Protocol for Evaluating the Impact of Pneumonia and Respiratory Syncytial Virus (RSV) Infections on Healthcare Systems in Bangladesh

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Prevent Infections, Save Lives

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1. Background

Over the last decade, the global community has achieved a notable reduction in childhood pneumonia through the introduction of vaccines against *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae*. Despite these advancements, pneumonia remains a significant global health challenge. In Bangladesh, pneumonia is estimated to cause 2.7 million illnesses and 21,274 deaths annually among children under five. The introduction of the Hib vaccine in 2009 had a significant impact on child health (1). Similarly, the introduction of the 10-valent pneumococcal conjugate vaccine (PCV10), locally known as the "pneumonia" vaccine, in 2015 is expected to further reduce disease burden, particularly pneumonia, in the coming years. However, the impact of PCV10, which targets 10 pneumococcal serotypes, may be less dramatic than that of the Hib vaccine due to the presence of approximately 50 invasive pneumococcal serotypes circulating in Bangladesh.

In addition to bacterial causes, pneumonia can also be caused by several viral pathogens, yet there is a paucity of credible estimates of their burden. Existing studies have attributed four viruses, RSV, Influenza, Parainfluenza, and Human Metapneumovirus, to half of all childhood pneumonia cases in Bangladesh, with RSV being the predominant cause (2,3). RSV is also a significant cause of sepsis in children under six months of age and the leading cause of neonatal sepsis, as documented in a recent publication from the largest community-based surveillance of neonatal sepsis (ANISA) (2).

Beyond the mortality, disability, and suffering caused by pneumonia and RSV infections, the burden on hospital resources, particularly bed occupancy, exacerbates healthcare challenges. In Dhaka Shishu Hospital (DSH), the largest pediatric hospital in Bangladesh, pneumonia is a leading cause of hospitalization and bed occupancy. Between November 2015 and October 2016, 3,448 out of 23,064 (15%) admissions were for pneumonia, illustrating its significant burden on the healthcare system. Additionally, approximately 3,500 admissions during this period were due to sepsis in children under six months of age. Concurrently, 5,879 cases requiring hospitalization were refused admission due to a lack of available beds. Among the

refused cases, the most common conditions included pneumonia (22%), severe perinatal asphyxia (17%), preterm birth complications (7%), and meningitis (2%), all requiring immediate care.

Timely access to appropriate care significantly improves survival rates for children with severe pneumonia. Similarly, children with intrapartum-related complications who receive care are more likely to survive without long-term sequelae. However, amidst fierce competition for hospital beds, pneumonia and RSV infections impose both direct and indirect burdens on the healthcare system.

In scenarios where hospital beds are perpetually full and patients are frequently denied admission, understanding the patterns of refusals and bed occupancy is critical. Such an analysis provides a clearer picture of the true burden (both direct and indirect) of a disease and enables an accurate evaluation of the impact of vaccines. Without this understanding, the reduction in disease incidence due to vaccines may not be adequately reflected in hospital admissions, leading to an underestimation of vaccine benefits due to the buffering effect of previously refused cases.

2. Study Objectives

i. To estimate the burden of RSV-infections in children hospitalized in DSH and hence on bed-occupancy
ii. To elucidate the impact of admission refusal on health of the children who are refused hospitalization due to lack of beds
iii. To elucidate the trend in pneumonia cases in IPD of DSH and cases refused admission since introduction of PCV-10
iv. To determine the nasopharyngeal carriage of pneumococcus in children with pneumonia

3. Methodology

A. Study Setting

This study will be conducted at Dhaka Shishu Hospital (DSH), the largest tertiary pediatric hospital in Bangladesh, which provides care for children from across the country. The hospital's high patient volume and limited resources make it an ideal setting for understanding the burden of RSV and pneumonia on healthcare systems. DSH is located in the capital of Bangladesh, and is a specialized hospital providing treatment of sick children at tertiary level. The hospital is under the administration of the Government of Bangladesh and consists of about 650 beds, of which 37% are free for poor patients. Department of Microbiology at Dhaka has state-of-the-art laboratory facilities which are capable of routine culture and detection of bacterial antigens by latex or BinaxNOW (Immuno-chromatographic test) or real-time polymerase chain reaction (PCR), and viral etiology by real-time PCR. The laboratory also has the capacity to do serotyping of pneumococcus. This laboratory also has facilities for the preservation of samples and isolates at -80^0C.

