

STUDY PROTOCOL

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# Screening and treatment of maternal genitourinary tract infections in early pregnancy to prevent preterm birth in rural Sylhet, Bangladesh: a cluster randomized trial

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## Abstract

**Background:** Approximately half of preterm births are attributable to maternal infections, which are commonly undetected and untreated in low-income settings. Our primary aim is to determine the impact of early pregnancy screening and treatment of maternal genitourinary tract infections on the incidence of preterm live birth in Sylhet, Bangladesh. We will also assess the effect on other adverse pregnancy outcomes, including preterm birth (stillbirth and live birth), late miscarriage, maternal morbidity, and early onset neonatal sepsis.

**Methods/Design:** We are conducting a cluster randomized controlled trial that will enroll 10,000 pregnant women in Sylhet district in rural northeastern Bangladesh. Twenty-four clusters, each with ~4000 population (120 pregnant women/year) and served by a community health worker (CHW), are randomized to: 1) the control arm, which provides routine antenatal and postnatal home-based care, or 2) the intervention arm, which includes routine antenatal and postnatal home-based care plus screening and treatment of pregnant women between 13 and 19 weeks of gestation for abnormal vaginal flora (AVF) and urinary tract infection (UTI). CHWs conduct monthly pregnancy surveillance, make 2 antenatal and 4 postnatal home visits for all enrolled pregnant women and newborns, and refer mothers or newborns with symptoms of serious illness to the government sub-district hospital. In the intervention clusters, CHWs perform home-based screening of AVF and UTI. Self-collected vaginal swabs are plated on slides, which are Gram stained and Nugent scored. Women with AVF (Nugent score  $\geq 4$ ) are treated with oral clindamycin, rescreened and retreated, if needed, after 3 weeks. Urine culture is performed and UTI treated with nitrofurantoin. Repeat urine culture is performed after 1 week for test of cure. Gestational age is determined by maternal report of last menstrual period at study enrollment using prospectively completed study calendars, and in a subset by early (<20 week) ultrasound. CHWs prospectively collect data on all pregnancy outcomes, maternal and neonatal morbidity and mortality.

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**Implications/Discussion:** Findings will enhance our understanding of the burden of AVF and UTI in rural Bangladesh, the impact of a maternal screening-treatment program for genitourinary tract infections on perinatal health, and help formulate public health recommendations for infection screening in pregnancy in low-resource settings.

**Trial registration:** The study was registered on ClinicalTrials.gov:NCT01572532 on December 15, 2011. The study was funded by NICHD: R01HD066156.

**Keywords:** Urinary tract infection, Bacterial vaginosis, Abnormal vaginal flora, Preterm birth, Stillbirth, Miscarriage, Cluster randomized trial, Bangladesh

## Background

About 15 million babies are born preterm (born <37 weeks of gestation) annually [1], and preterm birth is the leading cause of neonatal and under-5 child mortality globally, accounting for one million neonatal deaths annually [2, 3]. Eleven million preterm births and the vast majority of deaths due to preterm birth complications occur in low-income countries, where there are limited resources and capacity for prevention and management. One third of preterm survivors suffer from severe long term neurological disabilities (e.g., cerebral palsy or mental retardation) [4, 5], and preterm infants carry increased risk of behavioral problems, school learning difficulties, chronic lung disease, retinopathy of prematurity, hearing impairment, and lower growth attainment [5]. Few interventions effectively prevent preterm birth [6], and the incidence of preterm birth is rising, in both low- and high-income countries [1, 7–11].

Treatment of maternal infections is a critical target for the prevention of preterm birth, particularly in low-income settings. Genitourinary tract infections may affect up to 41 % of women of reproductive age globally, and as many as 60–80 % of these infections in pregnancy are asymptomatic [12]. Maternal genitourinary tract infections have been significantly associated with a wide range of adverse perinatal and maternal outcomes, including miscarriage, stillbirth, preterm birth, fetal growth restriction, neonatal and puerperal sepsis, neonatal encephalopathy and neonatal and maternal mortality [13–16]. In developing countries where antenatal care coverage is limited [17], maternal infections are inadequately diagnosed and treated. Lower genital tract infections may ascend the reproductive tract and seed the amniotic cavity, which can trigger an inflammatory cascade eventually resulting in a number of adverse outcomes including preterm birth, chorioamnionitis, fetal growth restriction, stillbirth, puerperal sepsis and early onset sepsis. Maternal infection accounts for an estimated 50 % of preterm births [18], thus timely diagnosis and treatment of maternal infections is a prime target for the prevention of preterm birth, as well as other adverse pregnancy outcomes [19].

Abnormal vaginal flora (AVF), including bacterial vaginosis (BV), is the most prevalent vaginal infection in

pregnancy, and is significantly associated with increased risk of preterm birth. AVF is triggered by an imbalance in the concentrations of endogenous vaginal microflora—a reduction in normal lactobacilli and the opportunistic overgrowth of *Gardnerella vaginalis* and other anaerobic organisms [20, 21]. Nugent et al. defined a scoring system (0–10) for vaginal flora based on a weighted combination of the relative concentrations of 3 bacterial morphotypes: *Lactobacillus*, *Gardnerella* or *Bacteroides*, and curved gram-variable rods (*Mobiluncus*) [22] [BV = Nugent score ≥ 7, intermediate flora: Nugent score 4–6, and AVF: Nugent score ≥ 4]. In a meta-analysis of 32 studies, asymptomatic BV was associated with a 6.32 times elevated risk of late miscarriage (95 % CI 3.65–10.94) and 2.16 times increased risk of preterm birth (95 % CI 1.56–3.00) [23]. Furthermore, intermediate vaginal flora is a heterogeneous condition which comprises ~15 % of all AVF [24], and has been associated with elevated risk of preterm birth and neonatal infections [25–28]. Among women with a prior history of preterm birth, Hauth and colleagues found that screening and treatment of asymptomatic BV with metronidazole and erythromycin at 22 weeks gestation significantly reduced the incidence of preterm birth from 46 to 31 % in the treatment group [29]. In the multi-center National Institute of Child Health & Human Development (NICHD) BV trial, 1953 average-risk women with asymptomatic BV between 16 and 24 weeks of gestation were randomized to receive two doses of metronidazole (2 g) or placebo. Treatment, however, did not significantly affect preterm delivery or other adverse perinatal outcomes [30]. In a more recent trial, Ugwumadu et al. reported that early (12–22 weeks of gestation) screening and treatment for AVF with 5 days of oral clindamycin resulted in a significant reduction in spontaneous preterm birth rate (12 % in placebo vs. 5 % in treatment group) and late miscarriage (13–24 weeks; 4 % in placebo vs. 1 % in treatment group) [24]. Lamont et al. randomized women with AVF between 13 and 20 weeks gestation to receive intravaginal clindamycin cream or placebo between 13 and 20 weeks gestation, and found that among infected women, there was a significant reduction in preterm birth incidence (10 % in control vs. 4 % in treatment group) [31]. Potential explanations for the treatment

effect in the two latter trials may include: 1) the earlier timing of treatment, prior to the amniotic membranes sealing the uterus at 20 weeks [32], which may prevent early ascension of bacteria into the intrauterine cavity; 2) antibiotic choice: 5–7 day course of clindamycin, which has greater activity against *Mobiluncus* and atypical *Mycoplasma* species vs. 2 days of metronidazole [33]; and 3) treatment of AVF in Ugwumadu et al. and Lamont et al. vs. treatment of BV only in the average-risk NICHD trial. A Cochrane meta-analysis including 15 trials ( $n=5888$  women) concluded that BV treatment across all gestational ages did not significantly reduce preterm birth, largely due to heterogeneity in definition, timing of treatment and choice of antibiotic [33]. However, the risk of preterm birth was significantly reduced by treatment of BV at <20 weeks gestation (5 trials in 2387 women; OR 0.63, 95 % CI 0.48–0.84) and treatment of AVF (2 trials, 894 women; OR 0.51, 95 % CI 0.32–0.81). Thus, in low-resource settings such as rural Bangladesh, where BV and preterm birth are prevalent, treatment of AVF in early pregnancy may hold promise in reducing the incidence of preterm birth. Evaluation in a well-conducted population-based randomized trial is needed.

Urinary tract infection (UTI) is also prevalent in pregnancy and associated with preterm birth. Uropathogens tend to be gram-negative bacteria with virulence factors and endotoxins, which may trigger the inflammatory cascade and preterm delivery. The prevalence of asymptomatic bactiuria in pregnancy may range from 7 % to as high as 86.6 % [34, 35]. Approximately 30 % of women with untreated bactiuria develop pyelonephritis [36], and before the era of antibiotics, 30–50 % of women with pyelonephritis delivered preterm [37]. In an early meta-analysis by Romero et al., asymptomatic bactiuria carried a 2-fold elevated risk of preterm delivery (95 % CI 1.43–2.77) [38]. In the Cardiff Birth Survey, asymptomatic bactiuria was not associated with all preterm births (OR 1.2, 95 % CI 0.9–1.5); however there was a significant association with medically indicated preterm birth (OR 2.0; 95 % CI 1.5–2.8) [39]. In a meta-analysis of randomized clinical trials for asymptomatic bactiuria, antibiotic treatment reduced the risk of low birth weight (RR 0.56, 95 % CI 0.43–0.73;  $n = 8$  studies) [38], however there are inadequate data on the effect on preterm birth.

## Methods/Design

### Trial design and preparation

#### Aim

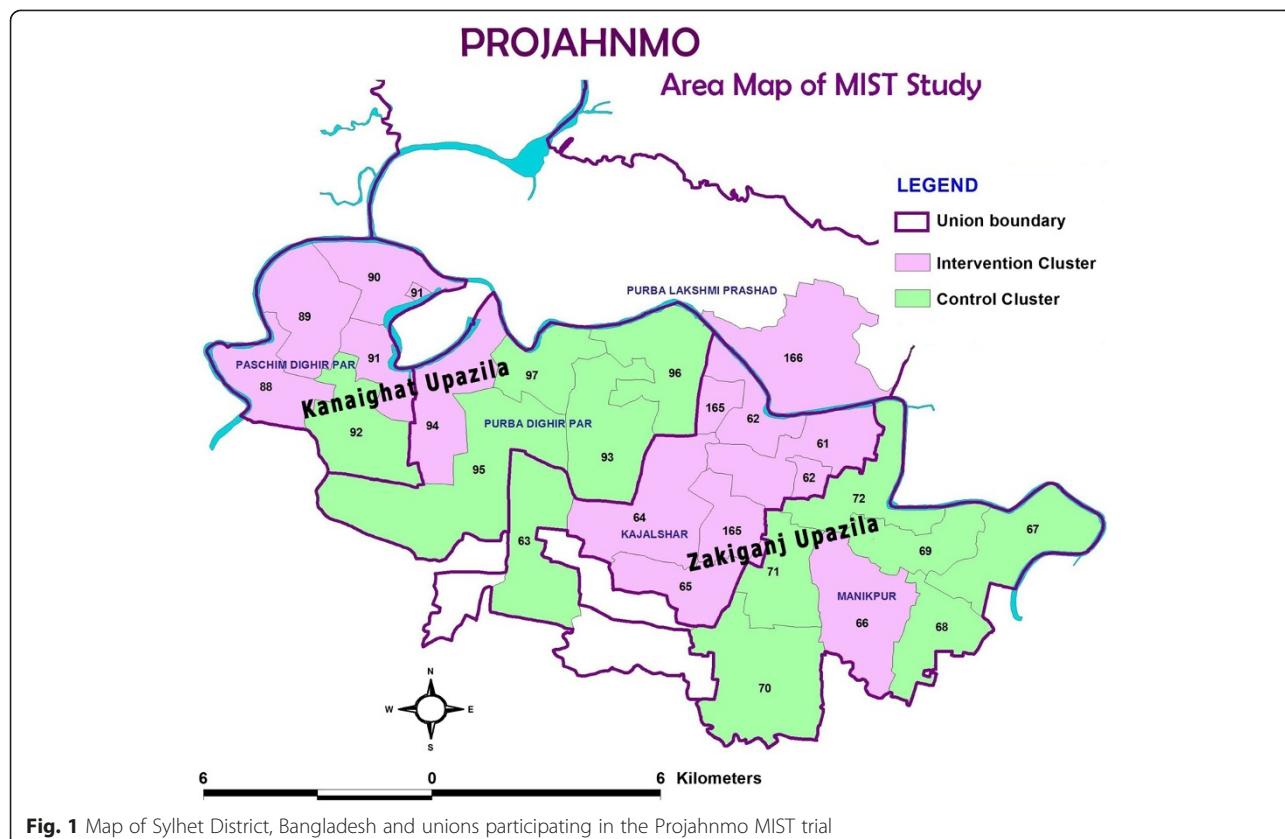
The **primary aim** of this study is to determine the impact of community-based screening and treatment of AVF and UTI in early pregnancy (13–19 weeks) on the incidence of preterm live birth in Sylhet district, Bangladesh.

