seasonal fluctuations corresponding to the changes in the prevalence of coryza and sore throats in the same person. This latter observation was also reported by Longcope and Fox (1905).

Gundel (1933) reported that repeated tests had been made every four weeks for one year on the upper respiratory tract of over 100 normal subjects. Type I occurred in 0.8 per cent of those tested; type II in 0.4 per cent; type III in 6.7 per cent; and group IV in 60.0 per cent.

Schleifstein (1938) made a study of 100 normal individuals in Albany, New York, who had not been in contact with pneumonia patients and found 73.0 per cent to be carriers of pneumococci.

Bliss, McClaskey, and Long (1934) made a study of 20 subjects in the John Hopkins University Medical School, on whom 1,016 cultures to be tested for Diplococcus pneumoniae were taken over a period of two years. Of these cultures 54.5 per cent were positive for pneumococci. All but one of the subjects yielded positive cultures of Diplococcus pneumoniae at least once during the course of the investigation.

Blacklock and Guthrie (1933) found pneumococci in the throats of 58.6 per cent of the healthy children examined.

Rosenau, Felton, and Atwater (1926) reported on the work of Sailer, Hall, Wilson, and McCoy (1917) who found, in 700 examinations, 16 per cent to be carriers of pneumococci.

Incidence of multiple-type carriers.--In the surveys which have been carried out to determine the ability of healthy individuals to carry Diplococcus pneumoniae in their throats, some investigators have found more than one type to be present at the same time.

Gundel and Okura (1933) investigated the occurrence of pneumococci of more than one serological type in the same subject. Of the individuals studied, 38 per cent carried organisms of two or more types. The appearance of the new types was attributed by the authors to infection from without or possibly to the development of a type which had been suppressed by the dominance of the first type found.

MacKenzie, Tepperman, and McKee (1940) found in a rural community that 41.3 per cent of the 250 people upon whom seven surveys were made during 14 months could be classed as multiple-type carriers.

Importance of contacts.--Dochez and Avery (1915) recognized that the existence of the carrier state among healthy persons and among those recently recovered from pneumonia established a basis for understanding the mechanism by which lobar pneumonia spreads and maintains its high incidence from year to year.

In a group of 270 contacts with 28 cases of pneumonia, Rosenau, Felton, and Atwater (1926) found a consistent excess of carriers over those found in the control group. The excess appeared only in those types which were present among the 28 cases, namely types I and III.

They found that contacts of type I cases were three times as likely to carry type I organisms as are controls (8.1 per cent against 2.3 per cent). Contacts with type III cases were twice as likely to carry type III organisms as controls (17.8 per cent against 9.9 per cent). It was also pointed out by these authors that type III organisms occurred in control populations more frequently than types I and II taken together, yet in most years type III causes not more than one-sixth as much pneumonia in the United States as types I and II. Type III apparently spreads from both carriers and cases more rapidly than does type I.

Rosenau, Felton, and Atwater (1926) showed that among 220 persons exposed to 28 carriers, 9 (4.1 per cent) carriers of the homologous organism were detected. They felt that the evidence presented corroborated the assumption that carriers, as well as cases, spread pneumococci, though, as they pointed out, the cases were more prolific sources than were the carriers.

MacKenzie, Tepperman, and McKee (1940) in a study of carriers in a village of 250 people in which 5 cases of type I pneumonia had occurred within 8 weeks, found that the carrier rate of all types of Diplococcus pneumoniae was 73.4 per cent. The carrier rate of type I was 24.3 per cent. Five months after the occurrence of the last case of pneumonia, the carrier rate dropped to 5.9 per cent.

Identification of types of Diplococcus pneumoniae.--Cooper and her associates (1929 and 1932) succeeded in showing by agglutination tests that Diplococcus pneumoniae in Group IV could be subdivided into 29 different types. Previous to this work, the identification of Diplococcus

pneumoniae to types was made as follows: All organisms which failed to agglutinate with antisera for types I, II, or III and which were able to ferment inulin were placed in group IV. Cooper's work greatly aided the identification of Diplococcus pneumoniae. But when Neufeld and Etinger-Tulczynska (1933) brought forth their method for type determination, it was possible for investigators to place their organisms into the correct types to the number of 32.