B. Study Design

This prospective observational study aims to collect demographic data (including name, age, gender, and clinical syndrome), clinical data (such as disease symptoms and prognosis), and laboratory data (identifying bacterial and viral etiologies) for patients meeting the inclusion criteria based on the WHO RSV hospital-based surveillance case definition. Data will be entered in real-time using a tablet-based system. Research physicians stationed in the pediatric wards will identify eligible children upon admission. Epidemiology and administrative coordinators will oversee all activities to ensure proper specimen collection, transportation, laboratory processing, and the accurate completion of specimen forms. Physicians will also record patient outcomes, including mortality, complications, treatment details, and hospitalization duration, using a standardized data collection tool. Additionally, research assistants stationed in the emergency room will gather information on hospital refusals and conduct follow-up phone calls with families to track disease outcomes.

C. Study Population

Children aged 0–59 months who are either admitted to the selected study wards or require hospitalization but are advised to seek care elsewhere due to unavailability of beds.

D. Recruitment of Study Patients in the Inpatient Department (IPD)

Children aged 0–59 months admitted to the selected study wards will be screened by study physicians using the WHO RSV hospital-based surveillance case definition (5). Children meeting the enrollment criteria (fulfilling at least one eligibility criterion and none of the exclusion criteria) will be enrolled in the study after obtaining written informed consent from their caregivers. Trained nurses will collect nasopharyngeal swab samples, which will be tested using established qPCR methods.

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E. Eligibility Criteria

Children 0 – 59 months hospitalized with a respiratory infection, defined as having cough or shortness of breath, with an onset within the last 10 days,

In addition, infants <6 months of age were also eligible if they were hospitalized with

- apnoea, which is characterized by a temporary cessation of breathing from any cause and/or
- sepsis - defined as
    - fever (temperature of 37.5 °C or above) or hypothermia (temperature less than 35.5 °C)
    - shock (lethargy, fast breathing, cold skin, prolonged capillary refill, or a fast, weak pulse)

F. Exclusion Criteria

- Age ≥ 59 months

G. Enrollment and Follow-up

Newly admitted patients will be tracked using the admission logbook from their respective wards. Physicians will take a detailed medical history and perform a clinical examination of each child. Cases will then be screened based on the inclusion and exclusion criteria outlined for the RSV study. Once a patient meets the inclusion criteria, the parents or guardians will be counseled about the study. During this counseling session, the purpose and procedures of the study will be clearly explained, and informed consent will be obtained. Written consent is mandatory for both data collection and nasopharyngeal swab sampling. Enrollment cannot proceed without this written consent. If the parent or guardian refuses to provide consent, the enrollment process will be immediately halted (Figure 1).

After written consent is obtained, the patient will be formally enrolled in the study, and the required data will be collected using a tablet-based data collection system. For each eligible case, the physician will record demographic information (such as age and sex), clinical diagnosis, and case definition onto a standardized data collection form. Physicians will sign the designated area on the consent form. Daily follow-ups will be conducted for each enrolled child in the hospital until discharge, death, referral, or discharge against medical advice (DORB). A research assistant or physician will conduct the follow-up. During these follow-ups, clinical signs, laboratory test results, additional diagnoses, outcomes, and antibiotic treatments will be recorded. The final diagnosis, date of discharge, and outcome will be documented within 24 hours of the patient's discharge.

