Revised copy = 22.10.81

Attachment 1. Date

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Leif Gothefors, Dr. Najaf S. Khalid — Trainee Investigator (if any) ____

Application No. 81-38 — Supporting Agency (if Non-ICDDR,B) ____

Title of Study Respiratory Infections as complications to diarrhoea in Hospital patients

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects Yes (No)
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes (No)
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used Yes No NA
   (c) Physical risks Yes (No)
   (d) Sensitive questions Yes (No)
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure NA Yes No
5. Will signed consent form be required:
   (a) From subjects (Yes) No
   (b) From parent or guardian (if subjects are minors) (Yes) No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ✓ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any change involving the rights and welfare of subjects before making such change.

Najaf S. Khalid (for Dr. Leif Gothefors) ____

Principal Investigator Trainee

SECTION I - RESEARCH PROTOCOL

1. Title: RESPIRATORY INFECTIONS AS COMPLICATION TO DIARRHOEA IN HOSPITAL PATIENTS

2. Principal Investigators: Dr. L. Gothefors and Dr. Nigar S. Shahid

   Co-Investigators: Dr. A.N. Alam and Dr. Imdadul Huq

3. Consultant/Advisor: Dr. W.B. Greenough III

4. Starting Date: November 1981

5. Completion Date: December 1982

6. Total Direct Cost: US$ ~~11750~~ 38810

7. Scientific Program Head:

   This protocol has been approved by the Host Defence Working Group.

   Signature of the Scientific Program Head: _____

   Date: 14/9/1981

8. Abstract Summary:

This study has the objective to provide data needed to plan a program for hospital - and later outpatient - care by which the impact of acute respiratory illness can be reduced.

Subject studied will be patients at the hospital as well as expatriates coming to the Traveller's Clinic with respiratory symtoms. Nasopharangeal secretions will be used to demonstrate viral and pertussis antigens with rapid immunefluorescens methods. Using classical bacteriological techniques, and if possible also counter immuno electro-phoresis,

we will examine "tracheal swabs", throat swabs, blood cultures and, when possible, post mortem lung aspirates for bacterial pathogens. Serology on paired sera will be done for some viral antigens, mycoplasma and some bacterial antigens.

8. Reveiws:

a. Research Involving Human Subject: _____

b. Research Review Committee: _____

c. Director: _____

d. B M R C: _____

e. Controller / Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: The objective is to provide the information needed to plan a programme for hospital and later outpatient care by which the mortality in acute respiratory infections can be reduced.

Although we suspect that the organisms causing these diseases are similar to those found in other parts of the world, there may be important deviations. Thus detailed studies of bacteria and viruses present are required for an intelligent approach to prevention and treatment.

2. Background:

2.1 Art and size of the problem

There has been a major progress in the treatment of especially watery diarrhoea and our hospital patients are now admitted due to more or less severe complications to their intestinal problems. A high number of the infants in our hospital have, for example, symptoms and signs of respiratory tract infections. In a retrospective analysis of patients age <3 admitted to ICDDR,B hospital in 1979 (n=1359) there were 732 pneumonias (54%) and of these children 5.3% died. (Dr. A.R. Samadi, pers. comm.).

If we look on the morbidity outside the hospital, a survey made in Bangladesh on outpatients during 1976 showed:

	No of cases 1st and 2nd visits	% of total (1198)
1. Intestinal worms, diarrhoea, enteritis, dysentery, typhoid and paratyphoid fevers	228	19.0
2. Scabies, with or without secondary infection	141	12.6
3. Diseases of respiratory tract (excluding tuberculosis) i.e., sore throat, tonsillitis, bronchitis, asthma, pheumonia	149	12.4
4. Dyspepsia, gastritis, pains, peptic ulcer	142	11.9
5. Anaemia, protein calorie malnutrition, avitaminosis, goitre etc.,.,	98	8.2
	768	64.1

(SEA/PHC/Fin.Met./CP/2 - June 1980)
(Meeting on Financing of PHC Programme in Asia, SEARO, New Delhi, 9-13 July 1979)

Thus respiratory diseases - in particular acute respiratory infections - constitute a major segment of diseases in tropical and developing countries.

Looking at mortality data from Matlab, the impact of respiratory diseases is even more obvious.

Number of deaths per 1000 population (all age groups) according to cause in Matlab, Bangladesh (1975-1977) (1)

Causes of death	Deaths	
	Number	%
1. Diarrhoea: watery	419	3.2
dysentery	2011	15.6
2. Tetanus	1276	9.9
3. Fever	1193	9.2
4. Respiratory	1041	8.1
5. Dropsy (hydrops)	891	6.9
6. Measles	607	4.7
7. Old age	607	4.7
8. Drowning	329	2.6

Diarrhoea still heads the list, of course associated with dirt, flies, contaminated water supply and faulty excreta disposal.