The **secondary aims** of this study are:

- To determine the population-based impact of community-based screening and treatment of AVF and UTI on the:
  - a. proportion of pregnancies with outcomes occurring prior to 37 weeks including late miscarriage (pregnancy losses 20 to <28 weeks) and preterm birth (stillbirth and live birth 28 to <37 weeks);
  - b. proportion of babies with early onset neonatal sepsis
  - c. proportion of pregnancies with maternal morbidity (clinical infections including pyelonephritis, puerperal sepsis)
  - d. proportion of babies born with low birth weight or small for gestational age
  - e. neonatal mortality rate
- To determine the prevalence of AVF and UTI, including asymptomatic bactiuria, among pregnant women in Sylhet district, Bangladesh.
- To evaluate the accuracy of simple, low-cost, point of care diagnostic tests for detecting BV and UTI by community health workers (CHWs) in a rural, developing country setting.

### Study site and population

The Maternal Infection Screening and Treatment (MIST) study is being conducted in the Projahnmo research site in two sub-districts (*upazillas*; Zakiganj and Khanaighat) of Sylhet District in Bangladesh (Fig. 1, Map). Projahnmo is a collaboration of the Ministry of Health and Family Welfare (MOHFW) of the Government of Bangladesh, the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Shimantik (a non-governmental organization), Child Health Research Foundation, Brigham and Women's Hospital (Harvard Medical School), and the Johns Hopkins Bloomberg School of Public Health. The estimated population of the MIST study area is about 100,000. The burden of prematurity, neonatal sepsis, neonatal death, and stillbirth is high in this population (preterm birth rate: 22 %, clinical neonatal sepsis: 9.9 %, neonatal mortality rate: 28/1000 live births, stillbirth rate: 38/1000 births) [40, 41]. Health services in Bangladesh are provided by the MOHFW, NGOs and private providers. In the government sector, two community-based workers, a family welfare assistant and a health assistant together serve a population of 6000–7000. First level outpatient clinics, called Health and Family Welfare Centres, serve a population of about 25,000, with one clinic per union (lowest local government entity in Bangladesh). Sub-district hospitals with both in-patient and outpatient facilities serve a population of about 250,000.



The study area is served by 24 project CHWs, who are women with at least a 10th grade education, are residents of the study community, and have received 6 weeks of training on basic maternal and newborn care. Within each CHW cluster area, there are additionally Village Health Workers (VHWs), one in each village with a population of ~1000. Each cluster area is assigned to 1 CHW and 4–5 VHWs to serve an estimated 120 pregnancies and 116 live births annually; this is feasible because the trial area has very high population density, and the same ratio of population to CHWs/VHWs has been used in our previous and current studies in Sylhet.

We conducted a complete census and socioeconomic survey of the study population in 2009 and continue to conduct health and demographic surveillance every two months. All households and women of childbearing age have unique current and permanent identification numbers, which allows individual tracking and longitudinal follow-up.

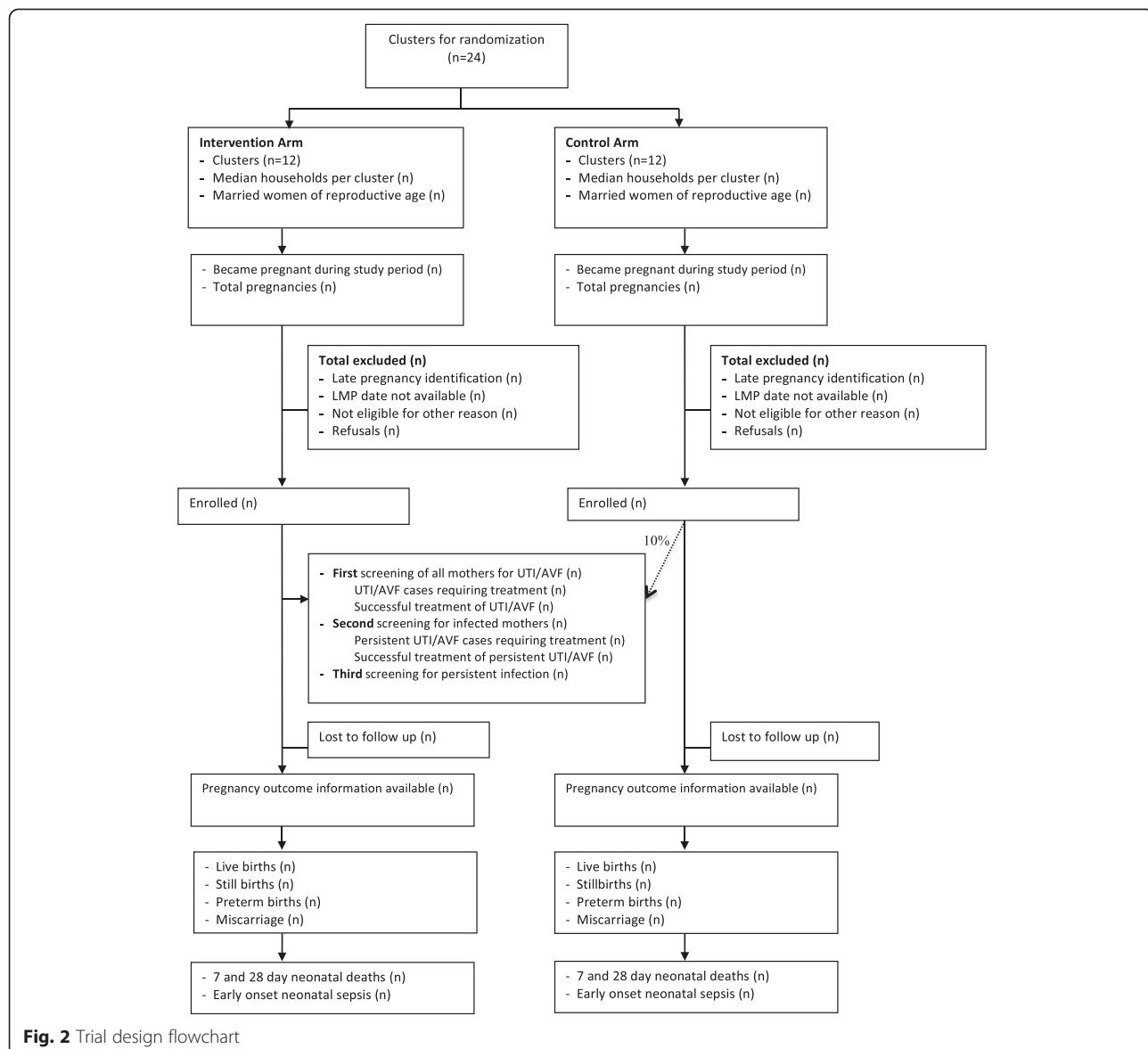
#### Pregnancy surveillance, eligibility, and enrollment (Fig. 2)

All women who become pregnant in the study area are potentially eligible to participate in the trial. Prior to the start of the study, all women of reproductive age

(15–49 yo) in the study area were provided study calendars with instructions and training by CHWs to prospectively circle the first day of each menstrual cycle. VHWs conduct monthly pregnancy surveillance visits where mothers are asked if their last menstrual period (LMP) was greater than 4 weeks ago. If LMP was >4 weeks, a CHW conducts a home visit to perform a urine pregnancy test. Enrollment may begin as soon as 5 weeks gestation and continues until 19 weeks gestation. At the time a missed period is detected during screening for study eligibility. Subjects are excluded from the study if they have no or uncertain recall of LMP (due to lactational amenorrhea, recent discontinuation of contraceptive or irregular menses), LMP >19 weeks, history of irregular bleeding due to injectable Depo-Provera, or self-reported history of severe chronic disease based on a medical history checklist. Participants are enrolled after final eligibility is confirmed by the CHW and oral consent is obtained.

#### Basic antenatal-postpartum care package

In all study areas, CHWs provide basic antenatal and postpartum care. This consists of two antenatal home visits (13–19 weeks and 28–32 weeks of gestation) and four postnatal home visits (1st, 3rd, 7th, and 28th day

**Fig. 2** Trial design flowchart

of life) to all enrolled pregnant women. During antenatal home visits, CHWs provide: counseling on birth and newborn care preparedness (importance of antenatal check up, use of a skilled/trained birth attendant, essential newborn care, danger signs, care seeking, birth spacing), demonstrations (hand washing, cord cutting, immediate newborn care), facilitation (emergency savings, selection of birth attendant and newborn care person), supply (clean delivery kit, iron folate tablets) and referral (for routine antenatal care, tetanus toxoid immunization, maternal danger signs). Postnatal visit components include: counseling (maternal and newborn danger signs, essential newborn care, and exclusive breast feeding), assessment of the newborn (morbidity and breastfeeding), referral (for newborn and maternal danger signs, postpartum contraceptive

methods), demonstration/support (breastfeeding techniques) and supply (contraceptives).

### Randomization

The units of randomization in this trial are the 24 CHW areas, each comprising several adjacent villages. Allocation to the intervention vs. control arms was done via a restricted randomization procedure. All  $\binom{24}{12}$  possible randomization sequences were generated where each sequence allocated 12 of the CHW areas to either intervention or control. Using data on births and preterm status from our prior study in this area [42], we restricted the set of eligible sequences to those where intervention vs. control ratios of predicted preterm birth

and predicted total births were within 0.975 to 1.025. An additional criterion for retaining a sequence as eligible was that the 13 CHW areas that had participated in a prior study on birth spacing ("The Health Fertility Study") were allocated equally to intervention and control (i.e. 6 and 7 or 7 and 6). Among the 2,704,156 sequences, 164 sequences met the criteria, from which a single sequence was randomly selected. Workers and participants in the study are not blinded to the intervention group but are not aware of the study aims/hypotheses.

### Screening and treatment intervention

The screening and treatment intervention is provided to all pregnant women enrolled in the intervention clusters. Furthermore, to examine the comparability of baseline infection prevalence between intervention and control areas, a random 10 % of mothers enrolled in the control arm are selected to receive the screening and treatment intervention. To select the 10 % of mothers from control areas, each CHW initially randomly selected a number between 1 and 10 which indicated the starting number of the women in her area to receive the intervention. From that starting point every 10th enrolled mother was included in the group to receive the screening and treatment intervention. The sample size was not adjusted for this allocation.