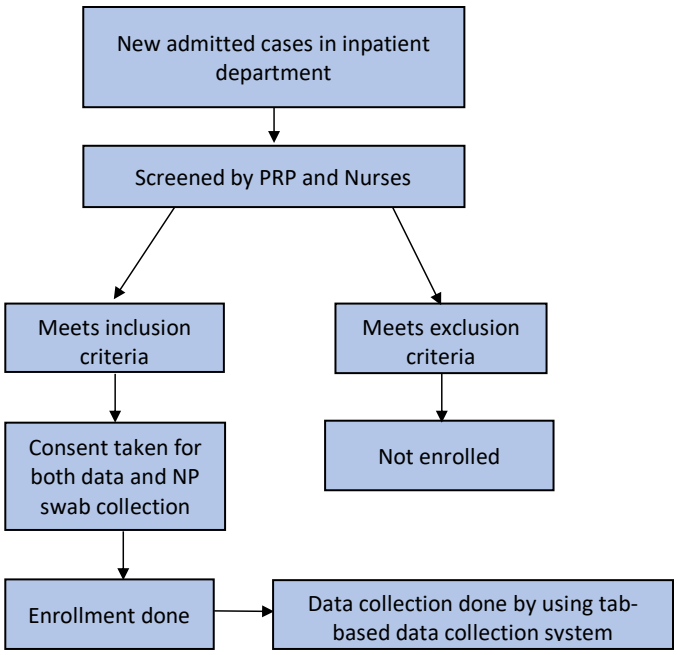

Figure 1: Participant enrollment flow in the IPD

H. Taking Informed Consent from Caregivers

Informed consent is a legal requirement for this study, in which parents or legal guardians provide consent (by signing or providing a thumbprint on a consent form) after gaining a clear understanding of the study protocol, research procedures, risks, anticipated benefits, confidentiality, and other relevant aspects of the project. Written consent must be obtained from one of the caregivers (primarily the mother) before enrolling any participants in the inpatient department (IPD). Caregivers will be provided with an information sheet detailing the study procedures, risks, and benefits. A study physician will explain the study in detail and address any questions from the caregiver. If the caregiver is unable to read or understand the

information sheet, a witness will explain the study and confirm the caregiver's understanding. In such cases, the thumbprint of the parent or legal guardian will be collected, and the witness's signature will also be required to validate the consent process.

For participants in the refusal cohort, verbal consent will be obtained over the phone. Caregivers will be provided with a simplified explanation of the study objectives, the value of the findings, and the participants' role in the study.

I. Enrolling Refusal Cases

Research assistants will approach the caregiver to document the emergency room (ER) physician's diagnosis for patients whose children are refused admission due to bed shortages, despite requiring hospitalization. The research assistant will also seek permission to obtain contact number, address and age of the child to make a follow-up call using the caregiver's telephone number to record the disease outcome of the patient. If the caregiver does not provide permission, no information will be collected. Everything will be done as quickly as possible to ensure minimal delays.

On day 14, research assistants will contact the caregivers of selected patients by telephone to document the child's health status. Before collecting any health information, the caregiver will be reintroduced to the study, including an explanation of the study procedures and assurance of data confidentiality. Verbal consent for the child's participation in the study will be obtained before collecting any information, and it will be noted down. Data will only be collected for the study if the caregiver provides verbal consent for their participation in the study.

J. Selection of Cases for Phone Follow-up

Given the team's capacity for follow-up, a maximum of 15 children admitted to the inpatient department (IPD) will be selected each day for phone follow-up. For refusal cases, up to 20 patients will be selected, as it is anticipated that fewer caregivers from the refusal cohort will

consent to participate compared to those from the IPD. A computer-based algorithm will be used to randomly select participants for follow-up, ensuring an unbiased selection process.

K. Phone Follow-up of the Enrolled Cases

All enrolled patients selected for phone follow-up will be contacted on Day 14 following their hospital admission or refusal to assess their health status. During the Day 14 follow-up call, caregivers will be asked about the child's condition, including any symptoms, recovery progress, complications, hospital readmissions, or other relevant health outcomes. For patients confirmed to be alive on Day 14, an additional follow-up will be conducted on Day 90 to capture longer-term health outcomes. Detailed information regarding the child's vital status, any persistent or new symptoms, illness duration, or other health-related developments will be gathered during the Day 90 follow-up.

L. Nasopharyngeal (NP) Swab Collection

The study will use Skim Milk Tryptone Glucose Glycerol (STGG) medium for collecting and storing nasopharyngeal (NP) swab samples from children enrolled in the IPD department. This medium consists of skim milk, tryptone, glucose, and glycerol, which ensure the viability of both bacterial and viral pathogens during storage and transport.

To collect a nasopharyngeal swab safely and effectively, the child should be positioned lying down. A research assistant will gently hold the child's hands and legs to minimize movement during the procedure. The sample will be collected using a FLOQSwab, which should be inserted straight back into one nostril (Figure 2). Care must be taken to avoid contact with the tongue, cheeks, or other areas that may introduce contamination.

