ACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 5-4-98
8-6-98

Principal Investigator Dr. M. Rahman, LSD Trainee Investigator (if any) ____

Application No. 98-012 Dr. S. Hossain, CSD

Dr. A.H. Baqui, PHSD Supporting Agency (if Non-ICDDR,B) USAID/WC

Title of Study Surveillance of invasive Haemophilus influence (Hi) and Streptococcus Pneumoniae (Spn) disease in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh

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
   (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 (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 ERC for review.

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

Principal Investigator(s)

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

1. **Title of protocol: Surveillance of invasive *Haemophilus influenzae* (Hi) and *Streptococcus Pneumoniae* (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh**

2. Investigators:

2a. Principal Investigators:

Laboratory component:	Dr. Mahbubur Rahman, Laboratory Sciences Division, ICDDR,B
Hospital component:	Dr. Shahadat Hossain, Clinical Sciences Division, ICDDR,B
Population-based component:	Dr. Abdullah H Baqui, Public Health Sciences Division, ICDDR,B

2b. Co-investigators:

Laboratory component: Dr. John Albert, ICDDR,B
Dr. Motior Rahman, ICDDR,B
Prof. N. Moazzem, Dhaka Medical College Hospital (DMCH)
Prof. Nurul Islam, DMCH

Hospital component : Dr. George Fuchs, ICDDR,B
Dr. Mujibur Rahman, ICDDR,B
Prof. Nazmun Nahar, DMCH
Prof. Abid Hossain Mollah, DMCH
Prof. Hosne Ara Begum, DMCH

Population-based component: Dr. Md Yunus
(ICDDR,B) Mr. J Chakrabarty
Dr. Shams El Arifeen
Dr. Hafizur Rahman Chowdhury
Dr. Nigar Shahid
One physician from Matlab
Prof. Patrick Vaughan

2c. Int. Collaborating Investigator: Dr. Mark Steinhoff , Johns Hopkins University, USA

3. Dates of Proposed Period of Support
(Day, Month, Year - DD/MM/YY) : 01 May 1998 to 30 April 2001

4. Cost Required for the Budget Period:

Total budget: Year 1: $329,843 Year 2: $268,904 Year 3: $277,566 Total: $876,312

5. Location of project: International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), GPO Box 128, Dhaka 1000, Bangladesh

6. Sponsor(s): International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B)

7. Approval of the Project by the Division Directors of the Applicant

Professor V. I. Mathan		6/4/98.
Name of the Division Director, LSD	Signature	Date of Approval
Dr. George Fuchs		
Name of the Division Director, CSD	Signature	Date of Approval
Professor Patrick Vaughan	pp.	6 April/98.
Name of the Division Director, PHSD	Signature	Date of Approval

8. Certification by the Principal Investigator(s)

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

9. Signature of PIs:

a) Laboratory component:

Date: 5/4/98

b) Hospital component:

Date: 5/4/98

c) Population-based component:

Date: 05 April 1998

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PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application.

Acute lower respiratory infections (ALRI) , primarily pneumonia, is a leading cause of morbidity and mortality in children less than 5 years of age in all developing countries including Bangladesh. About 25% of all deaths in under five year old children and about 40% deaths in infancy, in Bangladesh, are associated with pneumonia. To reduce mortality due to pneumonia, the World Health Organization(WHO) has developed an useful and relatively simple case management strategy whereby children with cough and difficult breathing are diagnosed as having pneumonia and are empirically treated with an antimicrobial agent. Several antimicrobial agents such as co-trimoxazole, procaine penicillin, amoxicillin, chloramphenicol and gentamicin are recommended by the WHO for therapy because their spectrum of activities includes *Streptococcus pneumoniae* (Spn) and *Haemophilus influenzae* (Hi) which are the principal bacterial pathogens that cause pneumonia. In addition, Spn and Hi frequently cause meningitis, bacteremia, and otitis media.

In Bangladesh, co-trimoxazole is recommended by the National ARI Control Programme for the treatment of acute lower respiratory infections. However, the recent emergence of resistance in Spn and Hi to recommended antimicrobial agents is threatening the success of the case management approach designed for decreasing the mortality associated with these infections.

Vaccination of children against Spn and Hi have been proposed to prevent deaths from severe pneumonia, meningitis, and other invasive infections. However, disease burden studies and careful evaluation of effectiveness and cost of such vaccines are needed for policy decisions. Furthermore, little is known about serotype distribution of Spn in Bangladesh. For optimal efficacy of the vaccine this data is also needed since there are more than 90 serotypes of Spn. Sufficient data on isolation rate, prevalent serotypes and antimicrobial resistance of Spn and Hi isolates are not available in Bangladesh.

The main aims of the proposed study are to set-up hospital-based surveillance in urban Dhaka and a population-based surveillance in rural Matlab of Bangladesh to study the epidemiology of invasive Hi and Spn infections; to determine the prevalence, pattern and trends in antimicrobial resistance among Hi and Spn isolates during the study period; to determine the prevalent serotypes of Spn and Hi in order to determine the preferable serotype compositions for vaccines against Spn and Hi diseases in Bangladesh; and to disseminate the relevant information in a timely manner and improve the use of such data for policy decisions, particularly in government's ARI control programmes.

The surveillance will initially be continued for three year. ICDDR,B-Dhaka hospital and the Dhaka Medical hospital will be included in the hospital component of the study. Relevant clinical and epidemiological data will be collected from all pneumonia and meningitis cases attending these hospital. One thouand six hundred eighty pneumonia and 316 meningitis cases will be enrolled in the microbiological surveillance. A population-based surveillance will be set up in

a population of 105,000 in rural Matlab to identify all ALRI and meningitis cases. An estimated 3,047 ALRI and 30 meningitis cases will be enrolled in the microbiological surveillance. Blood samples will be obtained from all patients enrolled in the microbiological surveillance and will be cultured for Spn and Hi using standard methods. All Hi and Spn isolates will be serotyped in order to determine the predominant serotypes in the study population. Since antibiotic resistant strains of both Hib and Spn are already quite prevalent in Bangladesh and knowledge of sensitivity patterns are needed for successful case management, we will monitor the sensitivity patterns of the Hib and Spn isolates. Laboratory methods will be periodically assessed throughout the study. To ensure quality, paired samples will be taken from a sub-set of the cases and the second set of specimens will be sent to a reference laboratory.

Nasopharyngeal aspirate will be cultured from 20% (665) of all hospitalized pneumonia cases. A multiplex polymerase chain reaction will be used to detect Spn and Hib from all CSF and 20% of blood samples from hospitalized pneumonia patients.

Relevant demographic, clinical, epidemiological, and microbiological data will be collected from all 'potential' cases using standardized forms. All data will be entered onto a standardized computer database. Data will be analyzed to quantify the magnitude of Hi and Spn infections and to determine the clinical and epidemiological characteristics of bacterial pneumonia, meningitis and other invasive infections caused by Hi and Spn.

Timely dissemination of the findings from the project, technical assistance to build the capacity of the national institutions and improved use of data for policy decisions will be important priorities of the project. To this end, mechanisms of coordination with the government of Bangladesh (GoB) will be developed. Timely dissemination of findings will be particularly useful in selecting appropriate antimicrobial therapy for Hi and Spn infections. The serotype pattern will help to select and formulate protein-conjugate vaccine, particularly against Spn and evaluate existing and new vaccines against these two important pathogens.

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested in the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. The burden of invasive diseases (pneumonia, bacteremia and meningitis) caused by *Haemophilus influenzae* (Hi) and *Streptococcus pneumoniae* (Spn) is high in Bangladesh.

2. Serotype distributions of Hi and Spn in Bangladesh are different from other countries and new vaccine formulations based on these serotypes are required for use in Bangladesh.

3. The prevalence of penicillin and co-trimoxazole resistant Hi and Spn isolates are significant in Bangladesh.

4. Multi-drug resistant (MDR) Hi and Spn have clonal distribution that are associated with certain serogroups.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods **(Type within limits).**

1. To set up hospital and population-based surveillance to study the epidemiology of invasive Hi and Spn infections (e.g., incidence, age distribution, clinical presentations, severity, drug use and resistance patterns, serotypes and seasonality) in children less than five years of age in Bangladesh;

2. To improve the referral and treatment of invasive Hi and Spn diseases in children <5 years of age;

3. To improve the laboratory diagnostic capabilities including development of rapid diagnostic methods (e.g., multiplex PCR) for isolation, antimicrobial susceptibility testing, and serotyping of Hi and Spn isolates in Bangladesh.

