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Pakistan

PROPOSAL FOR THE INVESTIGATION OF
ETIOLOGICAL AGENTS OF ACUTE RESPIRATORY INFECTIONS IN CHILDREN
PAKISTAN

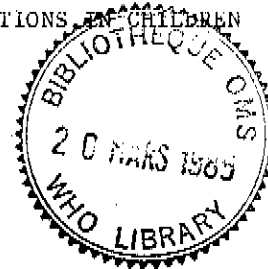


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PROPOSAL FOR THE INVESTIGATION OF
ETIOLOGICAL AGENTS OF ACUTE RESPIRATORY INFECTIONS
IN CHILDREN, PAKISTAN

1. Introduction

The knowledge of causative agents of acute respiratory infections in children in Pakistan is based on sporadic observations; systematic research into this matter is most important. In the routine clinical practice of even the most developed countries, it is not feasible to confirm the clinical diagnosis by isolation of the agent or identification of its antigens in the great majority of cases of ARI. Isolation of respiratory agents and the demonstration of specific antigens are time-consuming, expensive and require highly qualified staff and modern technology, which are not generally available. Special surveillance of the etiologies in a random sample of acute respiratory infections in children provides valuable information on the relative frequency of bacterial and viral agents, their association with clinical syndromes, the seasonal occurrence, the age distribution, and the pattern of bacterial sensitivity to commonly used antimicrobials. For the pediatrician this provides the information required in order to make an educated prediction of the causative organisms. For the public health administrator the survey data are extremely useful to define or adjust the standard plan of case management for ARI in children to be applied by general practitioners, health assistants, qualified health dispensers and primary health care workers in general.

The etiology of lung infections is of particular interest since they are frequent causes of death. Conventional methods of etiological investigation (bacteriological cultures, virus isolation and serology) pose many practical difficulties. Rapid immunological techniques offer operational advantages which make them more suitable to the conditions of developing countries. Large experience has been gathered in the use of immunofluorescence of nasopharyngeal secretions to establish the presence of respiratory viral pathogens in children. No such experience exists in respect of the use of the rapid bacteriological techniques such as counter-immunoelectrophoresis, coagglutination and latex agglutination. The only proven method for bacterial diagnosis of lung infections at the moment are based on culture of blood, which is only positive in one third or less of cases. Lung aspirates and trans-tracheal aspiration are often impractical or too dangerous. If rapid techniques can be properly validated in children, the collection of specimens and technical work for the diagnosis of ARI in children would be simplified. They might even give superior results. However, there is no information that rapid bacteriological techniques are sufficiently sensitive and specific for the diagnosis of ARI in children. A comparative study of the conventional and rapid techniques for the diagnosis of both bacterial and viral agents in children with ARI is proposed to be done as a research component of the etiological study.

2. Objectives

The objectives of the study are:

- (a) To establish the relative frequency of bacteria and viruses as causative agents of acute respiratory infections in children below the age of 6 who attend outpatient services.
- (b) To correlate the etiological findings with the clinical syndromes.
- (c) To determine the pattern of drug resistance of the most frequent respiratory bacterial pathogens.
- (d) To compare the results of rapid immunological techniques with those of conventional methods for the diagnosis of bacterial and viral agents.

The study will not provide epidemiological information on the incidence of acute respiratory infections, although the variations in the weekly attendances of children with ARI at the participating institutions will reflect the seasonality of the incidence of ARI.

3. Institution responsible for the research proposal

The National Institute of Health, Islamabad, will be responsible for the proposed study. The Laboratory of Virology has the equipment required for isolation of respiratory viruses. In the period from July 1981 to July 1982, 1354 specimens from patients suffering from ARI were processed. In 447, the results were positive, with the isolation of paramyxo-viruses, adenoviruses, echo and rhinoviruses. Influenza virus strain A/Bangkok/2/79 H₃N₂, was also identified. Respiratory syncytial virus was recognized by an immunofluorescence technique in samples of nasopharyngeal secretions.

The Laboratory of Bacteriology is equally well equipped and staffed. In the investigation of the 1354 specimens mentioned above, bacterial agents were identified in 701.

The duration of the study will be two years.

4. Selection of cases

4.1 Case definition

The study will be undertaken in children between the ages of 0 and 5 years (below 6 years of age) with a history of acute respiratory infection of seven days or less in duration who attend the pediatric services of the Central Government Polyclinic, Islamabad, the Clinical Research Centre, NIH, Islamabad, and the Rawalpindi General Hospital.