The swab should be guided slowly and steadily along the floor of the nose until it reaches the nasopharynx. If resistance or obstruction is encountered before reaching the nasopharynx, the swab should be removed, and a second attempt should be made in the other nostril. In cases where the nasal passages are blocked with mucus, a few drops of normal saline can be used to loosen the dried mucus and facilitate sample collection.

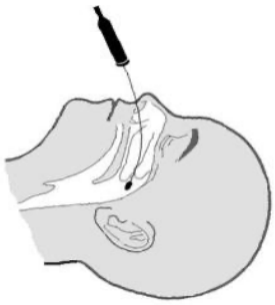

Figure 2: Nasopharyngeal specimen collection process

M. Sample Transportation to Laboratory

Specimen-containing tubes will be transported using a cool bag, and each sample tube will be placed securely in a rack (Figure 3). A thermometer should be included in the bag to monitor the temperature, which must be documented upon receipt of the samples in the laboratory. Specimen tubes can be stored in a refrigerator at 2–4°C for up to 24 hours for short-term storage.

N. Sample Receiving at Reception Laboratory

Figure 3: Specimen transportation procedure to laboratory using cool box

After receiving the sample, general information such as the name, age, sex, ward/bed number, and registration number will be recorded in the CHRF lab system by the receptionist. Two barcodes will then be printed: one to be affixed to the form and the other to be attached to the sample tube.

O. Sample Processing

After receiving the sample in the laboratory, the specimen will be vortexed for 20 seconds to disperse organisms from the swab stick. A 125 µL aliquot of the sample will be taken for culture. The remaining portion of the sample will be sent to the molecular laboratory for RSV detection and biobanking (Figure 4).

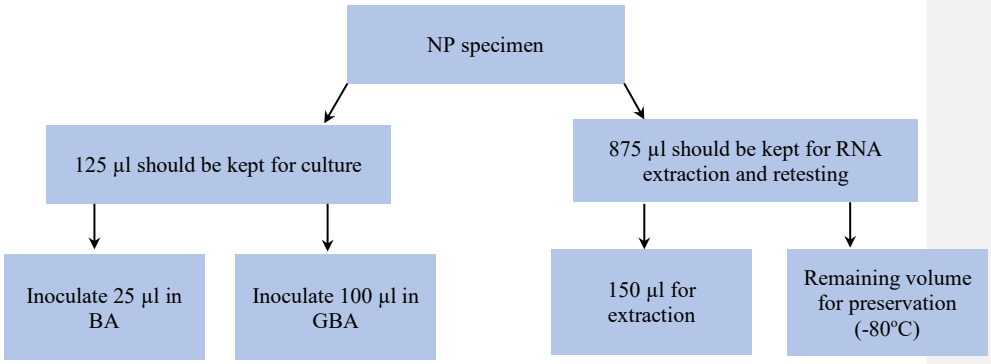

Figure 4: Distribution of a NP swab specimen for different lab procedures

P. Detection of *Streptococcus pneumoniae by* culture

A 100 µL aliquot of nasopharyngeal swab specimens will be inoculated onto each of Gentamycin Blood Agar (GBA) and Blood Agar (BA). GBA is a selective medium designed for *Streptococcus pneumoniae*, as it inhibits the growth of other bacteria, making pneumococcal colonies easier to observe. The BA medium supports the growth of gentamycin-sensitive *Streptococcus pneumoniae* colonies while also allowing for the detection of other organisms.

The plates will be incubated at 37°C and observed for growth after 24 hours. If no growth is observed, the plates will be incubated for an additional 24 hours and re-evaluated after 48 hours. Pneumococcal colonies will be identified based on morphological characteristics. In

cases of uncertainty, Gram staining and optochin susceptibility testing will be performed. For marginal zone diameters (approximately 14 mm), bile solubility tests will be used to confirm identification.

Identification of *Streptococcus pneumoniae*: *Streptococcus pneumoniae* will be identified based on colony morphology on both BA and GBA. On these media, pneumococcal colonies typically appear small and are surrounded by a greenish zone of hemolysis. Under a light microscope, Gram-positive pairs of cocci (diplococci) will be observed, though they may occasionally appear singly or in short chains.

