LABORATORY REPORT No. 311

RESPIRATORY DISEASE SURVEY

by

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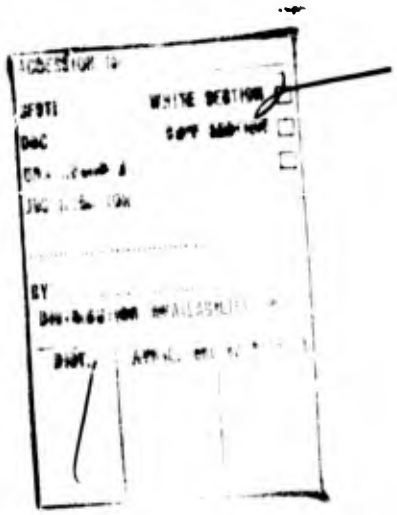
MICROBIOLOGY DIVISION

JULY 1967

USARMY MEDICAL RESEARCH
AND NUTRITION LABORATORY

FITZSIMONS GENERAL HOSPITAL

DENVER, COLORADO 80240



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RESPIRATORY DISEASE SURVEY

OBJECT:

A study was conducted to determine the incidence of specific etiologic agents associated with upper respiratory disease and pneumonia. Data was acquired from a series of surveys employing bacteriologic and serologic technics.

SUMMARY:

Results of these surveys indicate that antibodies to Mycoplasma pneumoniae can pass the placental barrier and are detectable at low levels in the serum of the newborn. Surveys for the incidence of Mycoplasma species show a rate of 86% from normal individuals, 46.6% from adults with chronic respiratory disease, and 12% positives from children with chronic respiratory disease. Approximately 3% of the normal individuals sampled over a seven-month period were positive for the important species, Mycoplasma pneumoniae.

APPROVED:

JAMES C. SYNER
Colonel MC
Commanding

66 Miscellaneous Microbiological Clinical Research

BODY OF THE REPORT

I. STATEMENT OF PROBLEM:

The control and prevention of infectious disease as related to accomplishment of a mission is self-evident. The fact that the general population is and can be maintained in a better state of health than formerly is due largely to medical research in this field. Further advances are needed in order to effectively control those diseases in which our knowledge is incomplete. It is estimated that approximately 75% of total respiratory diseases is of unknown etiology, also the etiology of 50% of acute respiratory disease (ARD) severe enough to require admission to the hospital, cannot be accounted for. Adenovirus has been shown to be a major identifiable cause of acute respiratory disease, and may play a major role in the production of bronchiectasis either as a primary or secondary invader. The relative importance of Mycoplasma sp. appears to be increasing; therefore further studies should be made to determine the incidence and control of these species, and the need for laboratory support in the diagnosis of pneumonia is becoming increasingly important. A small percentage of the clinically diagnosed pneumonias are associated with an identifiable etiologic agent, the majority being broadly grouped under the clinical diagnosis of primary atypical pneumonia (PAP). A portion of this latter group can be attributed to Influenza viruses, Adeno viruses, and cold-agglutinin positive pneumonias associated with Mycoplasma pneumoniae[1,2,3,4,5,6,7,8,9] (Eaton Agent). To determine the incidence of these specific etiologic agents a series of surveys were conducted.

II. APPROACH TO THE PROBLEM:

Population and Sampling Procedures

Survey 1. To determine the incidence of complement-fixing antibodies to Mycoplasma pneumoniae[11,12,13,14] present in the serum of infants. Military dependents hospitalized for reasons other than acute disease were selected for this survey. This group included the newborn and their mothers. Cord bloods and mothers' blood were obtained at birth. In addition to the cord blood, venous blood was obtained from one group of infants on the fourteenth day.

Survey 2. To determine the incidence of Mycoplasma sp. present in the sputum of patients hospitalized for chronic respiratory disease. Specimens were collected from 7 children and 16 adults and were cultured for the presence of Mycoplasma.

Survey 3. A field study to determine the viral etiology of respiratory disease seen in a hospitalized recruit population. Subjects for this study were selected at random from febrile patients having been admitted to the hospital 12 hours or less previous to sampling. An attempt was made to sample approximately 10% of the hospitalized respiratory disease patients. The sampling was conducted from December to March. Eleven sampling periods were accomplished during this period.