Acute respiratory infection is defined as any illness of onset within the past week with any of the following signs or symptoms: blocked or runny nose, sore or red throat, purulent exudates or membranes in the throat, hoarseness, stridor, wheezing, cough, expectoration, rapid breathing (more than 40 per minute), intercostal indrawing, cyanosis. Children with symptoms of otitis media only or ear discharge will not be included. Any child who has received an antimicrobial during the seven previous days will not be accepted into the study.

If the results are to be relevant for the case management of ARI in children at the health centres and rural health posts, they should be related to a categorization of cases which can be applied everywhere. For the purposes of this study the cases will be classified into mild, moderate and severe forms on the basis of the following key signs and symptoms:

- Mild forms: blocked or runny nose, sore throat, red throat, hoarseness, stridor, wheezing, cough.
- Moderate forms: purulent exudates or membranes in the throat, purulent expectoration, rapid breathing.
- Severe forms: intercostal indrawing (with rapid breathing or stridor), cyanosis

4.2 Sample size

The best sample procedure is to take every day a fixed proportion of children with ARI attending the selected institutions, since this method allows for qualitative and quantitative evaluation of seasonality. The disadvantage is operational. Since the daily workload follows the same trend of the respiratory infections peaks, in some periods there will be a large number of investigations to be performed to keep the fixed proportionality, while a few cases might be eligible in the low incidence period.

An analysis was made of the capability of the NIH to carry out the wide range of virological and bacteriological examinations which each patient would require. No more than three patients a day can be investigated, taking into account the availability of trained personnel, their current duties, the duration of the study and the need for laboratory methods to be performed within the acceptable limits of good quality. Since a high standard of performance is of paramount importance, it was decided to select every day three patients plus one control, that is eighteen patients and six controls a week. Considering that the average number of working days at the NIH is 266 per year, 800 patients will be investigated in one year and 1600 in two years. The total number of controls will be 532 in two years.

The number of children with ARI attending daily, including weekends, at the three institutions will be recorded. Later, in the analysis of the results, it will be possible to draw each week samples of the investigated cases as a fixed proportion of the weekly number of total attendances if a quantitative evaluation of seasonality according to syndromes and etiological agents is desirable.

Information on previous antimicrobial treatment among children attending with acute respiratory infections will be collected on a sample basis, for example, once a month in each institution any child with ARI will be questioned carefully by the paediatrician about any antimicrobial which could have been taken for the present illness.

For practical reasons the three cases and the control of each day will be selected from one of the three participating institutions. Considering the differences in the daily attendance of children, the Islamabad Polyclinic will collect the specimens three days a week, the Rawalpindi General Hospital two days a week and the NIH Clinical Research Centre one day a week (Table 1).

Table 1

Average daily number of children with ARI attending the participating institutions

	Winter Oct to Apr	Summer May to Sept	Participating in the Study
Polyclinic, Islamabad	80-100	30-40	Sun, Tues and Thurs
Gen. Hosp. Rawalpindi	40-50	10-15	Mon and Weds
Clinical Centre. NIH	15-20	4-8	Saturday
Total	135-170	44-63	-

Cases will be selected as follows:

- (a) Cases will be studied each week for 104 consecutive weeks.
- (b) Every day the designated institution will provide the specimens of the first children attending the pediatric service who meet the requirements of the case definition. However the patients will be classified into two categories: lung infections (pneumonia, bronchopneumonia and empyema) and other respiratory infections (bronchiolitis, tracheobronchitis, acute laryngitis, pharyngotonsillitis and rhinitis). In the selection process, every morning the first child with lung infection and the two first children with other respiratory infections who meet the requirements will be chosen for study. In this way the lung infections will constitute one-third of the children investigated.
- (c) It is most important to be able to define the proportion of the total number of cases that were investigated every week during the 2-year etiology study. Therefore a complete record must be kept, preferably in a record book at each institution, of the total number of children with ARI attending the pediatric services, whether hospitalized or not. In Annex 1 a model of a weekly report is presented, to be filled each Saturday with the data registered in the book during the last seven days. Attendances of children with ARI on Fridays and other holidays will also be registered.