Respiratory infection in all its varieties is also a common cause of death, being related to the ill-ventilated smoky housing and the cross-infection resulting from overcrowding.

In their five year plan for 1980-1985 the Government of Bangladesh has stressed the importance of controlling the communicable diseases, where the infections of the respiratory tract take a major part.

Disease pattern and target setting according to the Bangladesh five-year plan

Problems	Index	Level(1980)	Target (1980 - 85)
1. Smallpox	Incidence	-	-
2. Cholera	Mortality	3.5 / 1000	Reduction by 50%
3. Diarrhoea/ Dysentery	Prevalence	16.5 / 1000	Reduction by 15%
4. Tuberculosis	Prevalence (>10 years age)	7 / 1000	a. 100% immunization b. case detection and treatment of 100.000 cases
5. Malaria	Annual Parasite Incidence	0.1 / 2.5	Reduction to 01
6. Measles	Mortality Incidence	3 / 1000 36 / 1000	Reduction by 25% and treatmen
7. Worm infestation	Prevalence (<15 years)	80% of children	Deworming every 6 months
8. Tetanus	Incidence (<1 year) Mortality <5 years	271 / 1000 8.6 / 1000	- -
9. Diphtheria	Mortality	0.4 / 1000	-
10. Pertussis	Incidence	18.2 / 1000	-
11. Poliomyelitis	Prevalence	0.8 / 1000	-
12. Leprosy	Prevalence	2.6 / 1000	10-40% case treatment
13. Pneumonia	Mortality rate < 5 years,	5.3 / 1000	50% reduction

Many of the known causes of death are no doubt preventable by fairly simple means. Clearly, the immunization of pregnant mothers with tetanus toxoid and the wide spread use of oral rehydration therapy would reduce mortality.

Beyond that respiratory diseases seem to be an area where intervention could be important. Candidate interventions then would be immunization against tuberculosis, measles, pertussis and diphtheria as well as early and correct treatment of the commonest bacterial infections. In this context interestingly good results have been reported from India (2), where village level workers took the responsibility for treatment of lower respiratory tract infections with penicillin.

Discussing the different causes of disease and death we must keep in mind that it is, in the majority of cases, incorrect and impossible to assign a single cause to a child death in a tropical country. Jeliffe (3) gave his view of the situation, "Death is due to an accumulation of disease rather than to any simple entity. The immature, anaemic baby living in overcrowded unhygienic surroundings, becomes relatively malnourished in the second semester of life. His anaemia and nutrition deteriorate still further as a result of persistent malaria, leaving an attack of broncho-pneumonia or gastroenteritis to add the last straw to his pathological burden." This statement holds true also now, thirty years later.

2.2 Respiratory infections in a western society

More than 70% of infections of the upper respiratory tract (URI) are caused by virus and therefore not curable with antibiotics. Thus the major problem with URI is: when to refrain from antibiotics or postpone their use - as such therapy is useless - and which drug to use when treatment is motivated.

Careful Swedish studies of the etiology and treatment of upper respiratory tract infections are quoted in most recent literature in this field (4.). In acute otitis media the organisms found were: pneumococci 51%, Haemophilus influenzae 15%, Branhamella catharralis 9%, streptococci 5%; no growth 20%. The same organisms (plus Staph. aureus) are found in acute sinusitis only with some changes in the percentage. Tonsillitis is almose exclusively caused by ß-hemolytic streptococci.

Knowing the resistance pattern for these organisms, the drug of choice is penicillin-V, preferably as potassium-salt. Most H. Influenzae-strains are sensitive to a somewhat higher dose of pc-V. The lowest effective dose of pc-V (=the amount of antibiotics which is needed in order to give - in the infectionsite itself - a concentration of antibiotics exceeding MIC for the actual micro organism) is for URI 25 mg/kg bw which should be given twice a day. As an alternative, Ampicillin can be given in a dose of 50 mg/kg bw/24 hours.

The etiology of pneumonia (LRI) is much more variable between different studies. In a recent Swedish study (5) of hospitalized patients (age 11-92) using counter current electrophoresis, ELISA, immunofluorescens and serological methods as well as cultures, 70 of the 130 pateints had pneumococcal pneumonia, 12 mykoplasma, 6 influenza A and a few S. aurens, ß-hemolytic streptococci, RS-virus and Chlamydia. Some had mixed infections and in 36 out of the 130 (28%) no etiology could be proven.

The pneumococcus is supposed to be even more dominating in an outpatient population and again penicillin-V (or in severe cases penicillin-G i.v.) is the drug of choice. In hospitalized cases, other etiologies must be thought of and treated accordingly.

2.3 Respiratory tract infections in the developing world

In general it is believed that the diseases of this group are similar to those seen in developed countries of temperate zones. However, the form in which the disease presents, the course it follows, and the problems of management and treatment are constantly modified by several factors. Delay in seeking medical advice due to ignorance, apathy, poverty or the inaccessibility of medical services is one of the most important. Other factors are the high incidence of malnutrition and the frequency of coincident infections with parasites and other pathogenic organisms.