Survey 4. An upper respiratory disease survey of a unit attached to Fitzsimons General Hospital. This group was selected as ideal due to the nature of their training assignments they would represent all areas of the hospital community. The study was accomplished with 7 sampling periods from October - April and was designed to determine the morbidity of Mycoplasma sp., Influenza, Adenoviruses, Staphylococcus carrier rate, and Group A Streptococcus.

Cultural Methods

Specimens from the oropharynx were collected by a swab to 1 ml of Todd-Hewitt broth.

Material was dislodged from the swab into the broth using a vortex mixer. Excess broth was wrung from the swab and the swab discarded. If specimens were not to be inoculated immediately, they were placed in a refrigerator (4° - 6°C) until used. Results of prior studies have shown that specimens can be thus stored for a month with little or no loss of group A streptococci.

Throat specimens were inoculated to 5% sheep blood agar (streak plate), chocolate agar and into pour plates containing 5% sheep blood.

Inoculation to the sheep blood agar (SBA) streak plate and the chocolate agar streak plate was accomplished by placing a 3 mm loopful of the specimen to the agar and streaking it using a mechanized turntable and needle.

Pour plates were made by placing a loopful of specimen into a tube of melted, cooled (48°C) trypticase soy agar (TSA) containing 5% sheep blood. The contents were mixed thoroughly and transferred to a petri dish containing a thin base layer of TSA. All throat culture plates were incubated at 35°C and under increased CO_2 tension utilizing a candle jar (2-3% CO_2).

All plates were examined after 24 and 48 hours' incubation for the presence of beta-hemolytic streptococci, pneumococci, meningococci and Hemophilus influenzae or other bacterial pathogens. Suspect colonies were isolated for identification. Beta-hemolytic streptococci to be tested for susceptibility to low concentrations of bacitracin (BBL Taxos A Disc) for determination of group and final identification of Group A streptococci to be determined serologically.

Suspect pneumococcal colonies were tested for susceptibility to optochin (BBL Taxos P discs).

Suspect Neisseria meningitidis colonies were identified by their exhibiting a positive oxidase reaction, acid production in media containing glucose and maltose but not in sucrose. Final identification to be determined serologically. The technics for sampling for Staphylococcus and the media used[10] is the subject of a separate report.

Viral Isolation

Using throat washings Hela, HEP-2, monkey kidney cells and embryonated hens eggs were used to isolate and identify Adenovirus and Influenza. However, no isolates were made from the specimens collected at the first and third sampling periods and it was decided to attempt viral isolation only on selected cases of ARD. No hospitalization for other than injury was experienced by this group during the period of the survey. No positive isolates for Adenovirus or Influenza were obtained.

Mycoplasma Isolation and Identification

The throat washings obtained for viral studies were used to inoculate plates of yeast extract, horse serum-agar medium described by Chanock et al.[2,8] This agar, as modified by Crawford, was incubated at 36°C aerobically and anaerobically for 30 days before being discarded as negative. The aerobic plates were incubated in large candle jars which served the double purpose of a CO_2 atmosphere and retarded dehydration of the plates. The anaerobic plates were incubated in an atmosphere of 95% N_2 and 5% CO_2. All plates were examined at 48 and 96 hours and every 4th day following the initial 2nd and 4th day reading. Medium #1 which was formulated specifically for the isolation of the clinically significant Mycoplasma pneumoniae It was prepared from Difco PPLO agar base 23 8 gms , triple glass distilled water 700 ml , thallium acetate 0.35 gm. 25% Fleischman's 40-40 yeast extract 100 ml., methylene blue chloride 0.02 gm. horse serum 200 ml, crystalline penicillin 2 x 10^6 units, amphotercin B 5 mg. final pH of the medium was 7.8 The medium was dispensed into 60 x 15 mm. plastic dishes for use. This medium was prepared fresh weekly. Medium #2 consisted of Difco PPLO agar 34 0 gms. triple glass distilled water 900 ml, thallium acetate 0.5 gm, oxoid yeast autolysate 10 gms, horse serum 100 ml, crystalline penicillin 2 x 10^6 units, amphotercin B 5 mg, final pH 6 6. This medium was also dispensed into the 60 x 15 mm plastic dishes. This second medium is not ideal for the isolation of Mycoplasma pneumoniae and was used to isolate other species

harbored by man. From Medium No. I we expected to selectively isolate Mycoplasma pneumoniae, from Medium No. 2 we could expect to isolate M. salivarium, M. pharyngis, M. fermentans, M. hominis and others. No bacterial or the bacterial variant L forms were encountered. Fungal contaminants were encountered, however all throat washings were stored frozen and if contaminated were repeated. Using this technic no samples were lost due to contamination.