5. Selection of controls

For each child admitted into the study with a diagnosis of lung infection (pneumonia, bronchopneumonia or empyema) a control will be chosen in the same institution where the patient was selected. In this way six controls will be investigated in an ordinary week. The control will be selected from healthy children who attend the institution for periodic check-up or for immunization, or from children in the surgical or emergency department without any sign of respiratory disease and who have not received any antimicrobial treatment

during the previous seven days. The controls will be matched for sex and place of residence (village, quarter of Islamabad or Rawalpindi, rural area) with the cases (1). The age will be matched to within 30 days for cases less than 1 year old and within 90 days for older cases.

Control children will be used primarily as bacteriological controls, although for some viruses controls can also be used to evaluate the carrier status. A few risk factors can also be evaluated in relation to some syndromes (such as pneumonia) and for some etiological agents.

6. Data collection

For each case and control selected, a questionnaire will be completed, the Case Clinical Record (Annex 2) and the Control Record (Annex 3). One medical officer, called the "Survey Medical Officer", will be designated to carry out this activity on a full-time basis. The Survey Medical Officer (or an alternate when he is off duty) will spend the morning of each working day at the assigned institution. He will re-examine the patients referred by the pediatrician as candidate cases for the study to confirm that they meet the requirements to be a participant. If they do, he will fill in the Case Clinical Record, and will collect the required specimens. After the three cases of the day are taken, the Survey Medical Officer will look for a control with the required characteristics, will fill in the Control Record and will collect the necessary specimens. Not more than two hours after their collection, the specimens will be taken to the NIH laboratories by the Survey Medical Officer. There he will assist with the first steps of their processing.

The bacteriological and virological results will be registered in the Laboratory Form (Annex 4). The results of the sensitivity tests will be reported in the forms currently used by the NIH.

7. Collection of specimens

7.1 Cases

From all cases (lung infections and other respiratory infections) one throat swab will be taken for bacteriological examination. The posterior area of the pharynx will be rubbed with a sterile cotton (fibre covered) swab, avoiding contact with the tongue and buccal surfaces. The bacteriological swab will be placed in Amies' transport medium to be taken to the laboratory. In children without nasal discharge a second throat swab and a nose swab will be taken and pooled for virus isolation. The virological swabs will be transferred to a Bijou bottle containing 2 ml of transport medium (Hank's balanced salt solution with 10% bovine albumin Fraction V). The specimens will be taken to the laboratory on ordinary ice (4°C).

Caution: If there is a suspicion of epiglottitis or croup, the throat swabs are not to be obtained. In these patients the swabbing procedure may induce further obstruction of the airway and suffocation.

(1) The pediatric units of the participating institutions will be provided by the principal investigator with a map of the catchment area showing the urban quarters, the periurban sectors and the rural districts to facilitate the registry of the place of residence of cases and controls.

Nasopharyngeal aspirates will be taken only if the child has a nasal discharge. The best specimen is obtained by suction into a mucus collector. A sterile polyethylene feeding tube (size 8) is attached to one of the two outlets of a sterile plastic mucus collector. The other outlet is attached to a suction pump. The tube is inserted into one nostril of the patient and passed into the nasopharynx. A negative pressure not exceeding $2\text{kg}/\text{cm}^2$ (200 mgHg) is then applied and the secretion aspirated into the collector. The tube is withdrawn and the procedure repeated through the second nostril. If necessary, a small volume (not more than 0.5 ml) of physiological saline may be sucked through the tube to collect any mucus lining the tube. The nasopharyngeal aspirates will be divided into three portions:

- (a) for virus isolation;
- (b) for bacterial studies;
- (c) for rapid diagnostic techniques:
 - immunofluorescence for virus and Bordetella pertussis; CIE and coagglutination for bacteria (in cases of lung infection).

Blood samples will be obtained only from patients with a lung infection (pneumonia, bronchopneumonia or empyema) in whom the laboratory results (if positive) will be of benefit for their treatment. The blood will be obtained by venepuncture at a site determined by the physician based on the age and condition of the child. The skin at the venepuncture site is scrupulously cleaned by washing with tincture of iodine, waiting for two minutes, removing the iodine with a 70% alcohol swab, then allowing the alcohol to dry. A minimum of 8.0 ml, and if possible 12 ml of whole blood is obtained. After carefully changing needles two parts of 3.0 - 4.5 ml each are transferred to two bottles of blood culture medium (50 ml tryptic digest of soy broth with 0.025 to 0.05% sodium polyanethosulphonate). The bottles are shaken to disperse the blood and prevent it from clotting. The remaining amount will be used for virological serology and rapid bacteriological techniques. A second blood specimen of 2 ml will be taken from the same children after 10 days for serological virology. Pleural aspirates will be taken from children with pleural effusion or empyema for bacteriological study.