4. To compare two methods of isolation of Hi and Spn from patients with acute lower respiratory tract infections: blood culture and culture of nasopharyngeal aspirates;

5. To determine the prevalence, pattern and trends in antimicrobial resistance among Hi and Spn isolates during the study period;

6. To determine the prevalent serotypes of Spn and Hi in order to determine the preferable serotype compositions for vaccines against Spn and Hi diseases in Bangladesh; and

7. To disseminate the relevant information in a timely manner and improve the use of such data for policy decisions, particularly in government control programmes.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(Do not exceed 5 pages, use continuation sheets).**

The magnitude of the public health problem in terms of morbidity and mortality posed by Hi, particularly *Haemophilus influenza*e type b (Hib) as a cause of pneumoniae, meningitis and other invasive infections (e.g. septicemia, septic arthritis, cellulitis) in infants and young children has been amply documented in both developed and developing countries (1). Prior to routine use of Hib vaccines, Hib was the most common cause of bacterial meningitis in several developed countries. It was also a leading cause of other bacterial infections. In the 1970's and 1980's, results of carefully conducted population-based epidemiologic studies in developed countries documented Hib as an important cause of morbidity, mortality and health care expenditure. The annual incidence of invasive Hib diseases ranged between 20-100 cases per 100,000 children under 5 year of age. This led to the development of a series of conjugated polysaccharide Hib vaccines. The licensure and routine use of these vaccines in many developed countries has virtually eliminated the disease.

Several studies suggested that Hib is an important cause of invasive childhood diseases in many Asian countries as well, including Japan, Taiwan, Malaysia, Singapore, Korea and the Phillippines (2-7). In these countries, Hib is a leading cause of bacterial meningitis but the reported incidence rates have been much lower than noted in developed countries. It seems that the reported incidence rates of Hib meningitis from Asian countries were under-estimates. Many of these studies were hospital-based and high proportions of CSF specimens were culture negative. This is perhaps due to

widespread and indiscriminate use of antibiotics in sick children in these countries before they arrive in the hospitals and before culture specimens are obtained. The actual incidence of Hib and other invasive diseases in Asian countries is not known because of lack of population-based data.

Based on available data, it appears that the epidemiology of Hib diseases seen in developing countries differs from that seen in developed countries in several important respects. In developing countries, Hib diseases occur in younger children with most cases occurring before 12 months of age and about half before the age of six months (8). The clinical presentation of disease also differs; in developing countries, pneumonia is more common than meningitis, whereas epiglottitis is extremely uncommon (9). The outcome of invasive Hib diseases in developing countries is poor and neurological sequelae occur in a significant proportion of survivors with Hib meningitis (10).

Spn is also an important cause of pneumonia and meningitis in children in developing countries, including Bangladesh. Spn infections also account for significant morbidity, mortality and health care cost. The bacterial etiologies of pneumonia have been determined in several studies based on results of lung aspirate and blood cultures, and by non-culture methods such as antigen detection and serology (11-19). Results of these studies indicate that Spn and Hib cause most bacterial pneumonia mortality. A recent review estimated that globally Spn and Hib pathogens annually account for almost 1 million and 500,000 deaths, respectively (Schwartz, unpublished data). Promising polysaccharide conjugate vaccines for Spn diseases are now being evaluated in clinical trials. Disease burden studies of this infection which is part of this study will help to determine whether the vaccine should be used in Bangladesh. It will also provide the necessary background information for conducting a pneumococcal conjugate vaccine efficacy trial.

Data on invasive Hib and Spn diseases from Bangladesh are few. However, Acute Lower Respiratory Infections (ALRI), particularly pneumonia is the most important cause of morbidity and mortality of children in most developing countries including Bangladesh. About 12 million children in developing countries die every year before reaching their fifth birthday, many during the first year of life. Of this estimated 12 million deaths about 30% are attributed to ALRI (20). Although tracheobronchitis, bronchiolitis, and pneumonia each accounts for about one-third of ALRI cases, several studies suggested that pneumonia is responsible for most ALRI deaths. Three studies which reported the diagnosis in children who died of ALRI revealed that a median of 89% (range 71% to 100%) of ALRI deaths were associated with pneumonia (21-23).

In Bangladesh, about 25% of deaths in <5 year old children and 40% of deaths in infancy are associated with ALRI (24). A community-based cohort study in rural Matlab, Bangladesh observed an annual ALRI incidence of 30/100 child-years in children less than two years of age (25). A hospital-based study conducted in the ICDDR,B's Dhaka hospital between 1986-88 investigated 401 ALRI cases in children <5 years of age for respiratory pathogens. The most common manifestation was pneumonia (93%). A respiratory pathogen was identified in only 30% of the patients. Spn was detected in 7%

and Hib was detected in 3% of the cases. The case fatality rates were 14% in bacterial pneumonia and 3% in viral pneumonia (13).

A case management strategy developed by WHO has become the primary approach used by national ARI control programmes to decrease pneumonia mortality (21, 22). This strategy is based on diagnosis of pneumonia in children with cough or difficult breathing using easily discernible signs (tachypnea and chest in drawing), followed by empiric antimicrobial therapy. The successes of this approach depends on selection of an antimicrobial agent that is effective against the pathogens most likely to cause fatal pneumonia.

Acute bacterial meningitis is also a common cause of morbidity and mortality in Bangladesh among children less than five years of age. A laboratory-based study of diagnosed bacterial meningitis in the Shishu Hospital (a national pediatric hospital in Dhaka) identified 852 meningitis cases during the period 1987-94 (27). This 305 bed teaching hospital with a catchment population of about 16 million admitted about 1300 patients per month during the study period. Bacterial meningitis was diagnosed if a bacterial pathogen was isolated from CSF or if the CSF contained more than 100 white cells/cubic mm. Of the 587 culture-positive cases, Hi was isolated from 47% and Spn was isolated from another 32% of the cases. Sixty eight percent of the Hi cases occurred below one year of age and another 24% in the second year of life. Hi showed a remarkable increase of 700% during the study period. Ninety eight percent of the Hi strains were type b (Hib). Antigen detection in 72 culture negative cases in the last two years of the study also revealed the predominance of Hi (49%) and Spn (15%).

Co-trimoxazole, amoxicillin and procaine penicillin have been recommended as the first line therapy for children with pneumonia and because of their activity against both Spn and Hib. Co-trimoxazole is widely used because of its lower cost and greater ease in dosing compared to the other first line agents and is the drug recommended for the national ARI control programme in Bangladesh.

The emergence of antimicrobial resistance strains threatens the success of the case management approach since Spn and Hi isolates resistant to each of the first line agents have been reported throughout the world (28-33). The emergence and spread of penicillin resistant and multiresistant Spn and Hi appears to continue with accelerating speed in the 1990s (31-33). Studies in different countries of the world showed that a restricted number of serogroups of Spn (6,9,14,19 and 23) are generally associated with carriage in children and are frequently responsible for penicillin and multi-drug resistant infections (31,34,35). The close similarity of pulse-field gel electrophoresis (PFGE) patterns shared by multi-drug-resistant serotype 23F isolates of Spn from Spain, Portugal, USA, Croatia and South Korea suggested the wide geographic spread of this multi- drug-resistant clone (34-36).

The United States (US) national surveillance study detected that a significant increase in the prevalence of penicillin resistance among Spn isolates evidently took place in the US as shown by Thornsberry and colleagues who studied 524 isolates from 17 centres during 1991 to 1992. The study demonstrated an intermediate-level (Pen-I) and high-level

penicillin (Pen-r) resistance in 15.2% and 2.6% of the isolates respectively (37). Barry and colleagues one year later during 1992 detected even higher rates of penicillin resistance in a second United States national surveillance study with 14.9% Pen-I and 7.3% Pen-r among 799 isolates from 19 U.S. medical centres (38). In 1995, Doern and co-workers reported an overall penicillin resistance of 23.6% with 14.1 Pen-I and 9.5% Pen-r among 1,527 clinically significant outpatient isolates of Spn prospectively collected from 30 different medical centres between November 1994 and April 1995 (39). Multiply resistant Spn represented 9.1% of the organisms showing resistance to cephalosporin, macrolide, chloramphenicol, tetracycline and co-trimoxazole at different frequencies in this study (39). Penicillin resistant and multiply resistant Spn strains have been isolated in Pakistan (40), Korea (34), Australia (36), Spain (35) and other countries (41). Saha et al. in Bangladesh tested 51 Spn isolates from Dhaka Shishu Hospital and detected penicillin resistance in 12% of isolates. But during 1995-96, all 74 Spn isolates from blood and cerebrospinal fluid in Clinical Microbiology laboratory of ICDDR,B were susceptible to penicillin and chloramphenicol by the disc diffusion method (personal communication). The determination of MIC values for penicillin and other recommended antimicrobial agents might help to explore the real status of drug resistance among these two important pathogens in Bangladesh. The knowledge of antimicrobial susceptibility would be of great help in guiding the choice of therapeutic agent for optimum performance of ARI control programmes.