Serological Procedures

Venous blood was obtained to determine the antibody level to Influenza A, Influenza B, Adenovirus group and Mycoplasma pneumoniae. The antibody was measured by a quantitative complement-fixation technic based upon 50% hemolytic unit (C' H 50) of complement as determined by precise spectrophotometric methods. Serum dilutions eliciting 0 to 50% hemolysis were considered positive and the titer was expressed as the receiprocal of the highest dilution of serum yielding a positive reaction. The degree of hemolysis in the supernates was determined by visual comparison with simulated quantitative hemoglobin standards.[15] Commercially available C-F antigens were used for the Influenza A, Influenza B, and Adenovirus determinations. Mycoplasma pneumoniae antigens and antisera were prepared by methods outlined by Chanock et. al.[4]

III. RESULTS AND DISCUSSION OF RESULTS:

Survey Number 1. To determine the incidence of complement-fixing antibodies to Mycoplasma pneumoniae present in the serum of infants.

SEROLOGIC RESULTS OF 158 PAIRED MATERNAL-CORD BLOODS AND 132 SINGLE CORD BLOODS

C-F Titer	Mother Pos Infant Pos	Mother Pos Infant Neg	Mother Neg Infant Pos	Cord Blood Only
1-4 but not 1-8	2/158	19/158	2/158	4/132
1-8 but not 1-16	10/158	8/158	1/158	9/132
1-16	2/158	0/158	0/158	2/132

Fifteen (11.3%) of the single cord bloods tested for C-F antibodies to M. pneumoniae were positive. Twenty-six and six-tenths per cent (26.6%) of the positives had a titer of 1-4, 60% a titer of 1-8, and 13.3% a titer of 1-16. Seventeen per cent (17%) of the maternal blood samples were positive with the matched cord blood negative. Six and three-tenths per cent (6.3%) of paired maternal-cord bloods were positive at 1-8. Two of the specimens were positive at 1-4 and two at 1-16. Less than 1% (3) of the cord bloods were positive when the paired maternal blood was negative.

The testing of paired blood from the infants resulted in 11.6% positive reactions (cord blood and blood obtained at 14 days); 30% had a twofold drop in titer at the fourteenth day.

MYCOPLASMA ISOLATES FROM SPUTUM SPECIMENS
COLLECTED AT FOUR DAY INTERVALS
FROM CHILDREN WITH CHRONIC
RESPIRATORY DISEASE

Patient Number	Day Specimen Collected and Results of Culture							
	1	5	10	15	20	25	30	Totals
1C	+	-	-	-	-	-	-	1/7
2C	-	-	-	-	-	-	-	0/7
3C	-	-	-	-	-	-	-	0/7
4C	-	-	+	-	-	-	-	1/7
5C	-	-	-	-	-	+	-	1/7
6C	-	-	-	+	-	+	-	2/7
7C	+	-	-	-	-	-	-	1/7
Totals	2/7*	0/7	1/7	1/7	0/7	2/7	0/7	6/49

*No. pos/No. tested

Survey Number 2: To determine the incidence of Mycoplasma species present in the sputum of patients hospitalized for chronic respiratory disease. Initial sputa were collected from 7 children and 16 adults hospitalized with chronic respiratory disease and this was followed with sputa collections at 4-day intervals for a total of 7 specimens. Chemotherapy including tetracycline was instituted following the collection of the first or baseline specimen.