Organism	Media	Color	Size	Shape	Mucoid	Hemolysis
Streptococcus pneumoniae	BA/GBA	Green	1-2 mm	Round	No/Yes	Alpha

The optochin (ethyl hydrocupreine hydrochloride) sensitivity test will be used for the presumptive identification and differentiation of *Streptococcus pneumoniae* from other alpha-hemolytic streptococci. This test will follow the disk diffusion principle, as optochin is water-soluble and readily diffuses into the agar medium. Sensitive organisms surrounding the optochin-impregnated disk will be lysed, resulting in a clear zone of inhibition. An alpha-hemolytic strain with an inhibition zone of ≥14 mm in diameter will be considered *Streptococcus pneumoniae*. If the inhibition zone is less than 14 mm or no zone is observed, a bile solubility test will be performed. Colonies that are bile soluble will be confirmed as *Streptococcus pneumoniae*.

Serotyping of *S. pneumoniae* by Quellung Reaction: The Quellung reaction will be used for pneumococcal serotyping. In this method, a specific antibody will bind to the pneumococcal capsular polysaccharide, causing a change in the refractive index as a result of in-situ immunoprecipitation. This change will make the capsule appear "swollen" and more visible (Figure 5). Additionally, the bacteria may occasionally agglutinate, which will further confirm a positive result.

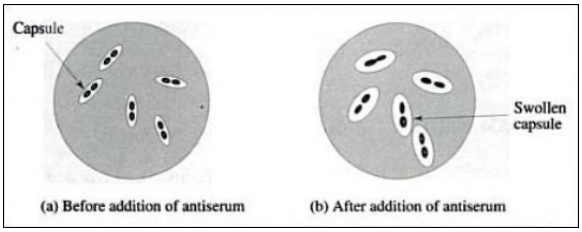

Figure 5: Quellung reaction of *Streptococcus pneumoniae*

Q. RSV Detection by qPCR

RNA Extraction

In this study, RNA from the NP swab samples will be extracted using the Qiagen QIAamp® Viral RNA Mini kit (Cat #: 529006). Manufacturer guidelines will be followed for RNA extraction, and PCR-grade, nuclease-free water will be used as an extraction control (EC) for each batch of extractions.

Real Time PCR for RSV

This study will use real-time PCR, also known as quantitative PCR (qPCR), to detect the presence of RSV in the collected NP swab samples. The qPCR will be carried out in a thermal cycler equipped with the capability to illuminate each sample with a beam of light at a specific wavelength and detect the fluorescence emitted by the excited fluorophore. The amount of fluorescence signal generated during PCR will be directly proportional to the quantity of the PCR product.The following primer and probes should be used to detect the target pathogens.

Target	Sequence (5'-3')	5'	3'	References

		chemistry	chemistry	
RSV	F-GGCAAATATGGAAACATACGTGAA			(6)
	R-TCTTTTTCTAGGACATTGTAYTGAACAG			
	Pb-CTGTGTATGTGGAGCCTTCGTGAAGCT	FAM	BHQ1	
RNaseP	F-CCAAGTGTGAGGGCTGAAAAG			(7)
	R-TGTTGTGGCTGATGAACTATAAAAGG			
	Pb-CCCCAGTCTCTGTCAGCACTCCCTTC	FAM	BHQ1	

R. Ethical Considerations

Ethics Approval: The study received ethical approval from the Bangladesh Institute of Child Health, with approval reference number BICH-ERC-02-04-2018.

Confidentiality: Data will be anonymized and stored securely. Only authorized personnel will have access to identifiable information. A data protection policy will be enforced to comply with international standards for research involving human subjects.

S. Statistical Analysis

Descriptive statistics will summarize demographic and clinical characteristics of the study population. The burden of RSV will be estimated using proportions and confidence intervals. Various statistical models will be used to compare mortality risk between admitted and denied children, adjusting for age, sex, and diagnosis and to assess the estimated impact of RSV vaccines. Analysis will also be conducted to summarize pneumococcal serotypes in the nasopharynx of children with respiratory illness, and any potential correlation with RSV.

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