Antibiotic resistance associated with beta-lactamase production by Hi is now a major problem . Ampicillin resistant Hib was first reported in 1972 and is now found worldwide in 15-20% of all isolates and in ~30% of strains of serotype b (32). Most ampicillin-resistant strains carry plasmids encoding TEM-1 beta-lactamase. Some of the plasmids are conjugative and carry an intact TnA-type transposon as well as other resistance genes. Resistance to chloramphenicol, tetracycline and co-trimoxazole exists but is less common in the U.S. and Europe (31, 32).

Vaccination of children against Spn and Hib have been proposed to prevent deaths from meningitis, severe pneumonia and other invasive infections. However, disease burden studies and careful evaluation of effectiveness and cost of such vaccines are needed for a policy decision. Furthermore, little is known about serotype distribution of Spn in Bangladesh. For optimal efficacy of the vaccine this data is also needed since there are more than 90 serotypes of Spn (41).

The formulation of the 23-valent pneumococcal polysaccharide vaccine was largely based on the serotypes found to be prevalent in Europe and North America (42). This vaccine does not cover common serotypes prevalent in many countries because of extreme diversity of pneumococcal serotypes or group distributions. For example, serogroup 19 was predominant in Pakistan but rare in Egypt and was never found in Brazil (43). Group 1 is most prevalent in Egypt (31.4%) but occasionally isolated in other developing countries such as Mexico (0.8%) and Pakistan (0.6%) and was never isolated in Finland (43). Saha and co-workers reported the isolation of two predominant serotypes (7F and 12F) among 165 invasive Spn isolates in a children hospital in Dhaka, Bangladesh during the period 1992 to 1995 (44). Serotype 12F is rarely isolated in any other country. Moreover, Mastro and colleagues reported the variation of serotypes pattern of Spn in

3 different seasons in Pakistan (40). This observation is very important particularly for the development of polyvalent pneumococcal vaccine such as protein-conjugate vaccine which can accommodate only a few serotypes (45). Therefore, knowledge of the regional distribution of pneumococcal capsular types is essential for the development of effective vaccine strategies.

In 1930, Pittman classified encapsulated Hi strains into six serotypes (a, b, c, d, e and f) and identified type b as the aetiologic agent of invasive diseases (46). Non-typeable (noncapsulated) strains were subsequently isolated as a common cause of pneumonia (40). As alluded earlier, the introduction of Hib conjugated vaccine in 1990 significantly reduced the incidence of invasive Hi infections in developed countries (47). Sufficient data on serotype distribution of Hi in Bangladesh are not available to determine the importance of typeable and non-typeable strains.

Thus, it is clear that there is an urgent need to determine the burden of infections caused by Spn and Hi and to study the prevalence of antimicrobial resistance and pattern of prevalent serotypes among the clinical isolates in Bangladesh. A systematic surveillance of Spn and Hi isolates for antibiotic resistance and serotyping should be an integral part of programme to control ARI and other invasive infections in a developing country like Bangladesh. Therefore, this study primarily aims at (I) the organization of a surveillance network to monitor antimicrobial resistance and developing guidelines for interpretation of antimicrobial resistance data; (ii) establishing and improving laboratory methods for collecting and testing clinical specimens for these pathogens; (iii) determining prevalent serotypes for inclusion in a vaccine for Bangladesh and (iv) finally providing important findings to national ARI control programme of Bangladesh.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(Do not exceed ten pages, use continuation sheets).**

The proposed study will have three components: A) a population-based surveillance of Hib and Spn diseases, particularly pneumonia and meningitis, B) a hospital surveillance of pneumonia and meningitis clinical cases in selected hospitals, and C) laboratory studies to isolate and characterize the respiratory pathogens from specimens collected as part of the hospital and population-based surveillance. The surveillance will be initially set up for a period of three years. The three study components together will describe and quantify the problem of invasive Hib and Spn diseases in Bangladeshi children including the antimicrobial sensitivity and serotype patterns of Hi and Spn isolates.

A. POPULATION-BASED SURVEILLANCE

Study Population, setting, and subjects:

The population-based surveillance will be carried out at the Matlab Health Research Program area of ICDDR, Bangladesh. Matlab is located about 55 km south-east of Dhaka, the capital of Bangladesh. The field area was originally developed for evaluation of cholera vaccines. To support field studies, a Demographic Surveillance System (DSS) was established in 1966. Over the years, the system has been refined and is currently operational in 144 villages containing about 210,000 population. The DSS gathers vital event information, such as births, deaths, migrations and marriages on a regular basis through home visits. The demographic database is computerized and updated regularly. In late 1977, ICDDR,B started implementing a comprehensive maternal and child heath and family planning (MCH-FP) programme in half of the DSS villages, called the "Intervention Area'. The remaining half has continued to receive MCH-FP services from the Government of Bangladesh and serves as the 'Comparison Area' for the MCH-FP program area. The proposed surveillance will be set up in the Intervention Area of ICDDR,B.

In the Intervention Area, a cadre of female community health workers (CHWs) provide education on signs and symptoms of ARI, management of less severe ARI cases, and referral services for moderate and severe ARI cases in the community by using the WHO guideline. A CHW is responsible for about 1,800 persons; she visits each household in her area once in a month and during home visits collects some selected information, including information on diarrhoea and ARI morbidity. This data collection system, known as Record Keeping System (RKS) is also computerized. Preventive and curative health services are provided from four first level health facilities, called Health Sub-centres (HC); the HCs are staffed by paramedics. Each HC provides care to about 25,000 population. Severe ARI cases are referred to the ICDDR,B Treatment Centre (TC) at Matlab. The TC is staffed by physicians and has X-ray and laboratory facilities.

Numerous clinical and epidemiological studies of infectious diseases, particularly diarrhoeal diseases, have been conducted at the Matlab field site of ICDDR,B. These studies have provided important insights into the epidemiology, etiology, and therapy of all types of diarrhoea. The Matlab infrastructure provides an unique opportunity to study the epidemiology and aetiology of ARI which has not yet been adequately studied.

Matlab is fairly representative of most parts of rural Bangladesh. In 1995, the crude birth rates in the MCH-FP and comparison areas were 25.2 and 27.8 per 1,000 populations respectively. The crude deaths rates and infant mortality rates in the MCH-FP and comparisons areas respectively were 7.3 and 8.4 per 1000 populations and 51.1 and 78.6 per 1,000 live births.

Surveillance Methods:

The existing ARI and other childhood illness surveillance and case identification systems at Matlab will be strengthened to characterize the incidence of clinically and microbiologically diagnosed cases of meningitis and pneumonia infections due to Hi and

Spn. The estimated number of <5 children residing in the Matlab Intervention Area is 12,500. All <5 years old children residing in the Matlab Intervention Area will be included in this component of the study. Systematic clinical and microbiological surveillance will be conducted to identify cases of suspected bacterial meningitis and pneumonia. The reported incidence of ALRI in <5 children in Matlab is 23/100 child years which is similar to other developing countries (25). The expected number of ALRI cases in three years of study period will be about 9,375. About 25 % of the cases or about 2,344 cases will have severe ALRI and the remaining 7,031 cases will have moderate ALRI. The severe cases will be referred to Matlab TC and the moderate cases to one of the HCs. Despite strengthening of surveillance and referral, it is unlikely that all these cases will come to one of the health centres. The experience from the Matlab ARI program of last three years suggest that about 70% of the ALRI cases identified and referred will comply with the referral (A de Francisco, unpublished document). In addition, some children with ALRI will receive antibiotics before coming to the health centres. The CHWs will collect information from all reported episodes of ALRI including information on disease severity, management and outcome.