MYCOPLASMA ISOLATES FROM SPUTUM SPECIMENS COLLECTED AT FOUR DAY INTERVALS FROM ADULTS WITH CHRONIC RESPIRATORY DISEASE

Patient Number	Day Specimen Collected and Results of Culture							
	1	5	10	15	20	25	30	Totals
1A	+	-	-	+	-	-	-	2/7
2A	+	+	-	-	-	+	-	3/7
3A	+	-	+	-	-	-	-	2/7
4A	-	-	-	-	+	-	-	1/7
5A	-	-	-	-	-	-	-	0/7
6A	-	-	-	-	-	-	-	0/7
7A	+	+	-	+	-	-	+	4/7
8A	-	-	-	-	-	-	-	0/7
9A	+	+	-	-	-	-	+	3/7
10A	+	-	+	-	-	+	-	3/7
11A	+	+	-	-	-	+	+	4/7
12A	-	-	-	-	-	-	-	0/7
13A	-	-	-	-	-	-	-	0/7
14A	-	-	-	-	-	-	-	0/7
15A	-	-	-	+	-	-	-	1/7
16A	+	-	-	-	-	-	-	1/7
Totals	8/16*	4/16	2/16	3/16	1/16	3/16	3/16	24/112

*No. pos/No. tested

Two (28%) had a sputum positive for Mycoplasma at the time of the baseline collection as compared to 8 of the adults (50%) during the same period. In the children's group 6 of 49 (12%) of the specimens collected during the study were positive with 24 of 112 (46.6%) of the specimens collected from the adults being positive.

Survey Number 3. This was a field study to determine the viral etiology of respiratory disease encountered in a hospitalized recruit population.

NUMBER OF PATIENTS SAMPLED AND SEROLOGIC RESULTS

Date	Percent of Total Patients Sampled	Adenovirus No. Pos/No. Patients	Influenza B No. Pos/No. Patients
7 Dec	7.0	1/1	0/1
14 Dec	5.4	2/2	0/2
21 Dec	35.0	4/4	0/4
11 Jan	17.0	7/9	1/9
18 Jan	8.8	8/10	2/10
25 Jan	11.2	10/11	3/11
1 Feb	7.3	3/10	2/10
8 Feb	11.9	6/12	3/12
15 Feb	10.5	6/13	1/13
22 Feb	7.4	3/9	0/9
1 Mar	7.2	4/10	0/10
	Avg. Sample 9.0	54/90	12/90

A total of 177 patients were sampled; convalescent sera were obtained on 90. The average sample was approximately 9% of the total number of cases hospitalized for respiratory disease.

Survey Number 4. This was a survey of a group of normal individuals attached to a general hospital for training and studied to determine the morbidity of Mycoplasma sp., Influenza, Adeno viruses, Staphylococcus carriers rate, and group A Streptococcus. Portions of the Staphylococcus study have been reported[10].

PERIOD OF SAMPLING AND NUMBERS SAMPLED

	Oct 83	Nov 70	Dec 73	Jan 72	Feb 72	Mar 67	Apr 64	Total 501
CULTURE RESULTS								
Group A Streptococcus	0/83*	0/70	0/73	1/72	1/72	1/67	1/64	4/501
Meningococcus	2/83	1/70	0/73	0/72	0/72	0/67	0/64	3/501
M. pneumonia	1/83	2/70	3/73	4/72	1/72	3/67	1/64	15/501
Mycoplasma	74/83	64/70	58/73	61/72	66/72	60/67	58/64	441/501
COMPLEMENT FIXATION RESULTS								
Influenza A								
No Titer	60/83	39/70	43/73	46/72	34/72	28/67	37/64	285/501
1/8 Titer	15/83	1/70	3/73	12/72	26/72	25/67	19/64	84/501
1/16 Titer	5/83	30/70	26/73	14/72	12/72	13/67	8/64	103/501
1/32 Titer	3/83	0/70	1/73	0/72	0/72	1/67	0/64	2/501
Influenza B								
No Titer	67/83	51/70	55/73	53/72	59/72	54/67	58/64	330/501
1/8 Titer	14/83	7/70	10/73	13/72	9/72	11/67	4/64	54/501
1/16 Titer	2/83	12/70	8/73	6/72	4/72	2/67	2/64	34/501
Adenovirus								
No Titer	77/83	63/70	15/73	54/72	42/72	51/67	48/64	386/501
1/8 Titer	5/83	8/70	15/73	11/72	8/72	10/67	13/64	70/501
1/16 Titer	1/83	0/70	4/73	7/72	12/72	6/67	3/64	33/501
M. pneumoniae								
1/8 or better	0/83	3/70	2/73	6/72	4/72	2/67	2/64	19/501