There are few studies of the aetiology of respiratory infection in tropical countries. Serological surveys (6) of acute respiratory infection in nine different countries of Africa, Asia, South America and the Caribbean suggest that RS, parainfluenza and adenoviruses caused illness with the same frequency as in Britain and North America. Mimica et al (7), reported a bacteriological study of pneumonia in Chile: cultures from needle aspiration of the lung yielded a pathogen in 44% of the 530 cases studied. The range of pathogens were similar to that in Europe and North America, except that S. aureus was found in 60% of positive cases in infants.

Some light on the aetiology of pneumonia has been thrown by a small but comprehensive study of acute non-tuberculous lower respiratory tract infections in children under 4 years of age in Calcutta (8). Evidence of a virus infection was found in 44% of children studied. Haemophilus influenzae was recovered from 25 to 30% of cases by percutaneous lung aspiration. The same workers cultured Mycoplasma pneumoniae from the nasopharygeal secretions of a child with pneumonitis.

An example of different etiologies in different parts of the world can be seen in the case of sore throat (9). In rural areas in West Africa, streptococcal throat infection is uncommon, and acute tonsilitis is rarely seen. However, this is not true of all hot areas, and in many acute rheumatic fever is a major problem. No satisfactory explanation has been given for this difference on incidence of streptococcal disease. It is certainly not purely climatic, as there are regions such as Singapore, with a similar climate to West Africa, where streptococcal infection is important and its complications, rheumatic fever and nephritis, are frequently met.

The spectrum of causative organisms thus seems fairly uniform to that of a developed country and indicate the relevance of Western experience to the tropical scence. Nevertheless, it seems likely that the relative importance of viruses and bacteria as cause of pneumonia will be different in tropical and temperate climate, bacteria still playing a rather larger role in the former than is now the case in the west. Still, as the large majority of acute respiratory infections in children are of viral origin, doubt must be raised about the near general advocacy of "covering" all such infections with an antibiotic. In the context of the low national income of the third world such use of antibiotics must be still more open to question. Combined clinical and microbiological studies of representative samples of childhood diseases in the tropics are clearly needed.

2.4 Immunity and malnutrition

The thymus is extremely sensitive to malnutrition. In children and adults with PCM there occurs variable reductions of both cellular and humoral immunity, deficiencies of phagocytosis, complement defects and decrease of secretory IgA antibodies.

In the malnourished child, studied by Chandra (10), phytohemagglutinin responses were deficient, but this deficiency was most profound when analysis of cell responses was carried out in the patient's own plasma; it was almost eliminated when the studies were carried out in the plasma of the well nourished child. T-cell numbers were reduced and their function was inadequate in a number of test systems. Measles vaccination induced poor IgA antibody responses in the nasopharyngeal secretion of malnourished children. The levels of many individual complement components were reduced by malnutrition, but children with malnutrition and infection had the lowest levels. Opsonization of fungi was found also to be defective in malnourished children.

Gilman and Brown (11) have shown that children living in Bangladesh have widely different frequencies of gram-negative colonization of the throat. Malnourished children have greater numbers of gram-negative isolates than a similar out-patient control group. However, this was a limited study on urban children and only throat swab was used for the isolation of colonizing micro organisms.

Other investigators like Keusch (12) have found that mild to moderate PCM did not inhibit responses to acute inflammation; CRP, IgM, IgA, C3 etc., were normal. Children with acute, severe amlnutrition however showed decrease in complement activation and opsonins.

2.5 Infections in the immuno compromised host

The term "immuno compromised" as used here includes individuals who have disorders of the different immune functions. Usually they are patients with severe maignancy, chronic kidney, liver and lung diseases or patients receiving heavy immunosuppresive or cytotoxic drug therapy. We could, however, also include the severely malnourished child as described above.

From the literature one can find some indications that one type of immune deficiency is correlated with certain pathogens (see Table). It must, however, be remembered that such a schematic Table is more didactic than real - there is almost always a question of complex immundefects.

Infections in immuno compromised patients

Predominating micro-organisms

Disorders of phagocyte functions	Defects in humoral immune functions	Defects in cell-medicated immune functions
Staphylococcus aureus	Streptococcus pneumoniae	Herpes simplex, herpes Zoster, CMV
Gram-negative enteric-bacilli	Haemophilus influenzae	Gram-negative enteric bacilli
Candida species	Neisseria meningitidis	Mycobacteria
	Pseudomonas sp.	
	Pneumocystis carinii	Listeria
		Candida sp.

It is seen that Gram-negative enteric infections are relatively more common in this group of infections, especially in patients with T-cell defects or disorders of phagocyte functions.