Urine specimens will be collected only from children with lung infection (pneumonia, bronchopneumonia and empyema) for rapid diagnostic techniques: CIE, coagglutination and latex agglutination for bacteria.

7.2 Controls

From each control, a throat swab for bacteriological investigation will be collected. A second throat swab and a nose swab will be taken and pooled in the transport medium for virological isolation.

Table 2

Specimens and Laboratory Procedures

SPECIMEN	Gram Staining	Bacterial Culture	GIE CAA LA	Virus Isolation	Virus IF	B. Pertussis IF	Viral Serology
<u>I. Lung Infection</u>							
Throat swab	X	X					
Pooled throat/nose swab (if <u>no</u> nasal discharge)				X			
Nasopharyngeal aspirates (if nasal discharge)							
part a	X	X					
part b				X			
part c			X		X	X	
Blood 1							
part a		X					
part b			X				X
Blood 2							X
Pleural aspirates (if pleural effusion)	X	X					
Urine			X				
<u>II. Other Respiratory Infections</u>							
Throat swabs	X	X					
Pooled throat/nose swab (if <u>no</u> nasal discharge)				X			
Nasopharyngeal aspirates (if nasal discharge)							
part a	X	X					
part b				X			
part c					X	X	
<u>III. Control</u>							
Throat swab	X	X					
Pooled throat/nose swab				X			

8. Processing of specimens

The laboratory procedures adopted for use in the study are limited to the diagnosis of the agents which are most commonly associated with ARI in infants and young children. Laboratory procedures will be carried out in order to detect the following recognized respiratory pathogens:

(a) Bacteria Streptococcus pneumoniae

Haemophilus influenzae

beta haemolytic streptococci

Staphylococcus aureus

Bordetella pertussis

Klebsiella pneumonia

Escherichia coli

Other enteric bacteria

(b) Viruses

Respiratory syncytial virus

Influenza A and B

Parainfluenza 1, 2 and 3

Adenoviruses

Rhinovirus

Enteroviruses

Measles

Herpes

Cytomegalovirus

Only an outline of the laboratory procedures is given here. The study will use the methods described in the WPRO/WHO Manual for Bacteriology of Respiratory Infections, 1984; the Manual for Rapid Laboratory Diagnosis, WHO Offset Publication No. 47, 1979, and Viral Respiratory Diseases, WHO Technical Report Series No. 642, 1980.

8.1 Bacteriology

The throat swabs, nasopharyngeal aspirates and pleural aspirates will be processed using essentially the same procedures for gram staining and culture.

(a) A smear is prepared for gram-stain. The cytology as well as the bacterial flora will be evaluated and the predominance of a morphological type noted.

(b) The specimen is streaked on to:

- two blood agar plates to be incubated in aerobic and anaerobic atmosphere;
- a supplemented chocolate agar plate to be incubated in 5-10% CO₂ atmosphere.

Each plate is streaked to yield isolated colonies and incubated at 35-37°C for 18-24 hours.

The bottles containing 3 or 4.5 ml of blood for bacteriological investigation are placed in the incubator at 37°C and are examined daily for 7 to 10 days. Routine sub-cultures from the blood culture bottle to a blood agar plate and chocolate agar plate in anaerobic atmosphere are made after 48 hours of incubation, and at any time when there is visible turbidity suggesting bacterial growth. The reliability of mothers' information about previous antimicrobial treatment will be checked by determining the antibacterial activity of serum samples against highly susceptible bacterial strains, such as Oxford staphylococcus strain in comparison with a control serum.

The blood agar and chocolate agar plates will be examined after 24 and 48 hours of incubation. Typical colonies of Beta-haemolytic streptococci with clear zones of haemolysis will be looked for with the aid of a hand lens. Grouping will be done by latex agglutination (Streptex and Wellcome).

Alpha-haemolytic colonies isolated on chocolate agar at 37°C in 5-10% CO₂ will be differentiated into Streptococcus pneumoniae and Streptococcus viridans by Quellung test and optochin sensitivity test. Final identification will be done by coagglutination test (Phaedebact).