### Screening

CHWs collect samples of vaginal flora and urine during home visits between 13 and 19 weeks gestation. Vaginal specimens are collected via sterile self-administered vaginal swabs. Self-administered vaginal swabs have been used with high acceptability and quality in diverse populations including in Bangladesh, providing cost-savings and cost effectiveness for STI testing [43–46]. Women are instructed by the CHW to insert a Dacron swab ~4–5 cm into the vagina, allow the swab to stand for 15 s, and rub the lateral walls of the vagina for 4–5 s prior to withdrawal [47]. The CHW gently rolls the swab onto a plain glass slide and allows it to air dry prior to transport to the Sylhet field laboratory. A clean catch midstream urine specimen is obtained for urine culture. The CHW instructs the mother to spread the labia

widely before collecting 20–30 mL of the midstream urine into a sterile wide-mouthed container.

### Specimen storage and transport

All specimens collected in the field by CHWs are immediately labeled and stored in a cooler refrigerated with ice packs (~2–8 °C) and transported to the Sylhet field laboratory. In the field laboratory, the vaginal smears are Gram stained within 1–2 days of plating [22]. Urine specimens are inoculated on sheep blood agar plates in the Sylhet field laboratory within 6 h of collection for incubation.

### Diagnosis of abnormal vaginal flora

Abnormal vaginal flora are classified by microscopic examination of a Gram stained sample of the vaginal smear and Nugent scored (Table 1) [22]. Nugent scores between 7 and 10 are classified as BV, and scores between 4 and 6 are classified as intermediate flora; all scores ≥ 4 are classified as AVF [48]. The time window from specimen collection to Nugent scoring is 4 days, and from Nugent scoring to first dose of treatment is 2 days, for a total of 6 days from sample collection to treatment. Treatment is based on a single reading by a trained and standardized microbiologist. The laboratory staff are trained and standardized in Nugent scoring on a non-study sample of slides ( $n = 250$ ) until there is high concordance of readings ( $\kappa > 0.8$ ) between laboratory staff and gold standard scorers, and high sensitivity and specificity of AVF and BV compared to the gold standard reader (>85 %) prior to the initiation of the study.

### Diagnosis of urinary tract infection

Urine specimens are plated on standard MacConkey and Blood agar plates and incubated for 48 h. Bacterial growth is speciated using standard microbial techniques, and antibiotic sensitivity patterns are determined. Bacterial growth is defined in the following categories: 1) **high-burden urinary tract infection:** bactiuria of  $>10^5$  colony forming units (CFU) per 1 mL of urine of a single uropathogen [49], 2) **intermediate growth:** bactiuria

**Table 1** Nugent scoring for vaginal flora

Lactobacillus Morphotypes <sup>a</sup>	Gardnerella / Bacteroides Morphotypes <sup>a</sup>	Curved gram-variable rods
Score 0 for >30	Score 0 for 0	Score 0 for 0
Score 1 for 15–30	Score 1 for <1	Score 1 for <5
Score 2 for 14	Score 2 for 1–4	Score 2 for 5+
Score 3 for <1	Score 3 for 5–30	
Score 4 for 0	Score 4 for >30	

<sup>a</sup>Average count per high powered field (1000× oil immersion), viewing at least 10–20 fields

with  $>10^3$ – $10^5$  CFU/mL of a single uropathogen, and 3) **contamination**: bacterial growth of  $>1$  organism OR growth of a non-urinary tract pathogen. At the time of the specimen collection visit, CHWs inquire about maternal symptoms of UTI (dysuria, urinary frequency, hematuria, abdominal pain, fever, flank pain). **Symptomatic intermediate growth** is defined as mothers with intermediate burden growth and UTI symptoms. **Cystitis** is diagnosed in women with positive urine culture (high burden or intermediate growth) and symptoms of dysuria, urinary frequency, hematuria, urinary urgency or suprapubic tenderness, without upper urinary tract symptoms (fever, chills, flank or back pain) [49]. **Pyelonephritis** is diagnosed in women with positive urine culture and systemic symptoms (fever, chills, flank pain or back pain) [49].

#### Treatment of abnormal vaginal flora and urinary tract infections

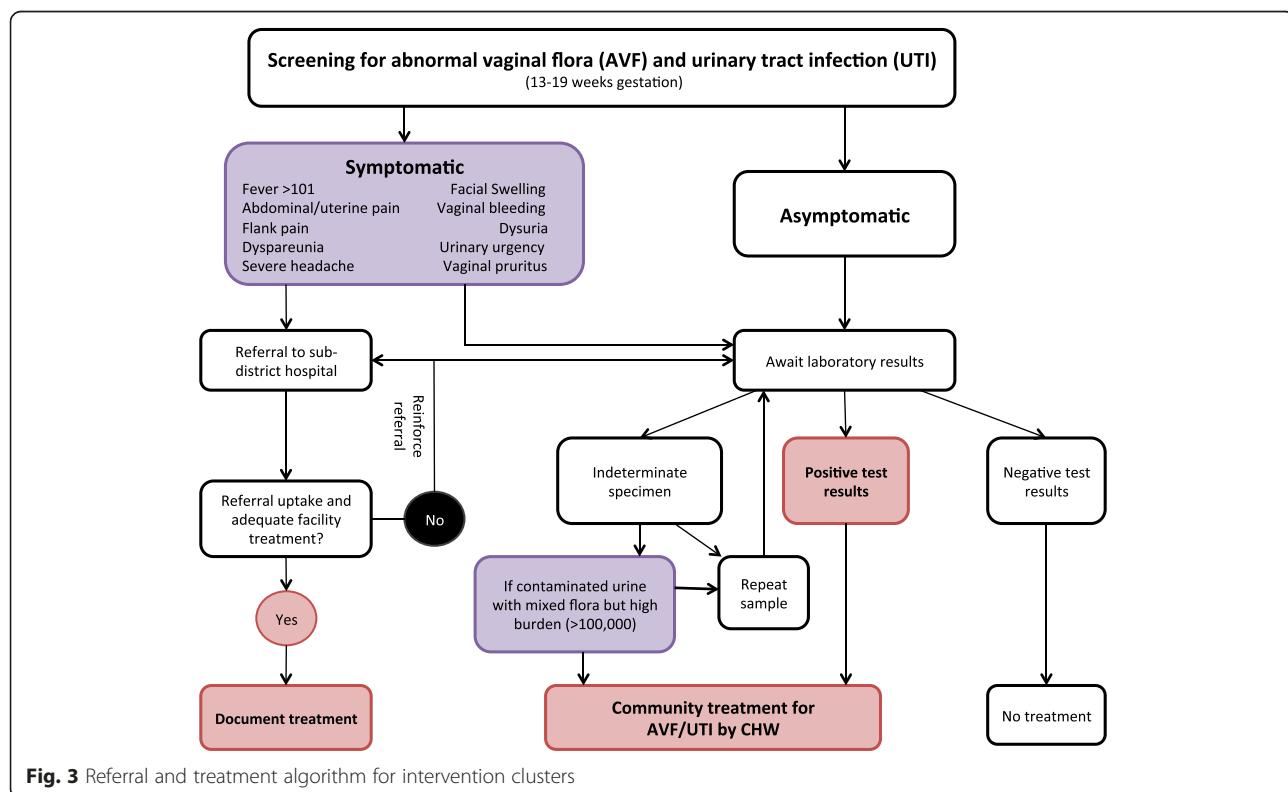
Women who are clinically symptomatic at any antenatal or postnatal visit are referred to the sub-district hospital for full evaluation and treatment (Fig. 3). Women with symptoms of illness are visited on the following day to follow her clinical status and ensure referral compliance. CHWs conduct a home visit to women with positive test results within 48 h of receiving the results to initiate treatment. AVF is treated with oral clindamycin 300 mg

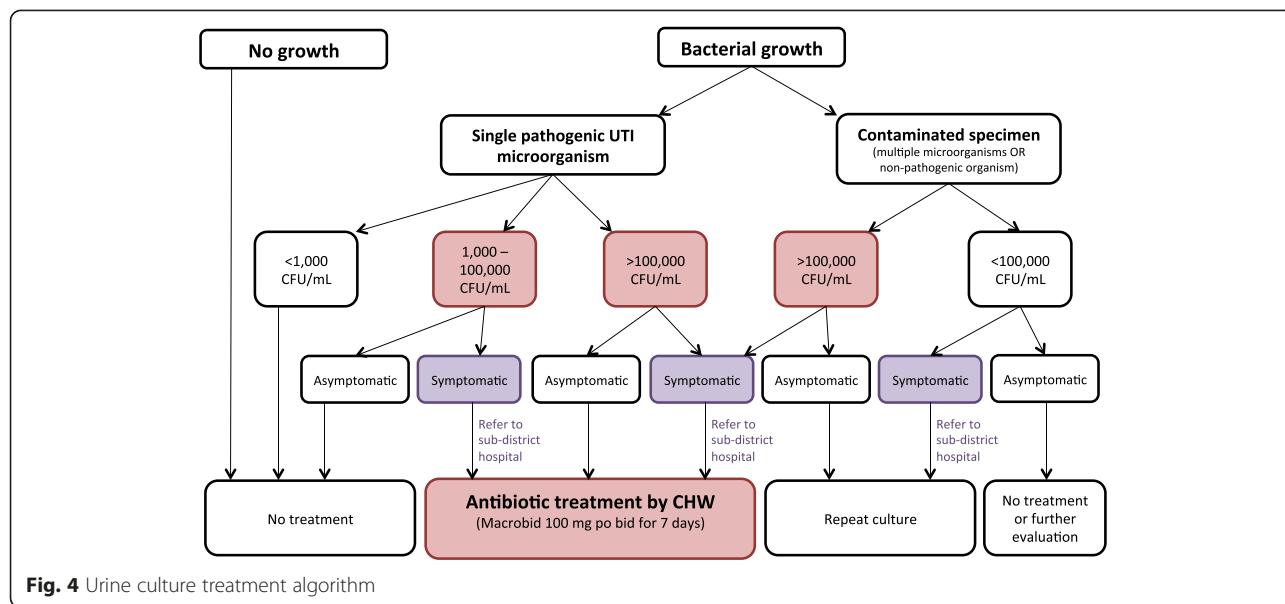
per oral (po) twice daily (bid) for 5 days per the regimen used by Ugwumadu [24]. After the initial treatment course, women are rescreened 3 weeks after the first treatment. If the second vaginal specimen has Nugent score  $\geq 4$ , the women are retreated with a second course of clindamycin 300 mg po bid for 5 days. For retreated women, a final vaginal sample is collected 3 weeks after the second course of antibiotics to document response to treatment.

The algorithm for treatment of positive urine cultures is shown in Fig. 4. All mothers with high burden UTI and symptomatic intermediate growth are treated. The initial antibiotic treatment is Macrobid/Nitrofurantoin 100 mg po bid for 7 days. Women diagnosed with pyelonephritis are referred to the sub-district hospital for further evaluation and management. For all positive urine cultures, a repeat urine culture is obtained 1 week after completion of antibiotics for test of cure. If the second urine culture is positive, the supervising field physician selects an appropriate antibiotic based on the prior culture's antimicrobial sensitivity pattern. Persistent UTIs are referred to Sylhet Osmani Medical College Hospital for evaluation and management.