In our hospital it has been claimed as a known fact that airways of malnourished children often are colonized - and thus later infected - with enteric organisms. Unpublished studies from the Nutrition unit are quoted in support. From that it is concluded that when pneumonia is suspected, a broad spectrum bactericidal regimen has to be instituted.

Recent therapeutic regimens for immuno-compromised patients consist of different combinations of an aminoglycoside with a beta-lactam antibiotic from the penicillin - or cephalosporingroup, and thus there may be a good rationale for the regimen suggested in our hospital.

3. Rationale (in general)

We propose to document the bacterial and viral aetiology of various classes of acute respiratory infection in patients admitted to the hospital in Dacca, Bangladesh and in expatriates treated in the Traveller's Clinic. Based on these data we will later extend the study to involve a rural setting like villages in the Matlab area.

These studies are proposed for several reasons. First, lower respiratory tract infections is a major problem in the hospital with high mortality in spite of treatment with massive doses of broad spectrum antibiotics. Proper knowledge of the relative frequency of viral etiologies and of the sensitivity pattern of the organisms found may give a sound basis for an optimal use of antibiotics.

Secondly, there is a close relation between diarrhoea, respiratory tract infections and malnutrition when causing high morbidity and mortality in a developing country.

In diarrhoea there is now a spreading knowledge among mothers to use ORS and its use has already led to a reduction of death rate for diarrhoea. In contrast the vast majority of children with respiratory infection still receive no treatment other than in the home or in what facilities there are available in their village. For this reason local availability of

antibiotics and personnel with a relatively simple understanding of their use appears to be the priority in reducing mortality from pneumonia. Such a development must be based on the knowledge of etiologic factors and, in case of bacteria, their sensivity to antibiotics.

Thirdly, some new techniques can be established at ICDDR,B through collaboration with other institutions. Examples are immunofluorescence for rapid diagnosis of viral infection, and counter current electrophoresis.

4. Rationale for the Choice of bacterial and viral laboratory methods

4.1 Bacterial: The bacteria most likely to cause severe respiratory disease in infants and children are:

- Streptococcus pneumoniae
- Haemophilus influenzae
- Bordetella pertussis
- Streptococcus, group A and B (and in some cases, group G)
- Staphylococcus aureus
- Mycoplasma pneumoniae

Because many patients will have previously been given antibiotics, leading to the secondary invasion of other bacteria, the following organisms are also included in the study:

- Pseudomonsa aeuroginosa and other pseudomonads
- Klebsiella pneumoniae
- Escherichia coli
- Proteus
- other enteric bacteria

Conventional microscopy and culture techniques are the methods of choice for the identification of these organisms. Immunofluorescence as well as ordinary culture can be used for the detection of B. pertussis and B. parapertussis.

Antigen detection (H. influenzae, pneumococci) by countercurrent immunoelectrophoresis (and/or ELISA) will be further investigated before it is adopted as a routine test.

Serological diagnosis using acute and convalescent phase serum samples may be used for H. influenzae, pneumococci and mycoplasma and L. pneumophilia.

4.2 Viral:

Immunofluorescence has been shown to be a sensitive method for indicating the presence of viral antigens in epithelial cells of the respiratory tract. It has the advantages of speed and simplicity over tissue culture techniques but it does not yield virus isolates for further study nor allow the distinction of serotypes in some virus groups. At present, immunofluorescence reagents are available from commercial sources for several of the more important respiratory viruses. These are respiratory syncytial virus, para-influenza virus types 1 and 3, influenza A, and adenovirus. Enterovirus and rhinovirus can at present only be recognised by isolation in tissue culture.

Serological tests may be used but have their limitations: one is the difficulty to obtain paired samples in children, another is that the antibody response may develop only poorly in these infants.

B. SPECIFIC AIMS

As a result of this study, it should be possible to describe the following:

1. The size of the problem of respiratory disease in association with diarrhoeal disease as presented to the ICDDR,B hospital.
2. Symptoms and signs to be elicited by those dealing with these patients.
3. The way the patients in the various categories are treated and whether and how soon they recover.
4. The apparent aetiology of various classes of acute respiratory infection.

C. METHODS OF PROCEDURE

Patients:

1. Patients admitted to the hospital who have been laid down with respiratory illness with or without diarrhoea for less than five days including prominent symptoms from the lower tract such as nasal flaring, intercostal indrawings, cough, dyspnoea, cyanosis, fever and RR > 40

(> 1 year) and > 50 (<1 year). Radiological confirmation will subsequently be made in each case during the course of illness. All admitted cases with respiratory illness above 10 years of age (expectedly 1-2 cases per day) and maximum 8 consecutive cases below 10 years per day will be included in the study. (Total number = 200). The virology

If necessary, a small volume (not more than 0.5 ml) of physiological saline may be sucked through the tube to collect the mucus lining the tube. Without delay, the specimen in the collector is put on wet ice and transported to the laboratory. Delays over one hour may cause damage to the infected cells and prevent the identification of specific viral immunofluorescence.