About 1,641 severe ALRI cases will come to the Matlab TC. All these cases will be enrolled in the study. Blood samples will be collected from all these cases for culture of Hi and Spn. Nasopharyngeal aspirates (NPA) will be collected and blood samples will be saved from every fifth cases (n=329). This number will be adequate to determine an estimated 50% isolation rate of Spn + Hi from NPA (13) with a $\pm$10% precision and 95% significance level. NPAs will be cultured for Hi and Spn and blood specimens will be tested to detect Hi and Spn using PCR.

About 4,922 moderate ALRI cases will attend the four HCs. Each HC staff will enroll every other case on a fixed day of every week in the microbiological component of the study. Blood samples will be collected by the Research Officer of the Matlab Laboratory who will attend the HCs on the surveillance day. The blood samples will be cultured for Hi and Spn.

Thus, although relevant clinical and epidemiological data will be collected from all reported ALRI episodes, an estimated total of 3,047 patients with ALRI will be enrolled in the microbiological surveillance.

It is difficult to estimate the number of meningitis cases. If we assume that the incidence of bacterial meningitis is 80/100,000 child years, about 30 cases are expected. It is assumed that most of these cases would present in the ICDDR,B's Matlab TC. All clinically suspected meningitis cases presenting to Matlab TC will be enrolled in the study. Both blood and CSF will be obtained from clinical meningitis cases. The specimens will be processed as appropriate including culture for Hi and Spn using standard methods, CSF gram staining, biochemical tests and antigen detection tests. Figure 1 summarizes the sampling method.

The clinical, epidemiological and microbiologic characteristics of pneumonia and meningitis cases caused by Hi and Spn will be determined. The surveillance will continue

for three years to identify an adequate number of cases to estimate the disease burden and to assess seasonal and annual variations.

Case definitions:

The following standardized case definitions will be used for case detection:

1. Definite Hi/Spn meningitis: Symptoms and signs suggestive of meningitis (clinical meningitis) *plus* CSF finding consistent with bacterial meningitis (>2 PMN per cubic mm) *plus* Hib/Spn cultured from blood or CSF.

2. Probable Hi/Spn meningitis: clinical meningitis+CSF finding consistent with meningitis (>2 PMN per cubic mm) + positive CSF antigen test for Hib/Spn.

3. Bacterial meningitis: clinical meningitis + CSF finding consistent with meningitis (>2 PMN per cubic mm) but negative cultures and antigen test for bacterial pathogens.

4. Hi/Spn pneumonia: Clinical pneumonia (cough or difficult breathing + elevated respiratory rate or auscultatory findings) or chest roentgenogram compatible with pneumonia + Hib/Spn cultured from blood

5. Probable Hi/Spn pneumonia: clinical pneumonia + chest roentgenogram compatible with pneumonia and Hib/Spn antigen detected in urine or blood or Hib/Spn cultured from nasopharyngeal aspirates.

6. Radiologically proven pneumonia: clinical pneumonia + chest roentgenogram compatible with pneumonia.

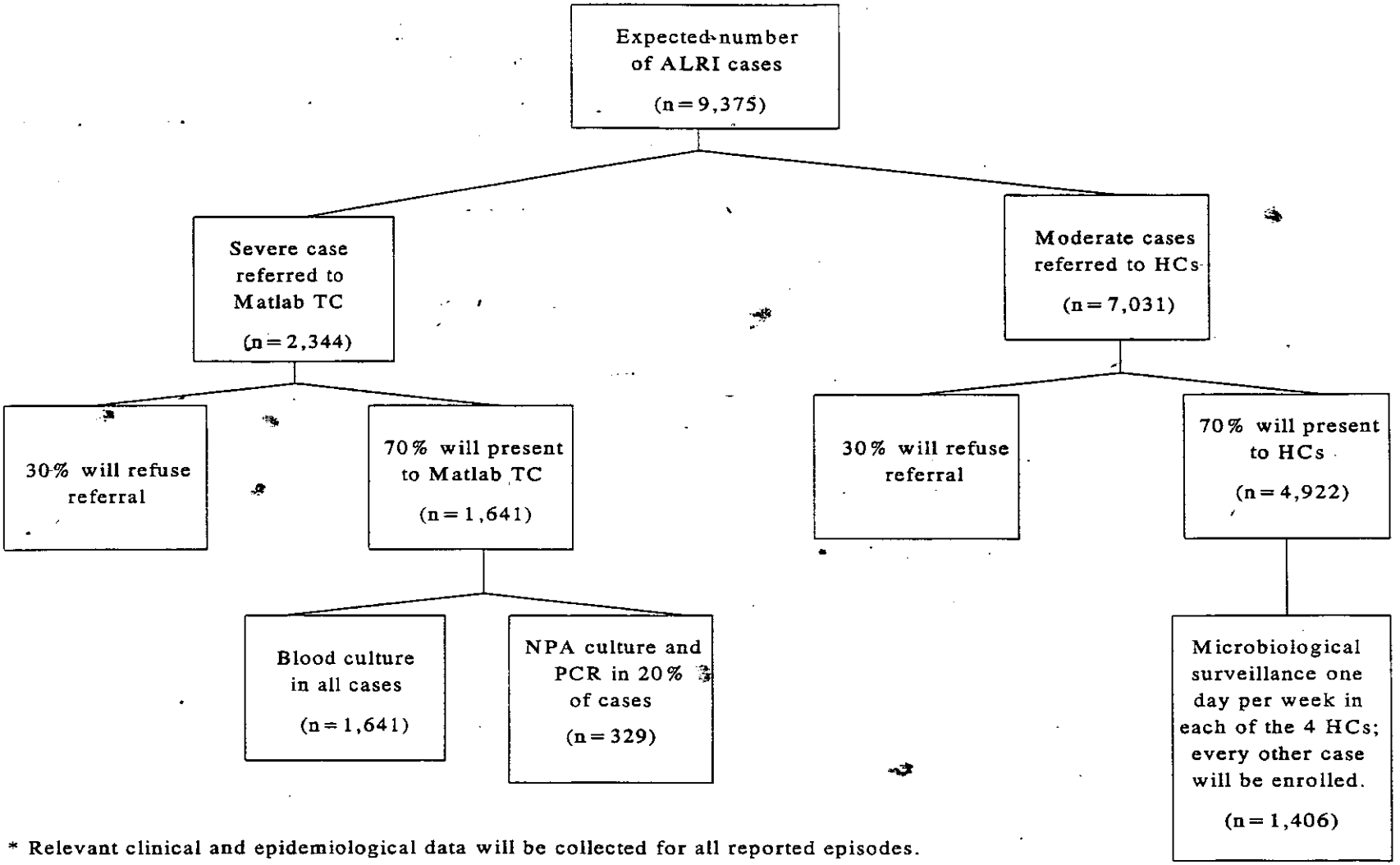
7. Clinical pneumonia: as determined by WHO criteria (cough or difficult breathing + elevated respiratory rates or auscultatory findings consistent with pneumonia)

Case detection, collection of specimens and laboratory procedures:

Pneumonia Surveillance:

Many of the pneumonia cases will be treated at the community or in peripheral centres where facilities are not available to perform chest x-ray studies. History of use of antimicrobial therapy will be collected in every cases. Urine will be collected from doubtful cases and tested for the presence of antimicrobial agents. Blood samples from clinically diagnosed ALRI cases enrolled in the study will be obtained for culture. Study physicians will periodically visit these facilities to encourage the providers to refer all suspected meningitis and severe pneumonia cases to the Matlab TC and to assess the quality of diagnosis made. All providers will be trained at the beginning of the surveillance to standardize the diagnostic and management practices. Need based training for the providers will be arranged periodically.

Figure 1. Diagram summarizing the sampling method for the population-based surveillance



* Relevant clinical and epidemiological data will be collected for all reported episodes.

Detection of meningitis:
Every child <5 years of age presenting with signs and symptoms suggestive of meningitis will have a lumbar puncture done. CSF will be visually inspected for turbidity and examined for total and differential cell counts, protein and glucose. Gram strains on smears of CSF, culture for bacterial pathogens, antigen detection test and PCR will also be performed. In addition, a blood culture will be performed to increase the likelihood of identifying the aetiologic agent.

Serotyping and antibiotic susceptibility testing:
All Hi and Spn isolates from blood, CSF and other body fluids will be serotyped in order to determine the predominant serotypes in the study population. Since antibiotic resistant strains of both Hi and Spn are already quite prevalent in Bangladesh and knowledge of sensitivity patterns are needed for successful case management, we will monitor the sensitivity patterns of the Hi and Spn isolates.