#### Point of care testing validation

On a subsample of women ( $n = 1386$ ), point of care tests are conducted in the field site at the same time as the first specimen collection. An additional vaginal swab is taken for





rapid testing with BVBLUE (Rapid Sialidase test, Genzyme, Cambridge, MA). An additional 2 mL aliquot of urine is tested with the URISCREEN test (rapid catalase screen, URISCREEN, Jant Pharmacal, Encino, CA).

#### Medication adherence and monitoring

The VHWs and CHWs monitor all women who are prescribed medication for medication adherence and adverse events. The CHW observes the mother take the first antibiotic dose and provides her with a medication punch card to indicate every dose taken. The CHW visits the mother 2–3 days after starting the antibiotic to screen for symptoms of *C. difficile* diarrhea/colitis (associated with clindamycin) and hemolytic anemia (associated with Macrobid). At the end of the antibiotic course, the CHW collects the medication punch card, conducts a pill count, and assesses compliance and response to therapy based on the mother's history.

#### Post-natal visits

CHWs in both intervention and control clusters are notified of all births through a community-based notification system which relies on families and VHWs. During the first postnatal visit, the CHW records the date and outcome of delivery, including stillbirths (pregnancy losses  $\geq 28$  weeks) or late miscarriages (pregnancy losses 20 to  $< 28$  weeks). If a stillbirth has occurred, the CHW notifies a supervisory staff member who returns to conduct a verbal autopsy module for stillbirth [50]. For all births, CHWs record the vital status of mother and baby, and basic information regarding

characteristics of labor and delivery, including data on timing and duration of labor, timing (before or after onset of labor) and length of rupture of membranes, maternal fever, and other morbidity before, during, and after delivery. For each live newborn delivered, the newborn's sex, weight, and care given during and immediately after birth (bathing, massage, cord care, breastfeeding practices) are recorded.

At the first postnatal visit, which occurs in most cases on the first day of life, CHWs weigh the infant with a digital weighing scale. At the start of the study, the KL-218 digital scale (precision 10 gm, Donghuan Electronic Manufacturing, Donghuan, China) was used. The scale was transitioned to the Tanita BD 585 digital pediatric scale in September 2015 (precision 10 gm, Tanita corporation, Toyota, Japan). Scales are calibrated daily prior to home visits. CHWs also measure head circumference, mid-upper arm circumference, infant length, and chest circumference. These measurements are repeated thrice (median value used for analysis). The general health status of the mother and infant are assessed by the CHW during postnatal visits. The mothers are assessed for vital status, fever, uterine tenderness and symptoms of postpartum hemorrhage. Women with symptoms of postpartum complications (fever  $> 38.3$  °C, abdominal/uterine tenderness, self-report of excessive hemorrhage) are referred to government sub-district hospitals for further management. Evaluation of the neonate is conducted according to an IMCI-type algorithm [51], and the CHW assesses the infant for temperature, respiratory rate, and signs and symptoms of severe illness. The CHWs conduct follow up postnatal visits at homes in which severe illness is detected in the mother or neonate

within the next 24 h to monitor the mother/infant for signs of illness and reinforce the referral. If the ill mother/newborn has not sought hospital care, the CHW facilitates referral by helping to arrange transportation and payment of hospital fees. The CHW returns on days 3, 7, and 28 after birth to reassess and inquire about signs of morbidity in the mother and infant.

### Outcome measures

The primary and secondary study outcomes and definitions are shown in Table 2.

#### Gestational age

The primary measure of gestational age is determined by maternal report of the first day of her LMP, reported during monthly pregnancy surveillance and with the aid of a prospectively completed menstrual calendar. An early ultrasound assessment (<20 weeks gestation) was originally planned for a random sample of 20 % of the enrolled women. In year 3 (2013), we began to conduct ultrasonography on as many enrolled pregnancies as possible.

#### Preterm live birth

Preterm live birth is defined as a baby born alive <37 weeks gestation defined by LMP. Early preterm live birth is defined as a baby born alive <34 weeks gestation defined by LMP. The denominator for preterm live birth rates is all live births.

#### Pregnancies with outcomes occurring prior to 37 weeks

This measure is defined as the spontaneous termination of pregnancy from 20 to <37 weeks resulting in: 1)

preterm live birth, 2) late miscarriage (spontaneous pregnancy loss 20 to <28 weeks) that is not due to induced abortion, or 3) preterm stillbirth (28 to <37 weeks). A preterm stillbirth is defined as an infant born without signs of life (no spontaneous crying, breathing, and/or movement) at 28 to <37 weeks gestation. The denominator for this outcome is all births ≥20 weeks.

#### Clinically suspected early onset neonatal sepsis

Early onset neonatal sepsis is defined using the neonatal IMCI algorithm for very severe disease [52] with onset in the first week of life.

#### Small for gestational age

Small for gestational age is defined as birth weight <10 % (measured within the first 72 h of life) for gestational age using the INTERGROWTH-21 newborn birthweight standard [53].

#### Low birth weight

Low birth weight is defined as birth weight (measured within the first 72 h of life) of <2500 g. We will also assess the outcome of birth weight <2000 g.

#### Maternal morbidity

Maternal morbidity outcomes are assessed primarily by self-report. We follow maternal history for clinical signs indicating probable pyelonephritis and endometritis.

#### Neonatal mortality

In the event of death of a newborn during the neonatal period, the CHW refers the case to a supervisory staff

**Table 2** Study outcomes for MIST trial

	Numerator	Denominator
Primary outcome		
Preterm live birth	Live births <37 weeks of gestation	All live births
Secondary outcomes		
Preterm birth and late miscarriage	Live births (28 to <37 weeks) Stillbirth (28 to <37 weeks) Late Miscarriage (20 to <28 weeks)	All birth outcomes ≥ 20 weeks
Late miscarriage rate	All spontaneous abortion/miscarriage (non-therapeutic abortions) occurring between 20 to <28 weeks	All birth outcomes ≥20 weeks
Stillbirth rate	Stillbirth (≥28 weeks) and no signs of life	All live births and stillbirths ≥28 weeks
Perinatal mortality rate	Stillbirth + early neonatal death (birth to 7 days)	All live births and stillbirths ≥28 weeks
Neonatal mortality rate	Neonatal death (birth to 28 days)	Live births
Early onset sepsis	Very severe disease by current IMCI guidelines on any day in first week of life	Live births

member who conducts an in-depth verbal autopsy using a revised version of the WHO standard verbal autopsy instrument which has been used extensively by our study team and CHWs at the study site [54–56]. Verbal autopsy data are reviewed by two independent physicians, and a consensus on proximate and underlying cause(s) is reached. Data are further analyzed for cause-specific mortality using a computer-based expert algorithmic approach [57, 58].

### Sample size and analysis

In order to calculate the sample size, we first hypothesized that a population-level reduction in the preterm rate in the range of 15–20 % would be of public health importance, given the importance of the outcome and the level of complexity of any future scaled-up programmatic implementation of the intervention. Once we established this desired effect size range, we worked through a series of calculations (with corresponding assumptions) to determine the number of babies required to achieve sufficient power (i.e. 80 %) for a population-level reduction in this range (i.e. 15–20 %).

Given that the intervention is directed only at a subset of the population, we first estimated the proportion of preterm birth among both infected and non-infected women. Estimation of these proportions requires three parameters:

1. The proportion of preterm in the population: we estimated this to be 20 %, based on our prior data from this population [51].
2. The proportion of women in the intervention clusters that actually receive treatment under the universal screening program, or equivalently an estimate of the total proportion of women with UTI and/or AVF (Nugent  $\geq 4$ ).
3. The ratio of preterm birth risk among infected and non-infected women; we estimated this ratio to be 2.5 from the literature.

From previous data and the literature, we estimated that about 15 % of women in this setting have either AVF, UTI, or both. Among those with AVF, some will fall into the intermediate flora score range (Nugent score 4–6) and some into the BV scoring range (Nugent score 7–10). Our best estimates of the relative proportions in these two subgroups was approximately 85 % within the BV (Nugent 7–10) range, with the remaining 15 % of women with AVF falling within the intermediate flora scoring range (Nugent 4–6) [24]. We based our prevalence estimates and treatment effect size on prior studies of similar screening and treatment programs for **all abnormal vaginal flora** (Nugent scores  $\geq 4$ ).

The last step in fixing our parameters and assumptions for sample size calculation was to examine the range of impacts that the intervention would need to afford **among infected women** in order to result in population-level impacts of 15 to 20 %. For a 50 % impact among infected women, we estimated a 15 % population-level reduction. For a 65 % impact on infected women, we estimated a 20 % population-level reduction. These impacts are consistent with those from prior studies that have demonstrated the impact of treatment of women with AVF (Nugent  $\geq 4$ ) is an approximately 60 % reduction in preterm births (Lamont et al.: 60 %, Ugwamadu et al.: 58 %). Assuming this impact (i.e. 60 %) among infected women to be similar to the impact in this population, the population-level effect size would be 18.4 %.

Having selected an effect size of 18.4 % (i.e. relative reduction in the population rate) we proceeded to estimate the sample size required. As the number of clusters available is fixed ( $n = 24$ ), we followed an approach where first the sample size required under a naïve assumption of individual randomization was calculated, and then inflated by a factor (“design effect”) in order to account for correlation within CHW areas arising from the cluster-randomized design. We estimated this design effect as  $(1 + \rho \cdot [\theta - 1 + \theta \gamma^2])$ , where  $\rho$  is the estimated intra-cluster correlation coefficient,  $\theta$  is the mean sample size per cluster under individual randomization, and  $\gamma$  is the coefficient of variation in the cluster size [59]. Among ~16,000 births between June 2007 and September 2008 in this trial area, the intra-cluster correlation coefficient in this setting was 0.0060 (95 % CI: 0.0025–0.0095) and the coefficient of variation in cluster size was 0.33, leading to a design effect of 1.95 for preterm live birth. Taking this design effect into account, the number of live births required in each group to observe the estimated 18.4 % relative reduction with 80 % power and allowing 5 % Type I error is approximately 3367. Finally, we assumed that 90 % of the women screened at 13–19 weeks would have a live birth and 10 % would be lost to follow up, requiring enrollment of a total of 8314 in both control and intervention areas at the 13–19 week visit. Given a total population size of ~100,000 and a crude birth rate of 27/1000, we project that this sample will be reached in approximately 37 months, with total field work and follow-up of about 4 years.

### Data management

Data are collected on paper forms by CHWs and other field workers during home visits with enrolled mothers and their newborn babies. Forms are checked for accuracy and completeness by field supervisors prior to transport for data entry in Dhaka. A data entry prioritization protocol enables time-sensitive data, such as results from screening tests or treatment follow up data, to be entered locally at the field

site/laboratory; this enables minimal turn-around time between screening and treatment, and allows real-time assessment of compliance and monitoring of treatment-related adverse events. All forms are eventually entered into a secure Oracle database using customized data entry screens, with built-in range and validation checks. The database is backed up daily and a de-identified version is transferred (using encrypted peer-to-peer transfer software) on a regular basis to Baltimore, USA for archiving and creation of merged analytic files.

### Analysis

Interim (i.e. for DSMB analyses) and final analyses will follow the same protocol. First, we will present descriptive information on the recruitment, enrollment, and follow up of women and their newborns (i.e. a participant flowchart). We will then assess characteristics of the enrolled pregnancies to determine the extent to which our randomization procedure achieved comparability; these characteristics will include maternal, paternal, household, and socio-economic variables. For clusters allocated to the intervention arm we will describe the coverage of the screening and treatment intervention, including the distribution of time (i.e. gestational age) at screening, results of the initial and follow up vaginal swab (overall AVF, and intermediate vs. bacterial vaginosis) and urine tests (normal, intermediate asymptomatic, intermediate symptomatic, and high-burden single growth), and compliance with treatment. For the 10 % sub-sample of women in the control clusters who are randomly selected for the screening and treatment intervention, we will generate a similar set of intervention/coverage related indicators and compare these with those of the larger group of women in the intervention clusters.