Occurrence of "suspicious" cultures.--Although antisera for types 1 to 33 are now available for typing Diplococcus pneumoniae, some investigators have been unable to classify as to type all of their cultures which by other tests seem to be Diplococcus pneumoniae.

Schleifstein (1938) found that of 132 strains of pneumococci only 84 could be classified. In 15 instances, a "Quellungs" reaction was obtained in pooled sera, but the pneumococci were present in such small numbers that identification was not attempted. Thirty-three cultures were bile soluble but could not be classified as to type.

MacKenzie, Tepperman, and McKee (1940) found that 18 per cent of the carriers harbored pneumococci which did not react with any of the specific sera of types 1 to 32.

Possibility of change from one type to another.--There have been, from time to time, suggestions from various sources that one type of Diplococcus pneumoniae might change to another type in vivo. However, there seems to be some divergence of opinion.

Megrail and Ecker (1924) stated that pneumococci had a type stability when placed under conditions which caused the typhoid and other organisms

to show variability in agglutination.

Barnes and Wright (1936) mentioned that their search of the literature had failed to produce definite evidence that a virulent pneumococcus could undergo spontaneous conversion from one type to another. They did, however, say that some of their work did not prove, but did strongly suggest, that under certain routine conditions a type V pneumococcus would spontaneously change into a type II organism. They were unable to give any factor or factors which might be responsible for this instability.

Dawson (1930) made the statement that it had not been conclusively demonstrated that transformation of types among Diplococcus pneumoniae actually occurred under natural conditions.

Gundel and Okura (1933) believed that the appearance of new types in the throats of individuals studied could be attributed to infection from without or possibly to the development of a type which had been suppressed by the dominance of the first type found.

Kinds of carriers.--It has been found that those individuals who carry pneumococci in their throats are not always consistent either in the length of time they carry the organism or in the type of organism they carry.

Stillman (1916) found that persons recovering from pneumonia remained carriers for 7 to 90 days. During that time they could act as "contact carriers".

Webster and Hughes (1931) found that some people were pneumococcus-free, some were "transient" carriers (positive on single and scattered

occasions between pneumococcus-free periods), some "periodic" carriers (those from whom pneumococci of one serological type were obtained for periods of 1 to 12 weeks between pneumococcus-free intervals), and some were chronic carriers (those from whom pneumococci of one serological type were obtained for periods of three months to three years or more). Evidence was presented showing that these differences were due to a variation in host-resistance to the pneumococcus organisms.

Bliss, McClaskey, and Long (1934) attempted a classification of carriers based on repeated bacteriological examinations extending over a period of a year or more, which was a slight modification of that drawn up by Webster and Hughes (1931). After a year's study of young adults the authors divided the subjects into non-carriers and chronic carriers, the latter group including those persons who intermittently exhibited pneumococci in their throat. While the so-called intermittent carriers might or might not yield positive cultures on repeated examination, the cultures when positive were consistently of the same type of pneumococcus in any given case, indicating to these authors a chronic condition with constant bacteriological findings only as to type. Furthermore, these authors considered that their demonstration added evidence in favor of the stability of pneumococcal types in the human body. It seemed more logical to these authors to designate healthy individuals who harbor pneumococci in the nose and throat for short periods of time as temporary carriers, and those in whom organisms persisted for longer periods of time as chronic carriers. It was suggested by these authors that a further subdivision of chronic carriers into "continuous" and

"intermittent" carriers would define more accurately the condition and the possible menace of the chronic case.