Case Ascertainment:
Data will be collected from all 'potential' or suspected cases of Hib and Spn infections using standardized forms. Each form will be reviewed by the study physician who will make the final categorization.

Data collection, management and analysis:
Relevant demographic, clinical, epidemiological, and microbiological data will be collected from all 'potential' cases using standardized forms. All data will be entered onto a standardized computer database.

Data will be analyzed to quantify the magnitude of Hi and Spn infections and to determine the clinical and epidemiological characteristics of bacterial pneumonia and meningitis. The serotypes and antimicrobial sensitivity patterns of Hib and Spn isolates will be described.

B. HOSPITAL SURVEILLANCE

Study Design:
Initially it will be a limited surveillance system and will be conducted in two major hospitals of Dhaka city, the capital of Bangladesh and therefore no attempt will be made that would reflect the population distribution of the country. It is assumed that antibiotic resistance will be highest in urban areas because of more antibiotic use and greater pressure on bacterial populations to develop resistance.

This study will address the following research questions:

1. What is the prevalence of invasive Hi and Spn diseases among children under five years of age attending an urban academic and a diarrhoeal diseases hospitals in Dhaka?
2. Do serotype distributions of Hi and Spn differ in the two selected institutions participating in the study?
3. Do antimicrobial resistance patterns of Hi and Spn isolates from the two institutions differ?
4. What are the clinical and epidemiological factors associated with invasive Spn & Hib infection among the under 5 children in urban Dhaka, Bangladesh?

Reference Population:
Children aged <5 years of age of Metropolitan Dhaka city.

Source Population:
Patients under 5 years of age attending the following two hospitals with pneumonia and meningitis:

1) Dhaka Hospital of ICDDR,B;
2) Dhaka medical college hospital.

Majority of the pediatric patients from Dhaka metropolitan city, the capital of Bangladesh attend these hospitals. Therefore, the source population will be a reasonably representative samples of under 5 population in Dhaka seeking medical care in the hospitals for pneumonia and meningitis.

Sampling frame:
This will be composed of a list of all pneumonia and meningitis patients aged under 5 years of age attending the ICDDR,B Dhaka hospital and Dhaka Medical College Hospital during the whole study period.

Study subjects:
Subjects who will be admitted in the hospital (ICDDR,B hospital and Dhaka Medical College Hospital) with symptoms and signs suggestive of meningitis or ALRI with no history of prior use of antibiotics will be eligible for enrollment in the study.

The following operational definitions will be used:

1. ALRI:
Cough and one or more of the following signs:

- respiratory rate >50/min in children of 2-12 months and 40/ min in 12 months to 5 years,
- chest in drawing
- rales on auscultation,
- stridor and
- cyanosis.

2. Meningitis:
Although the symptoms and signs of meningitis in children <5 years of age are non-specific and difficult to assess, however the following criteria will be used identify meningitis cases: A child with fever, irritabllity or lethargy with one or more of the followings:

- Bulging or full frontanel
- Convulsion (focal or generalized)
- Nuchal regidity
- Positive Kerning or Brudzinki sign
- Reduced level of conciousness
- Anorexia or poor feeding
- Perpura, petichiae or erythematous inacular rash

But the diagnosis will be confirmed by:

a) Positive CSF culture and/or presence neutrophil count in CSF (>2 per HPF) and reduced glucose (<30 mg per dl) and high protein in CSF (>100 mg per dl).

The final study subjects are:
Chest roentgenogram compatible with pneumonia will be the final study subjects for pneumonia and CSF finding >2 PMN per cubic mm for meningitis.

Case management: The cases will be managed as per WHO standard.

Data collection:
Data in this study will come from two sources:

1. From admission records
2. Laboratory:
   a) Blood culture and sensitivity test
   b) CSF culture and sensitivity test
   c) Nasopharegeal culture (from 15% of sub-sample).

The guidelines for specimen collection and laboratory procedures are mentioned in detail in the laboratory component of the protocol.

Sample size:
In a study with multiple objectives, sample size is estimated on largest sample needed to accomplish any of the objectives. If we assume a rate of resistance of 30% (co-trimoxazole resistance rate based on preliminary data obtained from sterile site isolates from Shishu Hospital and consider a difference of greater than +/-10% to be significant, then we need to collect 84 isolates. Data from previous studies from Shishu Hospital indicate that the yield of CSF cultures in bacterial meningitis is likely to be 32% for S. *pneumoniae*. Thus, about 263 children with bacterial meningitis will be needed.

If we assume a rate of isolation of 6% for Spn from blood cultures in pneumonia patients then 1,400 pneumonia cases will be needed. Assuming that 20% of the cases will be lost to follow-up, 316 meningitis cases and 1,680 pneumonia cases will be enrolled. These estimates are presented in Table 1 and 2.

Table 1: Sample size estimates for antimicrobial resistance surveillance study.

Disease	Clinical specimens	No. of isolates	# Cases
Pneumonia	Blood	84	1,680
Meningitis	CSF/Blood	84	316

Table 2. Distribution of study subjects in different study hospitals

Institute	Pneumonia	Meningitis
ICDDR,B	840	60
Dhaka Medical College Hospital	840	256
Total	1,680	316

Enrolment of study subjects:
Table 2 shows the number of pneumonia and meningitis cases to be enrolled in the study from the two participating hospitals. The study physician will enrol cases from the participating hospitals every other week. To ensure enrollment of the required number of pneumonia cases from each of the participating hospitals, eleven pneumonia cases will have to be enrolled per week from each of the two hospitals. For meningitis one case per week will have to be enrolled from the ICDDR,B hospital and 3-4 cases from the Dhaka Medical College Hospital. The study physician will enroll the first two cases of pneumonia that are admitted to the hospital everyday on the surveillance days and all the cases with meningitis from ICDDR,B and Dhaka Medical College Hospital until the weekly quota is fulfilled.

Data management and analysis:
Because of the large amount of data that will be generated by surveillance, a computerized system of record management using a database programme will be used. Data will be analyzed in two ways:

1. Analysis of antimicrobial resistance which will be done as follows:
    a) The overall yield of cultures and proportions positive for Spn and Hi.
    b) The overall rate of resistance (Spn and Hi) to each antibiotic tested.
    c) The separate rates of resistance for Spn and Hi to each antibiotic tested
    d) Rates of resistance between two urban hospitals.
    e) The clinical and epidemiological factors associted with positive cultures such as the subjects' age, time of year, severity of pneumonia, presence of fever, nutritional status.

C. LABORATORY METHODS:

Identification of bacterial pathogens:
Clinical specimens will be cultured on tryptic soy agar with 5% sheep blood and chocolate agar supplemented with X and V factors and relevant pathogens will be identified by standard methods (23). Blood (1-2 ml) will be cultured in 20 ml of tryptic soy broth (TSB) containing 0.025% sodium polyanetholesulfonate (SPS) and haematin (10 mcg ml). Spn will be identified by colonial morphology, Gram stain, ∝-haemolysis on blood agar plates, susceptibility to optochin and/or bile solubility. Hib will be identified by typical morphology by Gram stain and requirement of X and V factors. All CSF will be tested for Spn and Hib by latex agglutination test (Wellcome, UK). All CSF will be stored at -70°C for subsequent detection of Spn and Hib by multiplex PCR (48).

Antimicrobial susceptibility testing:
Antimicrobial susceptibility will be determined by disk diffusion method by inoculating a bacterial suspension of 0.5% McFarland standard on Mueller-Hinton agar (MHA) with 5% sheep blood for Spn and Heart Infusion agar with NAD (5ug/ml) plus 1% haemoglobin (or haemin 0.04%) for Hi. Penicillin, oxacillin, ampicillin, co-trimoxazole, gentamicin, chloramphenicol, erythromycin, rifampicin and ceftrioxone discs will be used.

Preservation and transportation of S. *pneumoniae* and *H.influenzae*:
Hi and Spn isolates will be preserved for short-term storage on chocolate agar slant and will be transported twice a month to ICDDR,B laboratory where long-term storage will be accomplished by lyophilization.

Determination of MIC:
It will be determined for all Hi and Spn isolates by E-test and agar dilution method in ICDDR,B's Dhaka laboratory using standard technique.

Serotyping of isolates:
Serogrouping will be done by Quelling reaction (antisera from Statens Serum Institut, Denmark) and further classification will be performed by capsular swelling with type-specific antisera with the help of a reference laboratory (WHO). Serotyping of Hi will be performed by slide agglutination test using reagents from Wellcome.