Staphylococcus aureus grows on blood agar and chocolate agar. Portions of suspected colonies will be smeared on slides for gram-stain. If the stain shows clusters of gram positive cocci the colony will be tested for coagulase production. It will also be confirmed by the slide agglutination method (Biomerieux).

Haemophilus influenzae grows on chocolate agar and its identity will be confirmed by subculturing the suspected colonies on blood agar plate and placing an X and V disc over the inoculated area. H. influenzae colonies grow heavily around the disc and more lightly with increasing distance from it. Colonies will be tested with type b H. influenzae anti-serum (Wellcome) and the organisms will be reported as type b or untypable.

Klebsiella pneumoniae, E. coli and other enteric bacteria may overgrow other bacteria if incubation is prolonged. Any suspect colony will be examined by gram's staining. They are best detected on MacCorkey agar which inhibits the growth of pneumococci, streptococci and H. influenzae.

All the bacterial pathogens isolated will be tested in vitro for susceptibility to the commonly used antimicrobials: penicillin, ampicillin, erythromycin, chloramphenicol and cotrimoxazole.

Nasopharyngeal, urine and serum specimens from children with lung infection will be used for rapid bacteriological techniques. Diagnosis of pneumococci will be attempted by coagglutination and counterimmunoelectrophoresis. Detection of H influenzae type b will be done by a new latex agglutination test.

An aliquot of nasopharyngeal secretions will be tested by immunofluorescence for Bordetella pertussis.

8.2 Virology

8.2.1 Isolation of viruses

Specimens in viral transport medium will be treated as follows:

0.1 ml amount of specimen will be inoculated into:

- the amnion of 4 fertile hen's eggs 10-12 days old,
- 2 tubes containing cultures of primary monkey kidney monolayers (PMK),
- 2 tubes containing culture of monolayers HeLa or Hep-2 cells,
- 2 tubes containing cultures of human lung fibroblast (e.g. MRC 5),
- 2 tubes containing MDCK with trypsin.

All these tubes of cultures will be incubated at 33-35°C for 48-72 hours. For PMK and MRC5 (or equivalent) the incubation tubes will be placed in a roller-drum at 32°C.

The inoculated eggs will be incubated at 33°C for 72 hours. These eggs will be used for the isolation of myxoviruses (influenza). The eggs will be chilled overnight after incubation, and the allantoic and amniotic fluids will be harvested separately. A spot test for haemagglutinin will be performed on each egg and, if it is positive, the influenza type will be identified by the haemagglutination inhibition test. If the spot test is negative, passage will be done in eggs.

The monkey kidney cultures will be examined daily for a cytopathic effect. The haemadsorption test with guinea pig erythrocytes will be done on the fifth day (or earlier if indicated by cytopathic effect). If the test is positive, passage into 4 monkey kidney cultures and identification of the virus will be done by haemadsorption inhibition test or immunofluorescence with specific antisera to influenza A, B, parainfluenza viruses and SV-5; for the haemadsorption inhibition test antisera having been treated with receptor destroying enzyme will be used. Negative tubes will be incubated for another 10 days. Before discarding them as negative, a haemadsorption test will be repeated.

If the haemadsorption test is negative, and the CPE is positive, an acid stability test will be performed to determine whether the agent is an enterovirus. Rhinovirus will be inactivated if incubated at 37°C for 3-4 hours at pH₃. If the agent is acid stable, then a neutralization test will be done using specific antisera for the identification of enteroviruses.

The HeLa or Hep 2 cultures will be examined daily for cytopathic effect. If grape like clusters are observed, identification of adenovirus will be done using the FA technique.

Enteroviruses will be recognized by observing the CPE on Hep-2 and HeLa cell culture. After doing a pH stability test to exclude rhinovirus, the enteroviruses will be identified by a microneutralization test.

Respiratory syncytial virus grows well on HeLa and Hep-2 cell cultures producing giant cell and syncytial formation. Definitive identification will be obtained by immunofluorescence.

8.2.2 Immunofluorescence

An immunofluorescence technique will be applied to detect RSV, influenza A and B, parainfluenza 1 and 3, adenovirus, herpes virus and measles virus.