1.2 Serum: 200 µl blood will be taken initially and at the follow up visit. The blood will be diluted in 1.8 ml of PBS and divided in 4 aliquots. If, venous blood is drawn for clinical reasons, an alternative would be to take 1 ml of that blood.

2. Immunofluorescence

2.1 Preparation of slides

In the laboratory the mucus collector is centrifuged at 350 g for 10 minutes at +4°C. The pellet is suspended in a slowly-increasing

laboratory cannot handle more than ten samples a day at the present time.

Patients who have had earlier antibiotic therapy will be excluded.

Records: Clinical documentation will be made for the hospital patients (Appendix 1) and follow-up will be done 10-14 days later, if necessary in their homes.

Viral studies:

1. Specimen collection

1.1 Nasopharyngeal secretions -

For the demonstration of virus or viral antigens by virus isolation or immunofluorescence, the quality of the specimen is of fundamental importance. The best specimen is a nasopharyngeal secretion obtained by suction into a mucus collector.

A sterile polythylene feeding tube (size 8) attached to one of the two outlets of a sterile plastic mucus collector. The other outlet is attached to suction pump. The tube is inserted in one nostril of the patient and passed down into the nasopharynx. A negative pressure is then applied (not exceeding 2 kg/cm^2) and the aspirated secretion passed into the collector.

2.2 Staining

Specimens are stained by the indirect immunofluorescence technique. An appropriate number of glass slides, each with two cell smears on etched areas, are put into a humid chamber and the different virus specific antisera, are applied, each to one smear. After incubation for 30 min at +37°C the slides are washed three times in PBS (10 min each) and dried. The appropriate FITC conjugated serum, is then dropped on all smears. After incubation for a further 30 min at +37°C, the slides are again washed, counterstained with Evan's blue and given a final rinse in distilled water. When dry, the slides may be examined directly under oil immersion or may be mounted and examined without oil.

To ascertain that the reagents are working, positive controls should be run in parallel with the tests once a week. These controls will be virus-infected tissue culture smears which could either be prepared at the local laboratory or be provided from the reference laboratory.

2.3 Interpretation of results: certain criteria must be used to classify the specimen as positive by the immunofluorescence method:

1. Only intracellular fluorescence is considered significant.
2. The appearance and localisation of fluorescence must be characteristic of the particular virus.
3. Fluorescence should be apple green.

3. Reagents

The laboratories will be provided with suitable reagents: virus specific antisera and appropriate FITC conjugated antispecies sera. A reliable

volume of PBS (up to 3-4 ml)by gentle pipetting with a wide-bore Pasteur pipette. When the fragments of mucus have been broken up, a further 4 ml of PBS are added. Large fragments still present may be discarded. The tube is again centrifuged at 350 g for 10 minutes and the supernatant cultured for B.pertussis. The deposit is resuspended in a volume of PBS sufficient to make the number of smears required to test for the different immunofluorescent antigens. Drops of the cell suspension are then placed on the etched areas of glass slides (two drops per slide). Of the remaining cell 1-2 drops may be treated as recommended later for tests for B. pertussis.

result is obtained only if the antisera react exclusively with their homologous antigen. All reagents used must therefore be tested for non-specific reactions on virus-infected cells and on clinical material according to the recommendations for quality control issued by the European Group for Rapid Viral Diagnosis.

4. Reference function

The accuracy of the diagnostic work will be monitored. The National Bacteriological Laboratory, Stockholm will act as reference laboratory. Reference activities will include confirmation of results. For this, duplicate slides with, preferably, a total of 6 cell smears from each specimen are stored at the local laboratory at the lowest temperature possible (preferably less than -20°C), for transfer to the reference laboratory at convenient intervals. They need not be transported frozen, but should reach the reference laboratory within 1-2 days.

5. Serological studies

These will be done later and perhaps in a limited scale in collaboration with laboratories in Sweden and USA.

Bacteriological studies

1. Specimen collection

Specimens for bacteriological examination will be collected at the same time as those for viral examination. In all instances if possible, specimens should be obtained prior to the initiation of antibiotic therapy.

1.1 Specimens from pneumonia

1.1.1 Pharyngeal aspirate

The collection of good pulmonary secretions or exudate from children with pneumonia is at best difficult and often non-productive. Furthermore, the pulmonary exudate is frequently contaminated with bacterial flora of the bronchi and upper respiratory tract, which complicates the attempts to isolate the bacterial pathogens that may be responsible for severe respiratory illness. Since nasopharyngeal secretion with a suction apparatus are to be obtained for viral studies it is felt that following this procedure the induction of coughing in the child will easily be acheived. If the child is not producing purulent or mucopurulent sputum that can be collected in a small sterile plastic cup with a cover, it is agreed that the physician will simultaneously insert two sterile swabs into pharynx during coughing spell to obtain whatever material may be forced upward by coughing. These two swabs are to be placed in a sterile test-tube and delivered to the laboratory at once - a delay of no longer than 30 minutes is permissible.