MATERIALS

For growing pneumococci it was necessary for the medium used to support growth but not to cause rapid autolysis. Beef heart infusion broth which contained 0.2 per cent Na_2HPO_4 as a buffer and 2 per cent peptone was found to be very satisfactory. This broth was prepared as follows: The fat was removed from beef hearts and the meat ground in an ordinary meat grinder. To each 500 g. of meat, 1000 cc. of distilled water was added. This mixture was infused in the icebox for 12 to 18 hours. After the fat had been skimmed from the top, the infusion was boiled for $\frac{1}{2}$ hour and the volume was restored with distilled water. With the aid of a meat press, the liquid was squeezed from the meat. This liquid was run through filter paper, and the volume again restored with distilled water. To each 1000 cc. of this infusion was added 20 g. of Difco peptone, 5 g. NaCl, and 2 g. Na_2HPO_4 and this mixture was heated to dissolve the peptone. After being titrated and adjusted to pH 7.6 to 7.8, the broth was boiled for $\frac{1}{2}$ hour. It was then allowed to cool to 30° C. before it was filtered, tubed, and sterilized in the autoclave at 15 lbs. pressure for 15 minutes. The final pH was approximately 7.4.

To insure the best growth of the pneumococci, the beef heart infusion broth was enriched by the addition of approximately 1 per cent fresh beef serum which had previously been sterilized by the use of a Berkefeld filter and which had 1 per cent of formalin added to it.

Sodium desoxycholate solution was used in the test for solubility of the cultures (Kolmer and Boerner, 1938). It was prepared as follows: To every 100 cc. of distilled water, 10 g. of chemically pure sodium desoxycholate was added. The mixture was shaken and stirred until completely dissolved and then autoclaved for 15 minutes.

METHODS

Methods of obtaining cultures for the 1939 to 1940 survey.--In the survey carried out during the school year 1939 to 1940, several methods were tried with varying degrees of success. The first method was as follows:

Using sterile swabs, cultures were taken from the tonsilar crypts, care being taken to avoid touching other parts of the mouth. The swab was placed in beef heart infusion broth enriched with approximately 1 per cent beef serum and incubated at 32° C. for 12 to 24 hours. This culture was then streaked on blood agar plates. Cultures were taken from the naso-pharynx region and treated in the same way as those from the tonsilar region. After a 24 to a 48 hour incubation period at 32° C., colonies which resembled those of Diplococcus pneumoniae were transferred to a 1 per cent dextrose broth. The dextrose broth was incubated at 32° C. for 12 hours and the Neufeld "Quellungs" reaction carried out. This method proved to be unsatisfactory since it yielded no pneumococci.

The second method, which proved to be more satisfactory than the first but not as satisfactory as the third, was as follows:

A swab was taken from the nasopharynx region and placed in beef heart infusion broth enriched with approximately 1 per cent beef serum and

incubated at 37° C. for 4 to 6 hours. One cc. of this culture was injected into the peritoneum of a white mouse. When the mouse became very sick or was just on the verge of death, he was autopsied. A culture of blood was taken from the heart and placed in a tube of enriched beef heart infusion broth. This culture was incubated at 37° C. until growth became visible. At that time, the Neufeld "Quellungs" reaction was carried out.

The third, and most successful method was as follows:

The student to be examined for the presence of pneumococci was asked to rinse his mouth, especially around the teeth, with 20 cc. of physiological saline solution (0.85 per cent). This washing was discarded. Then 20 cc. of the saline solution was used as a gargle for from 1 to 3 minutes and then expelled into a sterile container. This washing was centrifuged at 2000 r. p. m. for one hour. All the sedimented material was then suspended in 1 cc. of saline solution and injected into the peritoneal cavity of a white mouse. Six hours after injection, a sterile capillary pipette was inserted into the peritoneal cavity of the mouse and some of the fluid was withdrawn. One drop of this fluid was placed in beef heart infusion broth; another drop was smeared on a blood agar plate; and a third drop was smeared on a slide for staining.

After 48 hours incubation at 37° C., suspicious colonies were transferred to sterile beef heart infusion broth. When growth appeared in the tubes, 1 cc. portions were placed in each of three small tubes. To the first tube was added four drops of a 10 per cent aqueous solution of sodium desoxycholate; to the second tube was added $\frac{1}{2}$ cc. of a 10 per cent aqueous solution of Bacto ox-gall; and the third tube was used as a control.

Smears (for the Neufeld "Quellungs" reaction) were made on glass slides of all cultures which were soluble with either or both of the above tests. Blood agar plates were streaked from all positive cultures in order to obtain a pure culture of the pneumococci.