The next stage of analysis will focus on describing the impact of the intervention on the primary and secondary outcomes. The gestational age at birth will be defined for each pregnancy as the number of days between last menstrual period (reported and recorded at enrollment) and the date of the pregnancy outcome. The primary study endpoint is preterm live birth, and thus we will present, in each group, the number of live births born prior to the pregnancy reaching 37 complete weeks (numerator) and the total number of live births (denominator). The preterm “rate” will be estimated as this proportion with a 95 % binomial exact confidence interval. The impact of the intervention will be assessed by estimating the relative risk of preterm live birth and the absolute risk difference using binomial regression models with a log and identity link functions, respectively. The standard error of this estimate will be constructed using generalized estimating equations and a 95 % confidence interval calculated. We will follow a similar analytic approach to estimate live births prior to 34 weeks (by group and with relative risk) and to estimate

the average gestational age at birth for live births, for which we will use a linear regression model with an identity link function and GEE to estimate errors.

We will then expand the pool of analyzable pregnancies to include all spontaneous delivery (i.e. late miscarriages and stillbirths, in addition to live births) and follow an identical approach to estimate the impact of the intervention on this broader set of pregnancies. We will estimate stillbirth and neonatal mortality rates (very early: <1 day, early: <7 days, and overall neonatal mortality: <28 days), and compare across the groups using binomial regression models. Other secondary outcomes of interest that will be compared across the groups include early-onset neonatal infection (defined through use of a sign-based algorithm and restricted to the first 7 days of life) and maternal report of infection in the first 7 and 28 days after delivery.

These models will be presented without adjustment, and a second set of analyses will be conducted with adjustment for any variables found to be imbalanced between the groups (if necessary). Planned sub-analyses to examine for effect modification include stratifications by age at delivery and parity. All estimates of the intervention’s impact will be presented with confidence intervals constructed from standard error estimates adjusted for the cluster randomization using GEE.

Additional planned analyses that are not related directly to the impact of the intervention include descriptive analyses of the organisms isolated from urine specimens, antibiotic resistance patterns, contamination rates over time, and adverse event reporting.

### Data safety and monitoring board and interim analysis

An independent Data Safety Monitoring Board (DSMB) consisting of a clinical trialist and infectious disease specialist, an obstetrician-gynecologist and a biostatistician is established for this trial. The DSMB met prior to the start of data collection to review and approve the study protocol, and met in person to review interim analysis after 33 % and 66 % of the study outcomes were achieved. At both meetings (February 2014, February 2015), the DSMB indicated the study should continue as planned. The DSMB reviewed the data collected in the study for evidence of issues related to safety of study subjects and the efficacy of the study interventions. The DSMB may also request further meetings, either face-to-face or by teleconference. At all meetings, minutes are kept of the deliberations of the DSMB and provided to the JHU Institutional Review Board (IRB), the Ethical Review Committee of the International Centre for Diarrhoeal Diseases Research, Bangladesh, and Brigham and Women’s Hospital.

### Approvals

The study protocol was approved by the IRB of the Johns Hopkins Bloomberg School of Public Health, the

Ethical Review Committee of the International Centre for Diarrhoeal Diseases Research, Bangladesh, and the IRB of Partners Health Care (Brigham and Women's/Faulkner Hospital and Massachusetts General Hospital).

## Discussion

The Projahnmo MIST Study, expected to be completed at the end of 2015, is a cluster randomized trial that will evaluate the impact of an early pregnancy screening and treatment program for AVF and UTI on population-level rates of preterm birth, compared to a basic package of routine antenatal and postpartum care alone. Furthermore, we will evaluate the impact of the intervention on rates of neonatal and maternal postpartum infection, and determine the population-based prevalence of these important genitourinary tract infections in pregnancy in a rural low-income country setting in South Asia. Finally, we will determine the accuracy and potential utility of point-of-care diagnostics to diagnose these infections.

Maternal genitourinary tract infections are prevalent and substantially contribute to global maternal and neonatal morbidity and mortality, yet they are poorly quantified, detected, and treated in low-income settings. Few interventions have proven effective in the prevention of preterm birth at the population level in high-income settings. However, in low-resource settings, early detection and treatment of maternal genitourinary tract infections may hold promise as a potentially low-cost, high-impact intervention to prevent preterm birth. BV and UTI are the most common infections in pregnancy, and there is strong evidence of their association with preterm birth, low birth weight, and early onset neonatal sepsis [60, 61]. While treatment of asymptomatic AVF in pregnancy is not standard of care, two promising trials in the UK have shown that treatment of asymptomatic AVF may reduce preterm birth rates by up to 60 %. Treatment of asymptomatic bactiuria is considered standard of care in high-income settings, and may reduce low birth weight and maternal pyelonephritis. There are few studies assessing the impact of a community-based screening and treatment program for genitourinary tract infections in low-income settings, where the burden of disease and impact may arguably be the greatest [62]. The strengths of the proposed study are as follows: 1) this will be one of the first studies of the prevalence of AVF and UTI (including asymptomatic bactiuria) in a large, rural, community-based cohort of pregnant women in a developing country; 2) using a cluster randomized controlled design, we will determine the effect of treatment of AVF and UTI on preterm birth, because present data are heterogeneous and limited by study design; 3) we will utilize an established network of CHWs for the screening and treatment program, which may be a feasible

and low-cost approach to delivering antenatal health services in similar developing country settings.

If the intervention is shown to be efficacious, the data from this trial will be used to contribute to the design of global public health strategies and recommendations regarding routine antenatal screening and treatment of maternal infections in low- and middle-income countries, and further our understanding of novel pathways to prevent preterm birth and reduce newborn morbidity and mortality in the highest burden settings.

### Abbreviations

AVF: Abnormal vaginal flora (Nugent Score 4–10); BV: Bacterial vaginosis (Nugent Score 7–10); IF: Intermediate flora (Nugent Score 4–6); UTI: Urinary tract infection; TOC: Test of cure; BNCP: Basic antenatal-postpartum care package; CHW: Community health worker; POC: Point of care.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

AHB, ACL, LCM, AL, PC, MQ, DKM, EK, SS conceived and designed the study. All authors were involved in project implementation and conduct of study procedures. PA, AM, JU manage the field implementation of study procedures in Bangladesh. IR performed the microbiological testing, including Nugent Scoring and Urinary Cultures, and SS supervised the laboratory testing. SD developed and maintained the database with LM. ACL drafted the first version of the manuscript. All authors read and approved the final manuscript.

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### Projahnmo Research Group

The Projahnmo Research Group includes: Johns Hopkins University (Abdullah H Baqui, Dipak Mitra, Luke C Mullany, Alain Labrique, Parul Christian, Jennifer Applegate, Nazma Begum, Rashed Shah); International Centre for Diarrhoeal Diseases Research, Bangladesh (Mohammed Quaiyum, Parvez Ahmed, Jamal Uddin, Ashraf Eusufzi, Rahman Mahmood, Monir Zaman); Child Health Research Foundation (Samir Saha, Maksuda Islam, Roman Mortuza, Robel Partaway, Tarik Hassan, Mashuk Siddiquee, Zabed Ahmed); Brigham and Women's Hospital (Anne CC Lee, Rachel Whelan, Karima Ladhanji); Projahnmo Field team (Arif Mahmud, Salahuddin Ahmed, Ataur Rahim; Nasreen Islam; Sadia Naznin); Shimanik NGO (Kazi Moksed Rahman, Ahmed Al-Kabir).

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### Data Safety and Monitoring Board

The members of the DSBM include: Sameena Chowdhury (chair), Jalaluddin Ashraful Haq, Meerjady Sabrina Flora, Davidson Hamer, and Mahmudur Rahman.

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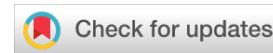
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## STUDY PROTOCOL

# Pneumococcal Conjugate Vaccine impact assessment in Bangladesh [version 1; peer review: 1 approved, 2 approved with reservations]

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## Abstract

The study examines the impact of the introduction of 10-valent Pneumococcal Conjugate Vaccine (PCV10) into Bangladesh's national vaccine program. PCV10 is administered to children under 1 year-old; the scheduled ages of administration are at 6, 10, and 18 weeks. The study is conducted in ~770,000 population containing ~90,000 <5 children in Sylhet, Bangladesh and has five objectives: 1) To collect data on community-based pre-PCV incidence rates of invasive pneumococcal diseases (IPD) in 0-59 month-old children in Sylhet, Bangladesh; 2) To evaluate the effectiveness of PCV10 introduction on Vaccine Type (VT) IPD in 3-59 month-old children using an incident case-control study design. Secondary aims include measuring the effects of PCV10 introduction on all IPD in 3-59 month-old children using case-control study design, and quantifying the emergence of Non Vaccine Type IPD; 3) To evaluate the effectiveness of PCV10 introduction on chest radiograph-confirmed pneumonia in children 3-35 months old using incident case-control study design. We will estimate the incidence trend of clinical and radiologically-confirmed

## Open Peer Review

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pneumonia in 3-35 month-old children in the study area before and after introduction of PCV10; 4) To determine the feasibility and utility of lung ultrasound for the diagnosis of pediatric pneumonia in a large sample of children in a resource-limited setting. We will also evaluate the effectiveness of PCV10 introduction on ultrasound-confirmed pneumonia in 3-35 month-old children using an incident case-control design and to examine the incidence trend of ultrasound-confirmed pneumonia in 3-35 month-old children in the study area before and after PCV10 introduction; and 5) To determine the direct and indirect effects of vaccination status on nasopharyngeal colonization on VT pneumococci among children with pneumonia.

This paper presents the methodology. The study will allow us to conduct a comprehensive and robust assessment of the impact of national introduction of PCV10 on pneumococcal disease in Bangladesh.

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Any reports and responses or comments on the article can be found at the end of the article.

## Keywords

Pneumococcal conjugate vaccine, invasive pneumococcal disease, pneumonia, impact assessment, radiograph confirmed pneumonia, ultrasound confirmed pneumonia, Bangladesh

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## Introduction

Pneumonia is the leading cause of death among children 1–59 months of age globally<sup>1</sup>. *Streptococcus pneumoniae* (pneumococcus) is a major cause of childhood pneumonia and other invasive pediatric diseases including meningitis and sepsis, accounting for approximately 14.5 million cases of invasive pneumococcal disease (IPD) and 826,000 child deaths worldwide<sup>2</sup>. Among these estimated deaths, 741,000 are pneumonia deaths and 60,500 are deaths due to meningitis<sup>2</sup>. Bangladesh is one of the ten countries with the highest number of IPD cases and IPD-related deaths among children under 5 years of age<sup>2</sup>.

Pneumococcal conjugate vaccines (PCVs) have been documented to be safe and effective for reducing illness and deaths caused by *S. pneumoniae* in numerous studies. In most high-income countries, PCVs are used routinely, with a concomitant and substantial reduction of pneumococcal diseases<sup>3–5</sup>. In 2006, the World Health Organization (WHO) recommended that PCV should be included in the routine infant immunization programs of all countries<sup>6</sup>. Despite this recommendation, many low- and middle-income countries (LMICs) have not introduced the vaccine. The Global Alliance Vaccine Initiative (GAVI) co-financing has resulted in an increase in PCV introduction into national immunization programs of LMICs. The proportion of the world's birth cohort living in countries with PCV in national immunization programs has increased from 1% in 2000 to 31% in 2012<sup>7</sup>. One hundred twenty eight countries have included PCV in their national immunization programs, however global coverage for completing all three doses of the vaccine were only 37% in 2015<sup>8</sup>. This finding suggests that efforts to increase PCV introduction and use globally have made substantial progress; however, gaps in PCV use remain, particularly in Asia and in countries with large birth cohorts, where concerted efforts are required<sup>7,8</sup>.