1.1.2 Post mortem specimens

As autopsies usually not are carried out and trans-tracheal aspiration and percutaneous needle aspiration of the lung has been considered unsuitable at this stage, the value of a post mortal lungpuncture cannot be overestimated. Lung material can be obtained by suction into a syringe through a long needle of wide bore. A suitable place for insertion of the needle is below the scapula to a depth of half the diameter of thorax.

1.1.3 Blood cultures

Blood cultures are often very useful in the diagnosis of bacterial pneumonia, and should be obtained whenever possible, and before therapy is started.

1.1.4 Serology

If possible, paired samples of sera are obtained for later analysis. ELISA techniques are available for pneumococci and capsulated H.influenzae. Other possible antigen are AST/anti DNAse, mycoplasma chlamydia, Legionela and viruses.

Tuberculosis.

When suspected from clinical and radiological findings:

1.1.5 Gastric aspirate for AFB, culture?

1.1.6 Skin test

2. Tests to be performed on swabs and spirates

2.1 A smear is prepared for Gram-stain - pulmonary cells?

2.2 The specimen - or swab - is streaked onto

- blood agar plate
- supplemented chocolate agar plate
- MacConkey agar plate
- B G medium or Laceys medium

The plates are examined using standard bacteriologic procedures.

Organisms to be considered:

Streptococcus pneumoniae

Haemophilus influenzae

Group A streptococci

Bordetella pertussis (ELISA on nasopharynx secretions)

Staphylococcus aureus

Klebsiella pneumoniae

Escherichia coli (and other enteric bacilli)

Proteus

Pseudomonas aeruginosa

Confirming tests are done like:

Str. pneumoniae	-	Optochin Serotyping
H. influenzae	-	X and V discs H. infl-antiserum
Group A Streptococci	-	Bacitracin Serogrouping
S. Aureus	-	Coagulase Mannitol

2.3 If a working method is available, countercurrent electrophoresis will be used to detect antigen from pneumococci and H. influenzae in blood sputum, and nasopharynx secretion. None capsulated H. influenzae and pneumococci type 7 and 14 cannot be demonstrated as they have no or non-charged capsule antigens.

2.4 New Pathogens

L. pneumophila will be sought in collaboration with Dr. K. Wachsmuth of CDC, Atlanta, USA.

2.5 Mykoplasma pneumonia will be searched for with coldagglutination and/or CF-tests.

2.6 In vitro antibiotic susceptibility tests will be done.

2.7 Strains would be freeze dried and stored.

D. ANALYSIS OF DATA

1. Patient Characteristics:

	Male	Female	Total
Age			
WT/HT			
arm cirmf/ HT			
degree of dehydration			

2. Causes of Diarrhoea

Organism	Male age	Female age

3. Each episode of diarrhoea

Causes of Respiratory illness	Etiology of diarrhoea

4. Cause of respiratory illness by Month or Season:

5. Distribution of Viral Respiratory Pathogens by cause of diarrhoea:

Causes of diarrhoea Organism	Respiratory pathogens

6. Bacterial diarrhoea:

Antibiotic sensitivity	Bacteria							

7. Respiratory Pathogens vs Clinical Observations:

8. Respiratory Pathogens vs X-ray:

9. Duration of diarrhoea vs Respiratory disease cause:

10. Prior antibiotics vs Cause of illness:

11. Outcome: Alive or Dead vs Cause of illness:

12. Duration of Hospitalization vs Cause and Course:

13. Bacteremia vs Causes of severity:

E. SIGNIFICANCE

Next to diarrhoea respiratory diseases is the second greatest killer as a group of diseases and when they overlap the combination is very serious. With increased knowledge of the aetiology and clinical appearence of these infections in our hospital patients, we will have a better basis for our clinical management of this problems.

Based on the results of this study an extension could be made where respiratory tract infections are studied in a rural setting like Matlab. The main objective then would be to provide information needed to plan a - nationwide? - programme by which the impact of acute respiratory illness can be reduced.

F. FACILITIES REQUIRED

1. Office space - already provided
2. Laboratory space - already provided but may need some reallocation to meet the increased load of respiratory tract cultures.

   Immunofluorescens microscope is already working.

   Space for counter currentimmuno electrophoresis may be needed.

   Handling of sera (and cold space) through immunology branch.
3. Hospital resources - no extra
4. For follow up visits - field workers and transport

G. COLLABORATION

1. In a SEARD collaborative study, coordinated by WHO, the incidence of viral respiratory tract infections in 8 countries of the subcontinent is studied using homogenous controlled reagents and reference controls for all laboratories.