Those mice which did not die were observed for 15 days, after which they were etherized, autopsied, and cultures taken from the peritoneal cavity and from the heart. The same procedure as described above was carried out with any cultures showing growth.

Selection of individuals for the 1940 to 1941 survey.--All but 14 of the individuals were chosen at random from those who were in Lewis Hall when the work on this survey began in October 1940. The group of 14 were girls who lived in a sorority house on the campus at Montana State College. (Individuals 1 to 9, 11 and 12, 14 to 16 in Table I)

Methods of obtaining cultures in the 1940 to 1941 survey.--In order that the two methods, swabbing and gargling, might be compared, each individual tested had his throat swabbed first and then was asked to gargle. The two procedures are described below:

The Swab Method.--With a sterile cotton swab a culture was taken from the nasopharyngial region of each individual. The swab was placed in a tube of enriched beef infusion broth and incubated at 37° C. for 6 hours. At the end of that time the broth was centrifuged at high speed (approximately 2000 r.p.m.) for 45 minutes. The supernatant fluid was poured off and the sediment suspended in 1 cc. of sterile physiological saline solution (0.85 per cent). One cc. of this suspension was injected intraperitoneally into a white mouse. Six hours later, by means of a

small capillary pipette, some fluid was withdrawn from the peritoneum. A drop of this liquid was placed in enriched beef heart infusion broth and incubated at 37° C. until sufficient cloudiness was present to run a solubility test with sodium desoxycholate. This test consisted in taking two 1 cc. samples of culture, adding 4 drops of sodium desoxycholate solution to one, and using the other as a control tube. The test was observed over a period of one hour and those cultures from which the cloudiness had disappeared were considered positive. Smears were made on glass slides from these positive cultures. A Neufeld "Quellungs" reaction was carried out on these smears.

The Gargle Method.--The individual was asked to rinse out his mouth with 25 cc. of sterile physiological saline solution (0.85 per cent) and to discard this washing. Another 25 cc. of sterile physiological saline solution was used immediately as a gargle for 1 to 3 minutes. This liquid was expelled into a sterile container and then centrifuged at high speed (approximately 2000 r.p.m.) for 45 minutes. The supernatant liquid was poured off and the sediment suspended in 1 cc. of sterile salt solution. One cc. of this suspension was injected intraperitoneally into a white mouse. The method from here was the same as that for the swab method, i.e., withdrawal after 6 hours, solubility test, and Neufeld typing.

As soon as possible after an inoculated mouse died, an autopsy was made. By means of a small capillary pipette blood was withdrawn from the heart. A drop of this blood was placed in enriched beef heart infusion broth and incubated at 37° C. until noticeable cloudiness developed. The culture was then tested for solubility using sodium desoxycholate and the

Neufeld reaction was carried out as for the others.

After 14 to 15 days the mice which did not die were chloroformed and cultures taken from the heart's blood. If growth occurred it was taken through the regular procedure.

The solubility test using sodium desoxycholate solution and the phenomenon of capsular swelling described by Neufeld (1902) and later used by Neufeld and Etinger-Tulczynska (1933) as a method for rapid type determination of Diplococcus pneumoniae, are the ones used at the present time by the author for the identification of types of diplococci. For this Neufeld "Quellungs" reaction, antisera of types 1 to 32, according to Cooper (1929), and antiserum for type 33, are placed into groups A, B, C, D, E, and F. Each group serum contains the pooled antisera for certain types of pneumococci.

The method for carrying out the Neufeld "Quellungs" reaction in this survey was as follows: Two smears of the soluble cultures were placed on each of six clean glass slides. A different group antiserum, obtained from the Lederle Laboratories, was placed on each smear. In the group antisera used there was a blue dye which stained the organisms. The smear was then covered with a clean cover glass and was examined microscopically with the aid of the oil immersion lens. A positive reaction was indicated by a clear, unstained area around the stained pneumococci. This area has a definite outline and it is the sharpness of this outline which is more important in type determination than the amount of swelling of the capsule. Halos may be seen in negative preparations, due, probably, to the presence of a small capsule around the organism or to the re-