In 2013, the Ministry of Health and Family Welfare (MOHFW) of the Government of Bangladesh (GoB) decided to introduce 10-valent Pneumococcal Conjugate Vaccine (PCV10) into the national routine immunization program beginning in March 2015 making it the second South Asian country to implement PCV into routine childhood immunization, after Pakistan. In Bangladesh, the first dose of PCV10 is offered at 6 weeks of age along with pentavalent vaccine. The second and third doses are given at 10 and 18 weeks of age. Additionally, when the vaccine was introduced in March 2015, all infants who were less than 12 months old were offered the first dose of PCV10 and routine PCV10 immunization continued for this cohort. The decision to introduce PCV in Bangladesh in this way offered a unique and time-limited opportunity to generate pre-PCV data on pneumococcal diseases, and to conduct an impact assessment of PCV10 in the country following its introduction to measure its effect. Although Bangladesh had pre-PCV data on IPD from urban hospitals<sup>9,10</sup> allowing for before-after studies, population-based data were limited on culture-positive IPD from rural areas where the majority of child deaths are expected to occur<sup>11</sup>.

We established community- and facility-based surveillance beginning January 1, 2014 to generate data on community-based pre-PCV IPD incidence rates in children 0–59 months of age in Sylhet district of Bangladesh. In addition to documenting the pre-PCV IPD rates in the study areas, the surveillance was designed to assess the feasibility of conducting an adequately powered IPD case-control study in our study population after vaccine introduction to estimate the impact of PCV10.

There are certain limitations of using IPD as the sole outcome when measuring the impact of PCV10. Many pneumococcal disease cases, particularly pneumonia cases, are not associated with bacteremia and therefore are not culture positive when testing for pneumococcus in the bloodstream<sup>12–14</sup>. Thus, the etiology of community-acquired pneumonia can only rarely be determined by blood culture and relying on blood culture substantially underestimates pneumococcal disease burden. Widespread use of antibiotics in the community may further reduce the likelihood of positive results from blood culture. Although the point prevalence of clinical pneumonia among young children in Sylhet as determined by maternal report is fairly high at 4.9%, we expect to detect only about 50 IPD cases in our study area which contains about 90,000 <5 year old children in the year prior to PCV10 introduction<sup>15</sup>. Therefore, it is important to consider other pneumococcal-related outcomes, albeit non-specific, when measuring the impact of PCV10 introduction. To provide a more complete assessment of the impact of the PCV10 vaccine, we are conducting a comprehensive impact assessment of PCV introduction in our population. We have designed this study to measure multiple outcomes including IPD, nasopharyngeal (NP) carriage, and both radiographically- and sonoraphically-confirmed pneumonia.

## Aims and objectives

The primary aim of this study is to conduct a comprehensive evaluation of the national introduction of the PCV10 in Bangladesh measuring multiple outcomes. To accomplish this aim, we have established the following objectives for the study:

**Objective 1:** To collect data on community-based pre-PCV incidence rates of IPD in children 0–59 months of age in Sylhet district of Bangladesh.

**Objective 2:** To evaluate the effectiveness of PCV10 introduction on Vaccine Type (VT) IPD in children 3–59 months of age using an incident case-control study design. Secondary aims include measuring the effects of PCV10 introduction on all IPD, regardless of serotype, in children 3–59 months of age using the case-control study design, and quantifying the emergence of Non Vaccine Type (NVT) IPD.

**Objective 3:** To evaluate the effectiveness of PCV10 introduction on chest radiograph-confirmed pneumonia in children 3–35 months of age using an incident case-control study design. An additional aim is to estimate the incidence trend of clinical and radiologically-confirmed pneumonia in 3–35

month-old children in the study area before and after introduction of the PCV10 vaccine.

**Objective 4:** To determine the feasibility and utility of lung ultrasound for the diagnosis of pediatric pneumonia in a large sample of children in a resource-limited setting. We also sought to evaluate the effectiveness of PCV10 introduction on ultrasound-confirmed pneumonia in children 3–35 months of age using an incident case-control study design and to examine the incidence trend of ultrasound-confirmed pneumonia in 3–35 month-old children in the study area before and after introduction of the PCV10 vaccine.

**Objective 5:** To determine the direct and indirect effects of vaccination status on NP colonization on VT pneumococci among children with pneumonia.

## Protocol

### Study design

This is a population-based prospective community- and facility-based observational study with a number of nested studies including incident case-control and incident trend studies to evaluate the impact of PCV10. To conduct the impact assessment, we are considering four outcomes: 1) IPD; 2) chest radiograph-confirmed pneumonia, 3) lung ultrasound-confirmed pneumonia; and, 4) NP carriage.

### Study site and population

The PCV Impact Assessment in Bangladesh study is being conducted in the Projahnmo study group's research site in the Sylhet district of rural Bangladesh. The Projahnmo study group is a research partnership of the Johns Hopkins University with the Government of Bangladesh's Ministry of Health and Family Welfare (MOHFW) and several Bangladeshi non-governmental organizations (NGOs): the International Centre for Diarrhoeal Disease Research, Bangladesh (iccdr,b); Shimantik; and Child Health Research Foundation (CHRF). The field site was established in 2001 to contribute to the improvement of maternal, newborn and child health by conducting clinical-epidemiological studies, intervention trials and program evaluations. The study area covers three *upazilas* (sub-districts) of Sylhet district of Bangladesh (Zakiganj, Kanaighat and Beanibazar) with an estimated population of 770,000 (data source: project census, 2011), yielding an annual birth cohort of approximately 19,725 (Figure 1). All households and women of childbearing age in the study area have unique current and permanent identification numbers, which allow for individual tracking and longitudinal follow-up. The study is being conducted among children 0–59 months old in this active surveillance area.

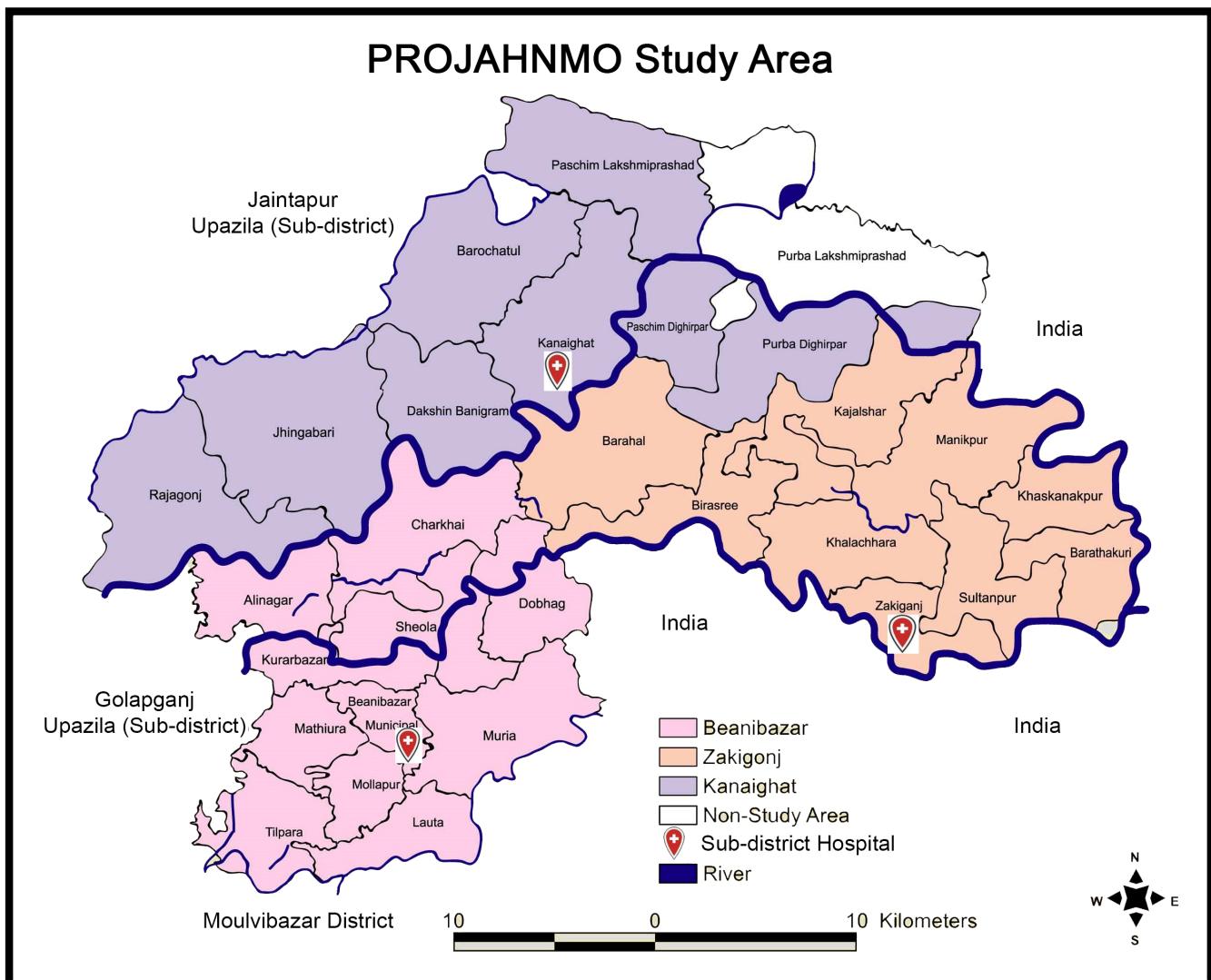
The substantial existing infrastructure includes a census; a Global Positioning System-based map and background characteristics of the entire population; community- and facility-based surveillance; mechanisms for community-based sampling and case identification; referral, specimen collection, transport; state-of-the-art laboratories; and a data center. We have established community-based surveillance, which involves

home visits by trained community health workers (CHWs) in the entire area in about 90,000 <5 year old children. Working closely with the Bangladesh MOHFW, we have established facility-based surveillance within the MOHFW sub-district hospital outpatient clinics and inpatient pediatric wards in the study area for potential IPD case detection, specimen collection, and transport. Project physicians who are trained and standardized to the study research protocols staff these facilities.

### Objective 1: Establish pre-PCV IPD rate

Trained female CHWs who are residents of the community and have at least a 10th grade education have been assigned to a population of about 10,000 individuals. Each CHW visits all households (about 2,000 households on average) in her area once every two months to update the population data by recording all new pregnancies, births, deaths, marriages and movements. Background data on age, parity, literacy, prior obstetric history and socio-economic status of all enrolled women in our study area were collected as part of previous or ongoing studies and are available in our database<sup>16–20</sup>. These data are collected for all newly identified pregnant women who did not provide them previously. Similarly, dates of birth of children born in previous or ongoing studies are available in our database and the data are prospectively collected for women and children <5 years of age who were not part of prior studies. In our efforts to provide a standard package of care to pregnant women and newborns in this research site, CHWs provide information to pregnant women about care during pregnancy, delivery and the postpartum period during regular visits every two months. Families are encouraged to notify CHWs about all births as soon as possible using the existing cell phone-based birth notification system, and are encouraged to seek care from study designated hospitals for any illnesses in newborns and children. All children are visited every two months until they are 60 months of age, and data on their vital status, illness history and care seeking are recorded. Mothers and family members are educated during home visits every two months on signs of pneumococcal diseases (pneumonia, meningitis, and other IPD) and are further encouraged to visit the designated study hospitals for clinical evaluation, enrollment and management of suspected illnesses of the children.