Dr. Nazrul Islam, Associate Professor of Virology, Institute of Post-graduate Medicine and Research, Dacca, is one of the participants in this study. In collaboration with him an immunofluorescene method is set up at the Centre for rapid diagnosis of virus in nasopharyngeal secretions. The slides for IFL will be prepared by the Immunology Branch but Dr. Islam will be responsible for their interpretation.

2. As our laboratory so far has its major interest in the field of intestinal pathogens, the diagnostic procedure to detect airway pathogens need a review. Professor Stig Holm, Department of Clinical Bacteriology, University of Umea, Sweden working in this field, has shown his interest to visit the Centre as a consultant during a 4-6 weeks sabbathical in October 1981 to check actual methods and suggest necessary changes. SAREC will be requested to cover the expenditure for his travel to Bangladesh. He will be welcome to avail the existing guest house facilities of ICDDR,B. A laboratory technician from Sweden will be traveling at ICDDR,B expenses for setting up new lab techniques for virological studies at the centre. Dr. K. Wachsmuth of CDC, Atlanta, USA will be requested to collaborate in this study for isolation of Legionella pneumophila.

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Abstract for ERC

1. All sampled patients admitted to the ICDDR,B Dacca hospital with respiratory illness in addition to diarrhoea will be included in the study. This is because at the moment a great clinical problem exists due to therapeutic failures with various antibiotics used in respiratory illness.

2. There is no potential risk to the subject.

3. Does not arise.

4-5. All efforts will be made to maintain confidentially and protection of anonymity. Written consent of the mothers will be obtained before taking the child into the study.

6. Not applicable.

7. The result of this study will provide data needed to plan a programme for health care by which the impact of acute respiratory illness can be reduced.

8. Blood and nasopharyngeal secretions will be used.

SECTION III - BUDGET

A. DETAILED BUDGET

1. Personnel Services:

	Effort	Time	Taka	Dollar
A.N. Alam	10%	6 mths.	6,000	-
N.S. Shahid	30%	6 mths.	11,000	-
W.B. Greenough		NO COST		
M.I. Huq		NO COST		
Two field workers		6 mths.	16,000	-
Lab staff: salary for microbiology is included in cost for the analysis: immunology can handle 10 samples/week with their actual staff				
Consultation X-ray 2 hr/wk			10,000	-
Virology			-	-
			43,000	0

2. Supplies and materials:

Plastics, glassware etc.	1,000
Suction utensils (US$1/Patient)	200
Reagents:	
1. antisera for IFL (∿ US$500/200 patients)	500
2. antisera for counter current electrophoresis (∿ US$200*200 patients)	200
Cultures: nasopharynx + trachea + blood	60,000 -
	60,000 1,900

3. Equipment
0

4. Hospitalization costs

2,500 hospital days x 150	375,000	-
	375,000	0

5. Outpatients care
0

6. ICDDR,B transport

For home-visits 15 miles/patient	6,000	-
	6,000	0

7. Travel

Two consultants (one month each) - bacteriology - virology/IFL		
Round-trip airticket 2 x 2000	-	4000
Guest house 2 x 30 x 30	-	1800
	0	5800

8. Transporation of things

Samples to different labs for serology	-	500
	0	500

9. Rent/Communication

0

10. Printing

	1,000	300
	1,000	300

11. Contractual service

0

12. Construction

0

Grand Total :	485,000	8500

or US $ ~~30312~~ 38,810

(16 Taka = 1 US Dollar)

B. BUDGET SUMMARY

	US Dollar
1. Personnel Services	2,687.00
2. Supplies & Materials	~~3,750.00~~ 5,650

APPENDIX I

1. Patient No.: _____ ____ ____ ____ ____ ____ ____ (1-6)

   Address: _____ ____ (7)

   _____

2. Age (years/months): ..... ____ ____/____ ____ (8-11)

3. Sex (0=F, 1=M) ..... ____ (12)

MEDICAL HISTORY (0=No)

		Number of days / hours	
4.	Fever	____ ____/____ ____	(13-16)
5.	Nasal discharge	____ ____/____ ____	(17-20)
5a.	1=bloodstained, 2=watery, 4=with pus...	____	(21)
6.	Earache	____ ____/____ ____	(22-25)
7.	Sore throat	____ ____/____ ____	(26-29)

3.	Equipment	-
4.	Hospitalization costs	23,437.00
5.	Outpatients Care	-
6.	ICDDR,B Transport	375.00
7.	Travel	5,800.00
8.	Transportation of Things	500.00
9.	Rent/Communication	-
10.	Printing	362.00
11.	Contractual Service	-
12.	Construction	-

Total US $ ~~36,911.00~~ 38,810

(Conversion rate:U$$1= 16 Taka)