Preparation of slides

In the laboratory, the mucus collector will be centrifuged at 350g at 4°C for ten minutes. The supernatant will be transferred to a small volume of transport medium to yield a final volume of at least 2 ml for virus isolation (see Virus Isolation). The pellet will be suspended in a slowly increasing volume of PBS (up to 3-4 ml) by a gentle pipetting with a wide-bore Pasteur pipette. When the fragments of mucus have been broken up, a further 4 ml of PBS will be added. Large fragments still present will be discarded. The tube will be again centrifuged at 350g for ten minutes and the supernatant discarded. The deposit will be resuspended in a volume of PBS sufficient to make the number of smears required. Drops of the cell suspension will be then placed on the etched areas of glass slides. Two ten spot slides will be prepared to allow for reference checking and controls. One to two drops of the cell suspension remaining will be tested for B.pertussis (see above, Bacterial Procedures).

Immunofluorescence staining

Specimens will be stained by the indirect immunofluorescence technique. An appropriate number of glass slides, each with ten cell smears on etched areas, will be put into a humid chamber and the different virus specific antisera, diluted in PBS as required, will be applied each to a separate smear. After incubation for 30 minutes at +37°C, the slides will be washed three times in PBS (10 minutes each) and dried. The appropriate FITC conjugated antispecies serum, diluted to its optimum in PBS containing naphthalene black (1mg/ml) as a counterstain, will then be dropped on to each smear. After incubation for a further 30 minutes at +37°C, the slides will be washed again, and given a final rinse in distilled water. When dry, the slides will be examined directly under oil immersion or will be mounted and examined without oil. Only intracellular fluorescence will be considered significant.

8.2.3 Serology

The first specimen of serum will not be investigated immediately, but will be stored until the convalescent specimen is obtained. A preliminary screening test by complement fixation will be carried out on the convalescent specimens. For this, a single dilution of 1 : 16 will be tested against all relevant antigens. Sera giving more than 50% fixation with an antigen will be titrated against that antigen, in parallel with the corresponding acute stage specimen.

9. Quality control

9.1 Bacteriology

The principal investigator will establish a programme for regular testing of media and reagents. A random sample of positive cultures will be stored frozen at -20°C for eventual confirmation by a reference laboratory.

9.2 Virology

The virus isolation and the serology will act as reference controls for the immunofluorescence staining. All the reagents used will have been tested for non-specific reaction with virus-infected and uninfected cells.

All positive virus isolates will be stored frozen at -70°C for eventual confirmation by a reference laboratory.

10. Reporting and analysis

A quarterly report will be prepared of the work done, the results obtained and the achieving of the operational targets. The difficulties encountered will be analysed and the necessary measures to overcome them will be recommended. The main data will be tabulated manually. However, the processing of data for the final analysis will be done by computer at the end of the study.

After the first semester of operations the protocol will be reviewed to introduce, if advisable, some simplification in the laboratory procedures which could make it possible to increase the number of cases investigated during the winter season.

The report will include a description of the personal and clinical characteristics of both patients and controls, and the pathogens isolated from them.

A statistical analysis will be made comparing the pathogens isolated from the cases and the controls. At the end of the study the relationship between pathogens and potential risk factors will be analysed. The etiological findings will be analysed by the method used (conventional or rapid) and by the different specimens, to estimate the relative value of each. If the bacteriological rapid techniques are valid in children, cases with positive blood culture should be positive for the same organisms by CIE or COA or latex agglutination. If they are more sensitive, cases clinically compatible with a bacterial infection may be negative by blood culture and positive by rapid techniques. Cases adequately explained by the results of virological testing should usually be negative by CIE, COA or latex agglutination.

Geneva, 4 April 1984

ALL ATTENDANCES AT THE PEDIATRIC UNIT
OF CHILDREN BELOW 6 YEARS OF AGE WITH ARI
Weekly Report

Institution

Week from to Year
day / month day / month

	No. of children with ARI (1)		No. of children with lung infection (2)		No. of children with other respiratory infection (3)	
	Total	Hospitalized	Total	Hospitalized	Total	Hospitalized
Saturday						
Sunday						
Monday						
Tuesday						
Wednesday						
Thursday						
Friday						

(1) ARI: any illness of onset within the past week in which the child shows signs and symptoms originating from infection in the respiratory tract, (with or without previous treatment): blocked or runny nose, sore or red throat, exudates in the throat, hoarseness, stridor, wheezing, cough, rapid breathing or respiratory distress.

(2) Lung infection: pneumonia, bronchopneumonia and empyema.

(3) Other respiratory infection: bronchiolitis, tracheobronchitis, acute laryngitis, pharyngotonsillitis and rhinitis.