*No. pos/No. tested

A total of 501 samples were collected and processed. None of the individuals sampled required hospitalization during the period of this study and none admitted during interview to more than slight colds, boils or injury. Even though this area, Denver, Colorado, has a reportedly high incidence of group A streptococcus, only 4 isolates were made, all from the same asymptomatic individual. Meningococcus was insignificant with 3 positives being reported. The increase in positive viral serology findings was probably due to the annual immunization program which was administered 1 week following the first sampling period. Approximately 3% of this group were positive for Mycoplasma pneumoniae on culture with 3.7% having a complement-fixing antibody titer of 1-8 or better.

IV. CONCLUSIONS:

In Survey Number 1, fifteen (11.3%) of the 132 single cord bloods tested for C-F antibodies to M. pneumoniae were positive. Twenty-six and six-tenths per cent (26.6%) of the positives had a titer of 1-4, 60% a titer of 1-8, and 13.3% a titer of 1-16. Seventeen per cent (17%) of the maternal blood samples were positive with the matched cord blood negative. Six and three-tenths percent (6.3%) of paired maternal-cord bloods were positive at 1-8. Two of the specimens were positive at 1-4 and two at 1-16. Less than 1% (3) of the cord bloods were positive when the paired maternal blood was negative. The testing of paired blood from the infants resulted in 11.6% positive; 30% had a two-fold drop in titer at the fourteenth day. Results of this study indicate that antibodies to Mycoplasma pneumoniae can pass the placental barrier and are detectable at low levels in the serum of the newborn. In Survey Number 2, two (28%) of the children had a sputum positive for Mycoplasma at the time of the base line collection as compared to 8 or 50% of the adults during the same period. In the children's group 6 of 49 (12%) specimens collected during the study were positive with 24 of 112 (46.6%) specimens collected from the adults being positive. In Survey Number 3 the results confirm previous reports of the Adenovirus group being the major viral entity in upper respiratory disease among recruits. The detection of a small outbreak of Influenza B was of interest in that it was generally limited to the recruit population and was not detected in either the surrounding military or civilian community. In Survey Number 4 significant findings were noted in the Mycoplasma studies. In this group 86% were positive for Mycoplasma species. Approximately 3% of this group were positive for Mycoplasma pneumoniae on culture with 3.7% of the total population having a complement-fixing antibody titer of 1-8 or better. Comparing the incidence of Mycoplasma species isolated from normal individuals in Survey Number 4, 86%, with the adult respiratory group with an incidence of 46.6%, it would appear that the "normal flora" Mycoplasma are reduced in numbers in the presence of chronic respiratory disease.

V. RECOMMENDATIONS:

It was shown in these studies that complement-fixing antibodies to Mycoplasma pneumoniae could pass the placental barrier. The persistence of these antibodies, however, was not determined. Longitudinal studies to determine the persistence of these antibodies will be initiated and reported later.

Surveys on various well defined populations will be continued to obtain added information on the microflora of man and the influence environment and interpopulation exposure may have on his indigenous microbiota.

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13. ABSTRACT

A study was conducted to determine the incidence of specific etiologic agents associated with upper respiratory disease and pneumoniae. Data was acquired from a series of surveys employing bacteriologic and serologic techniques.

Results of these surveys indicate that antibodies to Mycoplasma pneumoniae can pass the placental barrier and are detectable at low levels in the serum of the newborn. Surveys for the incidence of Mycoplasma species show a rate of 86% from normal individuals, 46.6% from adults with chronic respiratory disease, and 12% positives from children with chronic respiratory disease. Approximately 3% of the normal individuals sampled over a seven-month period were positive for the important species, Mycoplasma pneumoniae.

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Adeno viruses						
Mycoplasma sp.						
Influenza viruses						
cold-agglutinin positive pneumonias (Eaton Agent)						
complement-fixing antibodies						