PCR for Detection of *S. pneumoniae* and *H. influenzae* type b:
A multiplex PCR will be performed with blood samples collected from 20% (665) of hospitalized pneumonia cases and with all 346 heat-treated (15 minute boiling) CSF samples.

A multiplex PCR will be performed with target DNA extracted from EDTA mixed venous blood and finger prick blood. 250 μl. of venous blood will be collected from EDTA vial (which will be used for WBC counting), placed at 4^0C for 6-24 hours before being stored at -18^0C until processing for DNA extraction.

0.2 ml of finger-prick blood will be collected and processed by the same technique as venous blood for PCR.

Purification of whole DNA from blood sample: DNA will be purified for PCR with the QIAmp blood kit (QIAgen, Chatsworth, CA) and standard phenol chloroform extraction method.

Primers and Probes: A pair of primers P_1 and P_2 (48) will be derived from conserved areas of penicillin protein 2B gene of Spn. A probe (45 bp) derived from the PCR amplified product will be used to detect PCR product in the southern blot in addition to agarose gel electrophoreases.

Another pair (P^3 & P^4) of primers derived from Hib capsular gene will also be used to detect Hib. (48)

PCR amplification: All PCR amplifications will be performed in 0.1 ml volume using 2U of Taq polymerase (Perkin-Elmer) and primers (10 pmol each) for each tube. PCR will be performed according to standard protocol.

Detection of PCR products: PCR products will be detected by agarose gel-electrophoresis and auto-radiography of Southern gel hybridization.

Clonal relationship among the isolates:
Pulse field gel electrophoresis (PFGE) and PCR will be used to detect the clonal origin of similar serogroup/serotype, resistant/MDR isolates and non-typeable isolates (34).

Culture and nasopharyngeal aspirates:
Nasopharyngeal aspirates will be cultured from 15% (563 cases) of all pneumonia cases. The swab will be stored at -80^{0}C for further studies.

Quality Assurance of laboratory methods:
Laboratory methods will be periodically assessed throughout the study. To ensure quality, paired samples will be taken from a sub-set of the cases and the second set of specimens will be sent to a reference laboratory.

Ten percent of clinical isolates of Spn and Hi received from participating hospitals/clinics will be retested in ICDDR,B's central laboratory in Dhaka for identification and all will be subjected to antimicrobial susceptibility testing.

Reference laboratory:
An appropriate reference laboratory will be identified in consultation with the international collaborator, Dr. Mark Steinhoff of the Johns Hopkins University. The reference laboratory will be requested to perform serotyping and antimicrobial susceptibility testing of 10% of the isolates.

Establishment of Surveillance Network: ICDDR,B, Dhaka ARI laboratory will work as central (Referral) laboratory, will provide necessary training to personnel in other participating laboratories, and will coordinate the activities of all the laboratories in collection, processing, stocking and testing of all isolates for antimicrobial susceptibility (MIC) and serotyping. PIs and research physician, ICDDR,B will help the investigators to coordinate all the activities of other hospitals (centres)/laboratories for smooth functioning of the project.

DISSEMINATION AND TECHNICAL ASSISTANCE

Timely dissemination of the findings from the project, technical assistance to build the capacity of the national institutions and improved use of data for policy decisions will be important priorities of the project. To this end, mechanisms of coordination with the government of Bangladesh (GoB) will be developed. A Steering Committee involving concerned officials from the MOHFW, National CDD and ARI Projects, National Tuberculosis Programme, Bangladesh AIDS Prevention and Control Programme (BAPCP) and the Institute of Epidemiology Disease Control and Research (IEDCR), USAID, NIPHP and other development partners (e.g. WHO, UNICEF) with key ICDDR,B researchers in order to facilitate greater ownership by GoB and thereby facilitate timely application of the results of the studies into national programmes. A Core Group for ARI will be established by coopting appropriate representatives of all interested parties. The Core Group will organize regular workshops, seminars, and meetings with other health care providers, program managers and policy makers to share with them the various findings from the Project. The project will prepare quarterly summary reports with policy recommendations which will be shared with the Ministry of Health and Family Welfare (MOHFW), particularly with the ARI control program to adopt appropriate policy changes and pursue their program in an effective way. As professionals from national institutes are investigators of the project, the findings of the

project can easily be disseminated to teachers, students and other health professionals in Bangladesh and other countries for appropriate use. In addition, the findings will be published in regional and international peer-reviewed journals.

Timely dissemination of findings will be particularly useful in selecting appropriate antimicrobial therapy for Hib and Spn infections. The serotype pattern will help to select and formulate protein-conjugate vaccine, particularly against Spn and evaluate existing and new vaccines against these two important pathogens.

FACILITIES AVAILABLE

The International Centre for Diarrhoeal Disease Research, Bangladesh has all kinds of facilities to carry out research work in Clinical Microbiology, Molecular Biology, Immunology, Virology, Clinical Biochemistry, etc., in different laboratories (sections) equipped with modern instruments and carried out by expertise of the respective subjects. This laboratory will work as in-country reference laboratory in addition to one of the research centre for the proposed study. The serotyping, antimicrobial susceptibility testing and verification (retesting) of 10% of the isolates of Spn and Hi will be carried out in ICDDR,B.

The facilities for bacterial culture exists in the Dhaka Medical College Hospital, and ICDDR,B's Matlab centre, which will be improved further through the proposed study by supplying laboratory equipments, incubator, refrigerator small autoclave as required, training manpower and offering consultative service.

Time frame:

1st month: Visit of PIs to all hospitals & appointment of staff in ICDDR,B, meeting of PIs and Co-PIs, developing working protocol, laboratory manual, and ordering laboratory materials.

2nd month: Appointment of staff in other hospitals and meetings of PIs, Co-PIs and research physicians. Training of field, hospital and laboratory staff and research physicians.

3rd month: 1st week: Trial period for sample collection and processing.
2nd week: Start enrolling cases for the study.

4th month-36 month: Continue surveillance, laboratory activities, data processing, data analysis, report writing and regular dissemination of findings.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Ethical Implications

Surveillance results of detection and susceptibility testing of Spn and Hi isolates from clinical samples will provide direct benefit to the patient by facilitating specific therapy for the pathogen identified. No invasive procedure will be carried out on the patients in this study.

USE OF ANIMALS

No animal will be used in the proposed study.

COLLABORATIVE ARRANGEMENTS

It is a collaborative study involving ICDDR,B, and one Government Medical College located in Dhaka to collect representative data on infections caused by Spn and Hi and their pattern of serotype distribution and antimicrobial susceptibility. Within ICDDR,B, there will be a collaboration between the Public Health Sciences, Clinical Sciences and Laboratory Sciences Divisions. . It will be a collaborative study with Johns Hopkins University, Baltimore, Maryland.

Responsibilities of Principal Investigators:

Dr. Mahbubur Rahman will have overall responsibility for the laboratory component of the study and Dr. John Albert will provide consultative microbiological services.

Dr. Shahadat Hossain will have overall responsibility for the hospital surveillance component.

Dr. Abdullah H. Baqui will have overall responsibility of the community component of the study.

The PIs will meet once a week to plan and coordinate, and implement the study activities.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Surveillance of invasive Haemophilus influenzae (Hi) and Streptococcus Pneumoniae (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh

Budget Details

for Year-1

SALARY

Position	Pay Level	# of Staff	% of effort	Monthly Rate	May-Dec 1998	Jan-Apr 1999	Year-1 Total
LABORTORY							
Dr. Mahbubur Rahman	NO-C/6	1	75%	1,232	7,392	3,696	11,088
Dr. M. J. Albert	P-6	1	15%	10,188	12,225	6,113	18,338
Dr. Motiur Rahman	NO-B	1	10%	833	667	333	1,000
Research Officer (Lab)	GS-5/1	2	100%	347	5,552	2,776	8,328
Laboratory Attendant	GS-1/1	2	100%	170	2,720	1,360	4,080
Programmer	GS-6/1	1	100%	452	3,616	1,808	5,424
DMC (Lab)					2,313	1,157	3,470
Total Salary (Laboratory)					34,485	17,243	51,728
HOSPITAL							
Dr. Shahadat Hossain	NO-C/11	1	50%	1,437	5,748	2,874	8,622
Dr. M. M. Rahman	NO-C/6	1	20%	1,232	1,971	986	2,957
Medical Officer	NO-A/1	1	100%	632	5,056	2,528	7,584
Attendant	GS-1/1	1	100%	170	1,360	680	2,040
Health Assistants	GS-3/1	2	100%	224	3,584	1,792	5,376
Data Mgt.Assistant (Gr-II)	GS-3/1	1	100%	224	1,792	896	2,688
Secretarial services	GS-6/11	1	15%	675	810	405	1,215
DMC (hospital)					2,413	1,207	3,620
Total Salary (Hospital)					22,734	11,367	34,101
POPULATION-BASED							
Dr. A. H. Baqui	P-5/6	1	20%	9,633	15,413	7,706	23,119
Study Physician	NO-A/1	1	100%	632	5,056	2,528	7,584
Dr. Md. Yunus	NO-D/21	1	5%	2,125	850	425	1,275
Mr. J. Chakrabarty	NO-C/7	1	5%	1,370	548	274	822
Data Analyst/Manager	NO-A/1	1	50%	632	2,528	1,264	3,792
Research Officer (Field)	GS-5/1	1	100%	347	2,776	1,388	4,164
Sr. Health Assistant (LFPV)	GS-4/1	4	50%	268	4,288	2,144	6,432
Health Assistants	GS-3/1	1	100%	224	1,792	896	2,688
Secretary	GS-6/7	1	25%	600	1,200	600	1,800
Data Entry Technicians	GS-3/1	1	100%	224	1,792	896	2,688
Community Health Workers	Spl Level	60	20%	75	7,200	3,600	10,800
Total Salary (Population-based)					43,443	21,721	65,164

TRAVEL:

Local	2,667	1,333	4,000
International	0	2,500	2,500
TOTAL TRAVEL COSTS:	2,667	3,833	6,500

INTER DEPARTMENTAL COS	#	Unit Price ($)	May-Dec 1998	Jan-Apr 1999	Year-1 Total
Blood C/S	5000	5.6	6,222	3,111	9,333
CBC	3000	2.3	1,533	767	2,300
N/P	1000	3.5	778	389	1,167
CSF C/S	400	3.5	311	156	467
CSF: (Cytology gm. staining bio-chemistry)	400	6.0	533	267	800
x-ray chest	3000	2.0	1,333	667	2,000
Sensitivity test	780	3.0	520	260	780
Serotyping			2,444	1,222	3,667
	TOTAL INTER-DEPARTMENTAL COSTS:		13,676	6,838	20,513
SUPPLIES:					
Laboratory Supplies			8,000	4,000	12,000
Hospital Supplies:					
Syringe			111	56	167
T/A tube			222	111	333
Dupont			2,311	1,156	3,467
Drugs			7,289	3,644	10,933
Office Supplies:			889	444	1,333
	TOTAL SUPPLIES:		18,822	9,411	28,233
OTHER DIRECT COSTS:					
Printing			1,333	667	2,000
Xerox			667	333	1,000
Rent, comm. & Utilities			556	278	833
	TOTAL OTHER DIRECT COSTS:		2,556	1,278	3,833
CAPITAL ITEM:					
Computer	3	3,000	6,000	3,000	9,000
Refrizerator	4	625	1,250	1,250	2,500
Incubator	1	4,000	0	4,000	4,000
Culture Plate Dryer	1	4,000	4,000	0	4,000
Autoclave	1	5,000	5,000	0	5,000
Deep freezer (-80)	1	10,000	10,000	0	10,000
Electrical Balance	1	500	500	0	500
PH Meter	1	1,000	1,000	0	1,000
Centrifuse machine	1	4,000	0	4,000	4,000
Electronic Balance	1	1,500	1,500	0	1,500
Ophthalmascope	3	500	1,000	500	1,500
Software (statistical package)		3,000	1,500	1,500	3,000
Portable x-ray machine	1	6,000	6,000	0	6,000
Pulse oximeter	3	600	1,800	0	1,800
	TOTAL CAPITAL COSTS:		39,550	14,250	53,800
TOTAL DIRECT COSTS:			177,932	85,941	263,874
OVERHEAD @25%			44,483	21,485	65,968
TOTAL PROJECT COSTS:			222,416	107,427	329,842

Surveillance of invasive Haemophilus influenzae (Hi) and Streptococcus Pneumoniae (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh

Summary budget for 3 years

	Year 1			Year 2	Year 3	Total
	May'98- Dec'98	Jan'99- Apr'99	May 1998 - Apr 1999	May 1999 - Apr 2000	May 2000 - Apr 2001	May 1998 - Apr 2001
SALARY						
Salary (Laboratory)	34,485	17,243	51,728	54,315	57,031	163,074
Salary (Hospital)	22,734	11,367	34,101	35,807	37,597	107,506
Salary (Population-based)	43,443	21,721	65,164	68,422	71,844	205,430
TRAVEL:						
Local	2,667	1,333	4,000	4,000	4,000	12,000
International	0	2,500	2,500	5,000	5,000	12,500
INTER DEPARTMENTAL COSTS	13,676	6,837	20,513	20,513	20,513	61,540
SUPPLIES & MATERIALS	18,822	9,411	28,233	23,233	22,234	73,700
OTHER DIRECT COSTS	2,556	1,277	3,833	3,833	3,833	11,500
CAPITAL COSTS	39,550	14,250	53,800	0	0	53,800
TOTAL DIRECT COSTS:	177,934	85,940	263,874	215,123	222,052	701,050
OVERHEAD @25%	44,484	21,485	65,969	53,781	55,513	175,262
TOTAL PROJECT COSTS:	222,418	107,425	329,843	268,904	277,566	876,312

Surveillance of invasive Haemophilus influenzae (Hi) and Streptococcus Pneumoniae (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh

Budget Details

for 3 years

SALARY

Position	Pay Level	# of Staff	% of effort	Monthly Rate	1st Yr. 1998-99	2nd Yr. 1999-2000	3rd Yr. 2000-2001	Sub-total	Total (US $)
LABORTORY									
Dr. Mahbubur Rahman	NO-C/6	1	75%	1,232	11,088	11,642	12,225	34,955	
Dr. M. J. Albert	P-6	1	15%	10,188	18,338	19,255	20,218	57,812	
Dr. Motiur Rahman	NO-B	1	10%	833	1,000	1,050	1,103	3,153	
Research Officer (Lab)	GS-5/1	2	100%	347	8,328	8,744	9,182	26,254	
Laboratory Attendant	GS-1/1	2	100%	170	4,080	4,284	4,498	12,862	
Programmer	GS-6/1	1	100%	452	5,424	5,695	5,980	17,099	
DMC (Lab)					3,470	3,644	3,826	10,939	
Total Salary (Laboratory)					51,728	54,315	57,031	163,074	
HOSPITAL									
Dr. Shahadat Hossain	NO-C/11	1	50%	1,437	8,622	9,053	9,506	27,181	
Dr. M. M. Rahman	NO-C/6	1	20%	1,232	2,957	3,105	3,260	9,321	
Medical Officer	NO-A/1	1	100%	632	7,584	7,963	8,361	23,909	
Attendant	GS-1/1	1	100%	170	2,040	2,142	2,249	6,431	
Health Assistants	GS-3/1	2	100%	224	5,376	5,645	5,927	16,948	
Data Mgt. Assistant(Gr-II)	GS-3/1	1	100%	224	2,688	2,822	2,964	8,474	
Secretarial services	GS-6/11	1	15%	675	1,215	1,276	1,340	3,830	
DMC (hospital)					3,620	3,801	3,991	11,412	
Total Salary (Hospital)					34,102	35,807	37,597	107,506	
POPULATION-BASED									
Dr. A. H. Baqui	P-5/6	1	20%	9,633	23,119	24,275	25,489	72,883	
Study Physician	NO-A/1	1	100%	632	7,584	7,963	8,361	23,909	
Dr. Md. Yunus	NO-D/2	1	5%	2,125	1,275	1,339	1,406	4,019	
Mr. J. Chakrabarty	NO-C/7	1	5%	1,370	822	863	906	2,591	
Data Analyst/Manager	NO-A/1	1	50%	632	3,792	3,982	4,181	11,954	
Research Officer (Field)	GS-5/1	1	100%	347	4,164	4,372	4,591	13,127	
Sr. Health Assistant (LFPV)	GS-4/1	4	50%	268	6,432	6,754	7,091	20,277	
Health Assistants	GS-3/1	1	100%	224	2,688	2,822	2,964	8,474	
Secretary	GS-6/7	1	25%	600	1,800	1,890	1,985	5,675	
Data Mgt. Assistant Gr-II	GS-3/1	1	100%	224	2,688	2,822	2,964	8,474	
Community Health Workers	Spl Level	60	20%	75	10,800	11,340	11,907	34,047	
Total Salary (Population-based)					65,164	68,422	71,844	205,430	
	TOTAL SALARY:				150,994	158,544	166,471		476,010
TRAVEL:									
Local					4,000	4,000	4,000	12,000	
International					2,500	5,000	5,000	12,500	
	TOTAL TRAVEL COSTS:				6,500	9,000	9,000		24,500