The CHWs also recruit and supervise local resident village health workers (VHWs) in each village in the study area. The VHWs are provided with thermometers marked at a cutoff level of 101°F and trained on their use. They are also trained to recognize symptoms of pneumonia and meningitis. In addition, they visit and screen children of consented parents weekly for fever, respiratory problems and danger signs, and refer sick children to one of the study hospitals. The VHWs receive a monthly stipend and financial incentive payments for successful referrals of eligible children with clinical pneumonia, high fever, and meningitis. VHWs do not collect any study data but facilitate case detection and referral from surveillance population to the study hospitals. In case of refusal of referral advice by families, the VHWs call the CHWs for further facilitation of the referral process. The CHW referral criteria is in Table 1.



**Figure 1. Study area.** Pneumococcal Conjugate Vaccine impact assessment in Bangladesh.

In addition to family education for illness recognition and care seeking from sub-district hospitals, we have developed a network with the first-level facilities and private health care providers in both the formal and informal sectors within the study area and encourage them to refer sick children with suspected pneumococcal disease to the designated study hospitals. We have also included Sylhet Medical College and selected private tertiary level referral hospitals in Sylhet City in this network, which are frequent referral sites for children with symptoms of meningitis in our study area. We have mapped the first-level clinics and private health care providers in the study area including MOHFW's Family Welfare Centers (FWCs), Community Clinics, NGO-supported clinics, village doctors and drug sellers. All of these providers are given an orientation on the study and are requested to refer eligible cases to the

designated hospitals. The orientation includes standardized definitions of suspected pneumonia and meningitis cases and instruction on keeping a list of cases by village. Our study physicians periodically visit these providers and facilities to assess the quality of diagnoses made and reinforce adherence to the case definitions and protocols for referral of all suspected cases to designated health facilities. We identify community providers who see a particularly high volume of children <5 years of age with lower respiratory symptoms, and obtain the providers' consent to station mobile teams of trained health workers at their offices. We have established a quality control program to ensure the quality of screening to maximize enrollment of eligible cases and of blood specimen collection to avoid specimen contamination. Periodic and need-based refresher trainings are organized for all study staff.

For referred cases of meningitis being treated in non-study hospitals, a trained study physician travels to visit the patient in that hospital, obtain parental informed consent, clinically screen the patient for suspected meningitis, and perform lumbar puncture if appropriate and if the parent provides consent. The cerebrospinal fluid (CSF) specimen is transported to the study laboratory using the procedures described below.

Upon presentation at any of the participating study hospitals, a research assistant requests parents/caregivers of all children

**Table 1. Referral criteria for pneumonia and invasive pneumococcal diseases at the community level.**

High fever ( $\geq 101.0^{\circ}\text{F}$ )
Hypothermia ( $<95.9^{\circ}\text{F}$ )
Movement only on stimulation
No movement/unconscious
Reported/Observed convulsions
Unable to feed
Vomits everything
Bulging fontanelle
Stiff neck
Lower chest indrawing
Observed head nodding/use of neck muscles in breathing
Noisy breathing
Cough/difficulty in breathing PLUS Fast breathing
H/O Scanty micturition or high colored urine PLUS Puffy face/Ascites/leg edema

<5 years of age to give consent for screening for suspected IPD (clinical pneumonia, meningitis, or high fever) and for collection of basic demographic and clinical information. Suspected IPD cases are identified by physicians at the study hospitals and mobile team members in the community. After obtaining written informed consent from parents, detailed data on demographic characteristics, clinical assessment, and history of medication use are recorded in a case report form (CRF). The criteria used for blood or CSF collection are provided in [Table 2](#).

A blood sample (~3 ml) is collected from each eligible child and half of it is directly inoculated into a BACTEC (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) pediatric blood culture bottle and kept at room temperature until transportation to the Sylhet laboratory, where it is processed in a BACTEC machine. The culture bottles that show signs of bacterial growth are then subcultured. The other half of the blood specimen is transported to the Sylhet laboratory maintaining cold chain for molecular testing.

Lumbar puncture is performed for all consented hospitalized cases with signs of meningitis. Up to 2 ml of CSF is collected for immediate plating on chocolate, blood and MacConkey agar media and a portion of CSF is saved for cytology and measurement of biochemical parameters. The remaining CSF specimen is preserved for molecular testing. All specimens are transported to the study laboratory in Sylhet at least twice daily for identification and real-time assessment of biological parameters. Preliminarily identified isolates are transported to the CHRF lab at Dhaka Shishu Hospital in Dhaka for reconfirmation, serotyping, and drug susceptibility testing. All molecular testing is performed in the CHRF lab in Dhaka using real-time polymerase chain reaction (PCR) techniques. The study doctors record data on treatment given, status at discharge and laboratory findings in the CRF. An IPD case is identified using a pre-determined

**Table 2. Criteria for blood or CSF collection for testing for invasive pneumococcal diseases.**

<b>Inclusion criteria</b>	1. Any of following danger signs
	i. Hypothermia ( $<95.9^{\circ}\text{F}$ )
	ii. Movement only on stimulation
	iii. No movement/unconscious
	iv. Reported/Observed convulsions
	v. Unable to feed
	vi. Vomits everything
	vii. Bulging fontanelle
	viii. Stiff neck
	2. High fever ( $\geq 101.0^{\circ}\text{F}$ )
	3. Moderate fever (99.5–100.9) PLUS Any of the following signs of severe pneumonia
	i. Chest indrawing
	ii. Observed head nodding during breathing
	iii. Stridor (persists after bronchodilatation)
	4. Suspected Nephrotic syndrome or Glomerulonephritis
<b>Exclusion criteria</b>	i. History of scanty micturition or high colored urine AND Oedema (Puffy face/Leg oedema/Ascities)
	1. Previously enrolled in the preceding 7 days
	2. Had received antibiotics doses (confirmed by prescription OR bottle/strip) and the last dose within last 24 hours

criteria that take in to account the clinical findings, culture and PCR results. All sick children receive treatment based on the physicians' clinical diagnosis. For outpatients, a written report on blood culture results is provided to parents at the next routine household visit after the results become available.

#### Objective 2: IPD case-control and incident trend studies

We have maintained the IPD surveillance established for Objective 1 in our study area, including community surveillance,

health facility activities and laboratory procedures, in order to detect IPD cases. The inclusion criteria for the case-control and incident trend studies are shown in [Table 3](#) and [Table 4](#). The age eligibility for enrollment in the IPD case-control study is shown in [Table 5](#) and the sample size requirement for IPD case-control study is shown in [Table 6](#).

For each enrolled IPD case child, we randomly select and enroll two sets of four matched control children from the study area.

**Table 3. Inclusion criteria for the IPD case-control study.**

<b>Inclusion criteria</b>	1. Age 3–59 months meeting age-eligibility criteria under which they have had the potential to have started the PCV vaccine (not born yet or under age 12 months on March 25, 2015).
	2. Resident of the active surveillance area clinically eligible. <ul style="list-style-type: none"> <li>a. For cases – clinically suspected invasive pneumococcal disease (pneumonia or meningitis or high fever) and pneumococcus isolated from blood or cerebrospinal fluid</li> <li>b. For controls-without any signs/symptoms of invasive pneumococcal disease as screened by physicians (hospital controls) or data collectors in the community (community controls)</li> </ul>
	3. Written informed consent by parents/care givers.
<b>Exclusion criteria</b>	1. Refusal to join the study

**Table 4. Inclusion criteria for the incident-trend study.**

<b>Inclusion criteria</b>	1. Age 3–59 months meeting age-eligibility criteria under which they have had the potential to have started the PCV vaccine (not born yet or under age 12 months on March 25, 2015).
	2. Resident of the active surveillance area
	3. Diagnosed clinically as invasive pneumococcal disease and pneumococcus isolated from blood or cerebrospinal fluid
	4. Written informed consent for participation in x-ray study by parents/care givers.
<b>Exclusion criteria</b>	1. Refusal to join the study

**Table 5. Age-eligibility criteria for inclusion in invasive pneumococcal diseases case-control study by study months.** Age in completed months.

Year	2015												2016											
Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec							
Month of case-control study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17							
Minimum age of eligibility	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3							
Maximum age of eligibility	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31							
Year	2017																							
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct*	Nov*	Dec*												
Month of case-control study	18	19	20	21	22	23	24	25	28	29	30	31												
Minimum age of eligibility	3	3	3	3	3	3	3	3	3	3	3	3												
Maximum age of eligibility	32	33	34	35	36	37	38	39	40	41	42	43												

\*If needed for adequate sample size

**Table 6.** Sample size estimates for detectable levels of vaccine effectiveness in invasive pneumococcal diseases case-control study.

	90% Vaccine Effectiveness (VE)			95% VE		
	Average coverage	Number of VT IPD cases	Lower bound	Average coverage	Number of VT IPD cases	Lower bound
No vaccine	0%	45	N/A	0%	45	N/A
Scenario 1 (simulation)	45%	25	50.8	45%	24	72.7
Scenario 2	50%	25	54.3	50%	23	73.9
Scenario 3	60%	21	51.8	60%	19	72.1
Scenario 4	70%	17	44.2	70%	15	67.1

The control children are of the same sex as the case and within  $\pm 1$  month of age of the case child. The first set of controls is selected from the hospital from where the case is detected. Four age- and sex-matched children are selected from among those who visit the same health facility within 1 week of the identification of the case and are screened as not having any symptoms or signs of clinical pneumonia or meningitis. At our three sub-district hospitals, consent for screening of all children under 5 years of age is obtained at the time of presentation to the hospital. Children who screen negative for symptoms of suspected IPD are placed in a pool of possible controls. If a case is identified during a given week, the age- and sex-matched controls are then selected and visited at home to collect background data including immunization status data. For meningitis cases identified at tertiary hospitals in Sylhet, age- and sex-matched children are identified at the pediatric surgery clinic and at other services that are not treating children with infectious disease during the two weeks following identification of the case. Children of parents who consent for their children to serve as controls are recruited to the study.

The second set of controls is selected from the community. Four controls matched to the case child by age, sex, and road distance from their home village to the study hospital where the case was ascertained are selected within one week of identification of the case. To facilitate random selection, we use the complete list of 823 villages (including municipal wards) in our study area, each of which has a population of about 1,000 people on average. All the villages are uniquely numbered. Once a case is identified, we generate a complete list of villages within the hospital catchment area that are within one kilometer of the distance from hospital to the village where the case is ascertained (the “index village”). If fewer than four matching villages are generated, we then expand the matching distance range to two kilometers. If we have more than four villages in the list, we randomly rank the villages and attempt to select one control per village using the ranked list. The children within each village who meet the age- and sex-matching criteria for the case are also listed and randomly ordered. The health

worker then sequentially visits each child and asks the parent for their consent to screen the child as a control. The screening involves an examination for signs of IPD (pneumonia, high fever, and meningitis) and maternal recall of the presence of any such symptoms during the past seven days. Once one healthy control has been identified in a given village, we move to the second-ranked village to select the second control, and so forth.

Informed written consent is obtained from the parents of both hospital and community controls. After consent is obtained, the same information that we collect from a case child is collected from each of the control children. The information on the vaccination status of the control children are collected using the same method used for case children.