D. Present Illness History

	YES	NO	NOT KNOWN	
1. Fever	<input type="checkbox"/>
2. Running or blocked nose	<input type="checkbox"/>
3. Ear discharge	<input type="checkbox"/>
4. Ear pain	<input type="checkbox"/>
5. Difficulty in swallowing	<input type="checkbox"/>
6. Cough	<input type="checkbox"/>
7. Hoarseness	<input type="checkbox"/>
8. Stridor	<input type="checkbox"/>
9. Wheezing	<input type="checkbox"/>
10. Rapid breathing	<input type="checkbox"/>
11. Vomiting	<input type="checkbox"/>
12. Diarrhoea	<input type="checkbox"/>
13. For how many days has the child been ill?				<input type="checkbox"/>
14. Has the child received any medication for this illness in the past week?	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Not known <input type="checkbox"/>	
15. If YES, what medication			

E. Physical Examination

1. Weight Kg grams
2. Height cm
3. Temperature, axillary °C
4. Heart rate/minute
5. Respiratory rate/minute
6. Nasal Discharge None Clear Purulent Blood stained

7. Tonsils and Pharynx

Not examined Normal Red Purulent exudates

8. Ear Drum

Not examined Normal Red Discharge

9. Cervical Glands

Not examined Not enlarged

Enlarged, not tender Enlarged, tender

10. General appearance Alert Drowsy Comatose

Irritability or restlessness: Yes No

11. Skin rash None Morbilliform Vesicular Other

	<u>Present</u>	<u>Absent</u>	<u>Not Examined</u>	
12. Hoarseness	<input type="checkbox"/>
13. Stridor	<input type="checkbox"/>
14. Wheezing (without stethoscope)	<input type="checkbox"/>
15. Grunting	<input type="checkbox"/>
16. Nasal flaring	<input type="checkbox"/>
17. Chest indrawing	<input type="checkbox"/>
18. Cyanosis	<input type="checkbox"/>

Abnormal breath sounds
(with stethoscope)

19. Ronchi	<input type="checkbox"/>
20. Crepitation	<input type="checkbox"/>
21. Wheezing	<input type="checkbox"/>

F. Chest X-ray Examination

Not done Done

If done: Normal Consolidation lobar/segmental

Effusion Consolidation lobular/patchy

Hyperinflation Hilar adenopathy

Other

.....

G. Clinical Diagnosis and Outcome

Main diagnosis

Others

.....

Did the child receive an antimicrobial? Yes No

IF YES, which? For how many days?

..... For how many days?

..... For how many days?

Hospitalization Yes No

If hospitalized, number of days of hospitalization

Outcome: Discharge with improvement

Discharge without improvement

Absconded after improvement

Absconded before improvement

Death

Other

A. Control Identification

Institution Control No.

Name Date

Age: Years Months Date of Birth Sex M F

Father's Name

Address

.....

Matched with case number

Name of the case

B. Household Data

Location of house: Urban Periurban Rural

Total number of rooms Number of separate bedrooms

Number of persons living in the house

Number of children living in the house age 0-4 age 5-14

Occupation of head of house

Education of father: Illiterate Less than 2 years at school

2-5 years More than 5 years Unknown

C. Past Medical History

Feeding during first months of life: Breast Bottle Mixed

	BCG	D.P.T.				POLIO			MEASLES
		1st	2nd	3rd	4th	1st	2nd	3rd	
YES									
NO									
UNKNOWN									

Has the child been hospitalized for any acute respiratory disease during the past year? Yes No Unknown

If YES, give relevant information

.....

D. Reason for attending the institution

Immunization

Surgical problem

Accident or emergency

Other (specify)

Observations

.....

.....

LABORATORY FORM

I. Name of child Case/Control No. [][][][][]

Institution [] Laboratory No. [][][][][]

Case of Lung Infection [] Other Respiratory Infection [] Control []

		<u>Yes</u>	<u>No</u>	<u>Date</u>
<u>Specimens</u>	Throat swab
	Pooled throat/nose swab
	Nasopharyngeal aspirates
	Blood for culture
	Blood for serology, 1st
	Blood for serology, 2nd
	Pleural aspirates
	Urine
<u>Other</u> (specify)