INTER DEPARTMENTAL COS	#	Unit Price ($)	1st Yr. 1998-99	2nd Yr. 1999-2000	3rd Yr. 2000-2001	Sub-total	Total (US $)
Blood C/S	5000	5.6	9,333	9,333	9,333	28,000	
CBC	3000	2.3	2,300	2,300	2,300	6,900	
N/P	1000	3.5	1,167	1,167	1,167	3,500	
CSF C/S	400	3.5	467	467	467	1,400	
CSF: (Cytology gm. staining bio-chemistry)	400	6.0	800	800	800	2,400	
x-ray chest	3000	2.0	2,000	2,000	2,000	6,000	
Sensitivity test	780	3.0	780	780	780	2,340	
Serotyping			3,667	3,667	3,667	11,000	
TOTAL INTER-DEPARTMENTAL COSTS:			20,513	20,513	20,513		61,540
SUPPLIES:							
Laboratory Supplies			12,000	7,000	6,000	25,000	
Hospital Supplies:							
Syringe			167	167	167	500	
T/A tube			333	333	333	1,000	
Dupont			3,467	3,467	3,467	10,400	
Drugs			10,933	10,933	10,933	32,800	
Office Supplies:			1,333	1,333	1,333	4,000	
TOTAL SUPPLIES:			28,233	23,233	22,233		73,700
OTHER DIRECT COSTS:							
Printing			2,000	2,000	2,000	6,000	
Xerox			1,000	1,000	1,000	3,000	
Rent, comm. & Utilities			833	833	833	2,500	
TOTAL OTHER DIRECT COSTS:			3,833	3,833	3,833		11,500
CAPITAL ITEM:							
Computer	3	3,000	9,000	0	0	9,000	
Refrizerator	4	625	2,500	0	0	2,500	
Incubator	1	4,000	4,000	0	0	4,000	
Culture plate dryer	1	4,000	4,000	0	0	4,000	
Autoclave	1	5,000	5,000	0	0	5,000	
Deep freezer (-80)	1	10,000	10,000	0	0	10,000	
Electrical balance	1	500	500	0	0	500	
PH meter	1	1,000	1,000	0	0	1,000	
Centrifuse machine	1	4,000	4,000	0	0	4,000	
Electric balance	1	1,500	1,500	0	0	1,500	
Ophthalmascope	3	500	1,500	0	0	1,500	
Software (statistical package)			3,000	0	0	3,000	
Portable x-ray machine	1	6,000	6,000	0	0	6,000	
Pulse oximeter	3	600	1,800	0	0	1,800	
TOTAL CAPITAL COSTS:			53,800	0	0		53,800
TOTAL DIRECT COSTS:			263,874	215,124	222,051		701,050
OVERHEAD @25%			65,969	53,781	55,513		175,262
TOTAL PROJECT COSTS:			329,843	268,905	277,564		876,312
Assumed inflation rate:				5%	5%		

(1)

Title of protocol:
Surveillance of invasive Haemophilus influenzae (Hi) and Streptococcus pneumoniae (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh .

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget			
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification ☐

- on technical grounds ☐
- on level of financial support ☐

REPORT ATTACHED.

I do not support the application ☐

Name of Referee:

Signature:
Position:

Institution:

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title: Studies on the Capsule of Vibrio cholerae O139 Bengal

PI:

Reviewer: .....

See attached sheet

REFEREES REPORT
RESEARCH PROTOCOL

Title of protocol: Surveillance of invasive *Haemophilus influenzae (Hi)* and *Streptococcus pneuoniae* (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh.

The identification of the burden of disease caused by these two important pathogens of children is a high priority. The designed study should answer this question and provide Bangladesh with useful information about the serotypes and susceptibility patterns of the isolates. The success of the study is entirely dependent on the ability of the investigators to culture the pathogens and perform antimicrobial susceptibility testing of the highest possible standards. Prior treatment of children with antibiotics will greatly reduce the yield of organisms. All attempts should be made to take the cultures prior to administration of antibiotic. Before embarking on expenditure of this magnitude I believe it would be prudent to assure oneself of the quality control issues inherent is such a study. I am aware that there is a proposal for on-going quality control during the study, but I believe that the laboratory should ab initio document their ability to grow the pathogens from blood. They may already have done so. They also must document their ability to achieve susceptibility results equivalent to those in a reference laboratory. My recommendation therefore is that these quality aspects should be confirmed prior to the start of the study as well as during the course of the study.

The two primary end-points of the study are documentation of serotypes and the documentation of susceptibility patterns. The serotype data will allow a measure of confidence in the design of future pneumococcal conjugate intervention vaccine trials in Bangladesh and the identification of *Haemophilus influenzae* type B might give sufficient information to authorities to help them decide on the potential introduction of HIB vaccine in the future.

The interpretation of the antimicrobial resistance data is more problematic. Based on studies elsewhere there is inconclusive evidence about the relevance of resistance to cotrimoxazole in the management of pneumonia. There are more compelling data about the problems of treating pneumococcal meningitis and *Haemophilus influenzae* meningitis when the pathogens are resistant to penicillin. I would argue that consideration should be given to collecting outcomes as part of this study. It appears that a computerized database exists which may allow for the collection of these data and the study could well be strengthened by the ability of the investigators to correlate outcomes with the identification of resistant pathogens. The most important information required by the

managers of your ARI program would be the clinical impact of cotrimoxazole resistance on the management of pneumonia and the clinical impact of penicillin and/or chloramphenicol resistance on the outcome of meningitis.

Taking into consideration the comments that I have made above, I strongly support the performance of the study. The burden of ARI compared to the amount of funding in this area represents one of the largest gaps in the investigation of burden of disease in children and I would strongly support the ICDDR in its efforts to not only provide information useful to the managers of the ARI program, but also data that are potentially useful to help professionals dealing with ARI everywhere.

(2)

Title of protocol:
Surveillance of invasive Haemophilus influenzae (Hi) and Streptococcus pneumoniae (Spn) diseases in Bangladeshi children and the antimicrobial resistance and serotype patterns of Hi and Spn isolates in Bangladesh

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project			
Adequacy of Project Design			
Suitability of Methodology			
Feasibility within time period			
Appropriateness of budget			
Potential value of field of knowledge			

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification ☐

- on technical grounds ☑
- on level of financial support ☑

I do not support the application ☐

Name of Referee:

Signature
Position:

Institution

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title:

PI:

Reviewer: .....

See attached sheet

Fax to John Albert
Lab Sciences Division
May 1, 1998

Dear John,
You asked me to review the proposal on Surveillance of H.Flu and S. pneumoniae.

This is clearly an important area and ICDDR(B) are well placed to provide critical data to guide both clinical and public health policy. Therefore overall I strongly support the proposal. However there are a number of points that require attention.

1. The assumption that 50% of severe cases will be positive (I presume on blood culture) for one of the organisms seems extremely high. Most labs only manage to get a positive culture from less than 20%. If this refers to PCR then it is not the critical issue since one can not get antibiotic resistance or serotyping from PCR (at least not without some very sophisticated lab work). On this basis I can not see the justification for only culturing 20% of samples. If a more conservative estimate of isolation rates is used then you would need to culture more than half, and preferably all, isolates.
2. The issue of Septrin treatment of ALRI by CHNs is only briefly mentioned. This is standard practice in Matlab and is likely to have a major impact on isolation rates. It would be worth considering examining urine from cases for evidence of antibiotic activity - this can be done relatively simply using fully sensitive organisms as test strains. At least this would give some estimate of the likely under diagnosis due to antibiotic usage.
3. The use of the ICDDR hospital in Dhaka is likely to lead to a very biased selection of subjects for study. Essentially all children coming to that hospital have diarrhoea - hardly a typical presentation of pneumonia or diarrhoea.
4. The budget is very large. I can see that this is because so much of the Matlab infrastructure is being under-written from it.

I therefore support the application with qualification on both technical and financial grounds.

I will also mail this report

Reader