PREVIOUS TREATMENT

17. Has the child had by mouth or ..... _____ (65)
by injection - any antibiotics
the last 14 days?

0 = none received	5 = tetracyclin
1 = sulpha	6 = gentamycin
2 = penicillin	7 = trimetoprin/sulph
3 = ampicillin	8 = erythromycin
4 = chloramphenicol	9 = yes, but not defined

18. Other medicine (0=No, 1=yes, 2=unknown) _____ (66)

Which? _____

_____ _____ (67-68)

_____

19. Immunizations ..... _____ _____ (69-70)

0 = none	4 = BCG
1 = measles	8 = pertussins with/without diphtheria and tetanus
2 = polio	16 = smallpox

9. Dyspnoea ..... ____ ____/____ ____ (34-37)

10. Rash ..... ____ ____/____ ____ (38-41)

11. Conjunctivitis ..... ____ ____/____ ____ (42-45)

12. Vomiting ..... ____ ____/____ ____ (46-49)

13. Diarrhoea ..... ____ ____/____ ____ (50-53)

(0=none, 1=watery, 2=blood, 4=mucoid) ... ____ (55-56)

W+B=5, W+M=6, D+B+M=7

14. How many during last 24 hours? ..... ____ ____ (55-56)

15. Duration of diarrhoea? ..... ____ ____/____ ____ (57-60)

16. Convulsions ..... ____ ____/____ ____ (61-64)

APPENDIX II

PHYSICAL EXAMINATION:

Patient No.: _____ ____ ____ ____ ____ ____ ____ (1-6)

Address: _____ ____ (7)

_____

Weight (grams) ..... ____ ____/____ ____ ____ (8-12)

Height (cm) ..... ____ ____ ____ (13-15)

Arm circumference (mm) ..... ____ ____ ____ (16-18)

Dehydration ..... ____ (19)
(0=no, 1=mild, 2=moderate, 3=severe)

Temperature (Fahrenheit) ..... ____ ____ ____/____ (20-24)

Respiratory rate per minute ..... ____ ____ (25-26)

Rash ..... ____ (27)

Fluttering nostrils ..... ____ (28)

Cyanosis ____ (29)

Respiratory retractions ..... ____ (30)
(0=none, 1=intercostal, 2=suprasternal)

Diminution of breath sounds..... ____ (31)
(0=none, 1=unilateral, 2=bilateral)

Rhonchi ..... ____ (32)

Crepitations ..... ____ (33)

Pleural friction ____ (34)

Cervical glands ..... ____ (35)
(0=normal, 1=enlarged, 2=tender)

Monilia ..... ____ (36)

Tonsillitis ..... ____ (37)
(0=normal, 1=hypermia, 2=pus)

A. COLLECTION OF SPECIMENS:

Nasopharynx

Pharyngeal aspirate

Bloodculture

Acute serum/conv.serum

FOLLOW UP (14 days):

Recovered within _____days ____ ____

Dead (0=no; withing "n" days after admission) ____

Cough for _____ days ____ ____

Wheezing for _____days ____ ____

Diarrhoea for _____days ____ ____

RESULT OF: (Code this)

Bacteriological examination _____

Virological examination _____

Blood culture _____

Serology _____

TREATMENT:

_____

_____

_____

_____

Organism	Sample		
	Nasopharynx	Throat swab	Blood
N. Catarrhalis			
Strepto viridans			
Diphtheroid bacilli			
Staph aureus			
Staph albus			
Pseudimonous			
Klebsiella			
E. coli			
B- haem strepto			
Haemophi Bordetella			
Pneumococus			
Shigella shiga			
Shigella flexneri			
Shigella dysentery			
Salmonella			
Legionella pneumophilia			

	Peni-cillin	Tetra-cyclin	Eryth-romycin	Ampi-cillin	Metha-cillin	Chloram-phenicol	Kana-mycin	Genta micin	Septrin	Polymy xin Carb
N. Catarrhalis										
Strepto viridans										
Diphtheroid bacilli										
Staph aureus										
Staph albus										
Pseudimonous										
Klebsiella										
E. coli										
β-haem strepto										
Haemophylus Bordetella										
Pneumococcus										
Shigella shiga										
Shigella flexneri										
Shigella Dysentery										
Salmonella										
Legionella pneumophilia										

CONSENT FORM

Respiratory disease is a very important cause of morbidity and mortality in Bangladesh. In association with diarrhoea the rates rise many fold. The International Centre for Diarrhoeal Disease is interested in identifying the organisms.

If you agree to let your child participate in the study we will collect 1 cc Nasopharangeal aspirate for identification of the organisms and 2 cc of extra blood for serology at the time blood is being drawn for clinical indication during the child's stay in the hospital.

The treatment offered to your child will not be affected if you do not wish to let your child join the study. If you agree to do so now but change your mind later you may withdraw your child from the study at anytime.

If you agree to let your child join the study, please sign here.

_____

Signature/L.T.I. of the parent