II. Rapid Bacteriological Techniques

	<u>Sample</u>	<u>Result</u>
Counterimmunoelectrophoresis

Coagglutination

Latex agglutination

Immunofluorescence

III. Bacteriology

Result	Gram Stain				Culture				
	TS	NPA	PA	Other	TS	NPA	Bl.	PA	Other
Not done									
No growth (or comments only)									
<u>Strept. pneumoniae</u>									
<u>Strept. β-haemolyticus</u>									
<u>Staph. aureus</u>									
<u>Haem. influenzae</u>									
<u>Klebs. pneumoniae</u>									
<u>Esch. coli</u>									
Other									
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Observations

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IV. Virology

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Result	Virus Isolation		I.F.	Other
	TS/NS	NPA		
Not done				
Negative				
RSV				
Influenza A				
Influenza B				
Parainfluenza 1				
Parainfluenza 2				
Parainfluenza 3				
Adenovirus				
Rhinovirus				
Enterovirus				
Herpes				
Cytomegalovirus				
Measles				
Other				
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Observations

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V. Serology

Result	Screening test Convalescent Serum	Acute Serum		Convalescent Serum	
		CF	HI	CF	HI
Not done					
RSV					
Influenza A					
Influenza B					
Parainfluenza 1					
Parainfluenza 2					
Parainfluenza 3					
Adenovirus					
Rhinovirus					
Herpex					
Cytomegalovirus					
Measles					
Mycoplasma pneumoniae					
Other					
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Observations

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Work load of main activities of the Study

Activity	1st year	2nd year	Total
No. of children to be investigated:			
- Cases of lung infection	266	266	532
- Other respiratory infections	534	534	1 068
- Controls	266	266	532
No. of forms to be filled in:			
- Case Clinical Record	800	800	1 600
- Control Record	266	266	532
- Laboratory Form	1 066	1 066	2 132
Bacteriological Investigations:			
<u>Gram staining:</u>			
- Throat swabs	1 066	1 066	2 132
- Nasoph. aspirates (50%)	400	400	800
- Pleural aspirates	30	30	30
Sub total:	1 496	1 496	1 496

Activity	1st year	2nd year	Total
<u>Culture:</u>			
- Throat swabs			
Blood agar	2 130	2 130	4 260
Chocolate agar	1 065	1 065	2 130
Subculture (20%)	640	640	1 280
- Nasopharygeal aspirates (50% of patients)			
Blood agar	800	800	1 600
Chocolate agar	400	400	800
Subculture (20%)	240	240	480
- Blood			
Culture	532	532	1 064
Subculture (10%)	27	27	54
- Pleural aspirates			
Blood agar	60	60	120
Chocolate agar	30	30	60
Subculture	18	18	36
Sub total:	5 942	5 942	11 884

Activity	1st year	2nd year	Total
<u>Antibiotic Susceptibility Tests</u>			
- Throat swab cultures (70% of patients and controls) x 2	1 492	1 492	2 984
- Nasopharyngeal asp. cultures (40% of patients) x 2	800	800	1 600
- Blood cultures (15% of lung infections) x 2	80	80	160
- Pleural aspirates	50	50	100
Sub total:	2 422	2 422	4 844

Rapid bacteriological techniques

CIE: Serum	266	266	532
Urine	266	266	532
NPA	133	133	266
COA: Serum	266	266	532
Urine	266	266	532
NPA	133	133	133
IF: NPA	400	400	800

Activity	1st year	2nd year	Total
<u>Virology</u>			
<u>Pooled throat/nose swabs (50% of patients plus controls)</u> <u>and nasopharyngeal aspirates (50% of patients)</u>			
- Egg inoculation (1 066 x 4)	4 264	4 264	8 328
Passages (20%) x 4	852	852	1 704
<u>Cell Lines</u>			
Monkey kidney monolayers	2 132	2 132	4 264
Passages (20%) x 8	1 704	1 704	3 408
Monolayers of Hela or Hep ²	2 132	2 132	4 264
Passages (20%) x 4	852	852	1 704
Culture of human lung fibroblasts (MRC 5)	2 132	2 132	4 264
Passages (20%) x 4	852	852	1 704
Monolayers of MDCK	2 132	2 132	4 264
Passages (20%) x 4	852	852	1 704
<u>Immunofluorescence (50% of patients)</u>	400	400	800
<u>Serological Investigations (cases of lung infection)</u>			
- Complement Fixation Test (CF)			
Screening Test	266	266	532
Paired tests for antibody titles	532	532	1 064
- Haemoagglutination Inhibition Test (HI)			
Screening test	266	266	532
Paired tests for antibody titles	266	266	532