**Exposure ascertainment.** The following epidemiologic information is collected for each case and control child using a standardized data collection instrument:

- Risk factors for IPD: age, family structure, antibiotic use, season/date, comorbid illness status of the individual, crowding measures, smoke exposure variables, geographic location, nutritional status, birth weight, gestational age at birth, and socio-economic status;
- Vaccination status: Immunization status of each child including PCV, oral polio vaccine, and pentavalent vaccine is recorded. The mother is asked to provide the child’s immunization card for examination and it is extracted onto a standard data collection form. We also take a digital photograph of the immunization card. If the immunization card is not available, the caregiver is asked if the child has been vaccinated, and if so, at which immunization center. The register of the identified immunization clinic is then reviewed and the child’s immunization history is abstracted. Only children who received at least two doses of the vaccine at least fourteen days before the date of case identification or control selection will be considered as vaccinated for the purposes of the study analysis;

- Anthropometric measurements: Current weight, height and mid-upper arm circumference are also collected.

#### Objective 3: Chest radiograph confirmed pneumonia case-control and incidence trend studies

We have established pediatric computed radiology (CR) units in the three sub-district hospitals through procurement of portable analogue radiograph units (POLYMOBIL® Plus, Siemens, Erlangen, Germany) with accompanying CR Fujifilm™ cassette readers, which digitize the image. Trained radiography technologists have been recruited by the project and are responsible for carrying out supine antero-posterior chest radiographic imaging for children meeting the study clinical pneumonia criteria. Training on the use of the radiograph unit and CR cassette readers was conducted online via teleconference with the vendors. The radiographers have been provided with hands-on training by radiology faculty and technologists from Sylhet Medical College and Dhaka Shishu (pediatric) hospital. We have a service, inspection and maintenance agreement for the radiograph units with the vendor (Siemens/Bangladesh) to ensure that the radiograph machines are in good working order at all times and meet all safety requirements. The machines are calibrated and the performance of the machines are evaluated immediately following installation, immediately following any repair, parts replacement or maintenance, and annually. Radiography technicians are trained to capture radiograph images according to a standardized protocol and a random sample of images are assessed for quality weekly by the study pediatric pulmonologist (EDM).

Study physicians at the study hospitals are also trained to assess image quality. We aimed for a proportion of less than 5% “uninterpretable” images assessed by a panel of chest radiograph readers. For children 3–35 months of age who meet the inclusion criteria as a potential case either in the case-control study or incidence trend study, the local radiographers use the CR cassette reader to visualize the image and translate the image to PC-readable format and send it electronically to the trained panel of chest radiograph readers. We created a pool of eight primary chest radiograph image readers comprised of consultant pediatric radiologists and pediatric consultants who are based in Dhaka. They were trained and standardized to the WHO chest radiograph interpretation definitions for pediatric vaccine effectiveness studies by an international WHO-certified trainer for two days ([Table 7](#))<sup>21,22</sup>. After this initial standardization process was completed the study pediatric pulmonologist (EDM) is facilitating ongoing, twice-annual re-standardization of the readers to the WHO protocol, in consultation with the WHO-certified trainer, for the duration of the project and also serves as the panel’s expert reader and provides quality control oversight of the panel.

Each digital image of a chest radiograph from a child with clinical pneumonia is independently read by two trained primary readers, selected randomly from the pool of eight readers. Readers are blinded to the clinical data and also the ultrasonographic images and interpretations. For interpretable radiographic images, if the interpretation by the two primary readers is identical for the presence or absence of WHO primary

**Table 7. World Health Organization-defined pediatric antero-posterior chest radiograph findings used in the Bangladesh Pneumococcal Conjugate Vaccine Impact Assessment.**

Quality	Interpretable	Image is interpretable for the presence or absence of endpoint consolidation or pleural effusion.
	Uninterpretable	Image is not interpretable for the presence or absence of endpoint consolidation or pleural effusion.
Classification	Endpoint Consolidation	A dense or confluent opacity that occupies a portion or whole of a lobe or the entire lung that may or may not contain air bronchograms.
	Other infiltrate (non-endpoint)	Linear and patchy opacities (interstitial infiltrate) in a lacy pattern, featuring peribronchial thickening and multiple areas of atelectasis; it also includes minor patchy infiltrates that are not of sufficient magnitude to constitute endpoint consolidation, and small areas of atelectasis that in children may be difficult to distinguish from consolidation.
	Pleural effusion	Presence of fluid in the lateral pleural space between the lung and chest wall that is spatially associated with a pulmonary parenchymal infiltrate (including other infiltrate) or has obliterated enough of the hemithorax to obscure any infiltrate; in most cases, this will be seen at the costophrenic angle or as a layer of fluid adjacent to the lateral chest wall; this does not include fluid seen in the horizontal or oblique fissures.
Conclusion	Primary endpoint pneumonia	Presence of endpoint consolidation or pleural effusion, as defined above.
	Other infiltrate	Presence of other (non-consolidation) infiltrates as defined above in the absence of a pleural effusion.
	No consolidation/infiltrate/effusion	Absence of consolidation, other infiltrates or pleural effusion.

endpoint pneumonia, then the classification is considered final. If the classifications of the image by the two primary readers are discordant, the image is then sent to a third reader who is randomly selected from the remaining readers in the pool. The third reader is blinded to the initial classifications by the first two readers and the fact that they are serving as the third reader for that image. If the classification of the third reader agrees with one of the initial two readings, then the third reader interpretation is considered final. However, if the third reader does not agree with either of the first two readings, the image is sent to the study expert reader (EDM) and his reading is then considered final. In addition, a 20% random sampling of all the images are read by the expert reader (EDM) as a quality control measure and regular performance reports are generated as feedback to the radiograph reading panel. Initial management of the children is done based on clinical and bacteriological findings since radiological findings take 24–48 hours to be finalized. Once finalized, the image results are also shared with the treating physicians and may also be taken into consideration for further patient management given the initial treatment decision is made on the patient's clinical presentation only. Individual reader performance is monitored throughout the study and refresher trainings and reader re-standardization to the WHO protocol are conducted every six months under the facilitation of the expert reader (EDM), in consultation with the WHO certified trainer. All images are archived at the site and catalogued by study ID and date. A written report on the chest radiograph result is provided to parents at the next routine household visit after the results become available or sooner if the child returns to the hospital clinic.

We initiated the incident chest radiograph case-control study two months after introduction of PCV10 in the national

vaccine program in our study area. The age eligibility for the chest radiograph case-control study is shown in [Table 8](#) and sample size requirement based on different PCV coverage scenarios is shown in [Table 9](#). Cases are age-eligible children diagnosed with radiographically confirmed pneumonia at a study hospital. Control selection procedures are the same as the IPD case-control study.

**Data collection from cases and controls.** An additional written informed consent is obtained from the parents of the selected case and control children. After consent is obtained, case and control households are visited to collect vaccination status data and other information that is required as covariates or potential confounders in the analysis, such as socio-economic status, as detailed for the IPD analysis.

#### Objective 4: Lung ultrasound confirmed pneumonia case-control and incidence trend studies

In contrast to chest radiography, the use of lung ultrasound for the diagnosis of pneumonia is not yet considered standard of care or even part of medical curricula or guidelines from pediatric societies. A recent meta-analysis and several randomized clinical trials, however, suggest that lung ultrasound performs well as a diagnostic tool for the identification of pneumonia when compared to chest x-ray<sup>23,24</sup> and that outcomes are similar when if lung ultrasound were to replace chest x-rays<sup>25</sup>. Recent studies by our research group conducted in Peru<sup>26</sup> and Nepal<sup>27</sup> suggest that lung ultrasound is a feasible and appropriate technology for the diagnosis of pediatric pneumonia in resource-poor settings. However, since these studies only used one or two study physicians, we were unable to assess the role of our standardized training program on the conduct and interpretation of lung ultrasound findings when using a

**Table 8.** Age eligibility criteria for inclusion in radiographic and lung-ultrasound confirmed cases in case-control studies by study months.

Year	2015						2016											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Month of case-control study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Minimum age of eligibility in completed months	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
Maximum age of eligibility	15	16	17	8	19	20	21	22	23	24	25	26	27	28	29	30	31	
Year	2017																	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec						
Month of case-control study	18	19	20	21	22	23	24	25	26	27	28	29						
Minimum age of eligibility in completed months	3	3	3	3	3	3	3	3	3	3	3	3						
Maximum age of eligibility	32	33	34	35	35	35	35	35	35	35	35	35						

large number of study physicians. Three portable ultrasound machines (Sonosite Edge, Bothell, WA) were placed at each of the three sub-district hospitals (Zakiganj, Kanaighat and Beanibazar) and used for lung ultrasound assessments. Study physicians underwent a standardized training course to learn the use of lung ultrasound in the diagnosis of pediatric pneumonia. As per the study protocol, the seven-day training course consisted of both theoretical and practical training. The first three days of training consisted of classroom learning of ultrasound basics and recognition of pathologies, and the next

four-days were direct ultrasound training in a pediatric ward to properly identify lung ultrasound patterns in children consistent with pneumonia, other respiratory abnormalities or with normal findings ([Table 10](#)). After completion of the seven-day training, the trainees undertook both a theoretical and practical competency assessment. All study physicians were required to achieve a passing grade of 80% to be considered standardized.

In contrast to chest radiography where findings are consistent with anatomical changes, findings on lung ultrasound are

**Table 9. Detectable odds ratio with 80% power and 0.05 type 1 error for radiographic and lung ultrasound studies.**

Cases	Vaccine coverage	Matched controls per case	Detectable OR
1130	46%	1	0.766
1130	46%	2	0.800
1130	46%	3	0.813
1130	46%	4	0.820

**Table 10. Lung ultrasound findings used in the Bangladesh Pneumococcal Conjugate Vaccine Impact Assessment.**  
Description of findings on lung ultrasound and definition of endpoint pneumonia, interstitial abnormality and atelectasis.

Quality	Interpretable	Ultrasound is interpretable for the presence or absence of endpoint consolidation, atelectasis, or interstitial abnormalities.
	Uninterpretable	Ultrasound quality is not interpretable for the presence or absence endpoint consolidation, atelectasis, or interstitial abnormalities. Ultrasound does not have all 24 clips recorded.
Classification	Endpoint Consolidation	Hypoechoic area or tissue pattern with loss or attenuation of distinct pleural lines.
	Air Bronchogram	Fluid or inflammation along the bronchial walls. This is visualized on ultrasound as punctate hyperechoic or hypoechoic images.
	B-Lines	Well defined hyperechoic comet-tail artifacts arising from the pleural line, spreading down, indefinitely erasing A lines and moving with lung sliding when lung sliding is present.
	Pleural Abnormality	Disruption along the pleural line that is not large enough to be measured as a consolidation.
	Shred Sign	Disruption of the pleural line, caused by consolidation or pleural effusion, that forces the pleural line to become discontinuous and move below the level of the consolidation.
	Pleural effusion	Presence of fluid in the lateral pleural space between the lung and chest wall. This is visualized on ultrasound as hypoechoic images in the pleural space.
	Primary Endpoint Pneumonia	Presence of consolidation that measures $\geq 1$ cm or greater than 1 intercostal space, or a pleural effusion with any of the following; consolidation $< 1$ cm, $\geq 3$ B-lines, air bronchograms.
	Interstitial Abnormalities	Presence of artifacts consistent with $\geq 3$ B lines or pleural abnormalities.
	Atelectasis	Presence of consolidation $< 1$ cm or smaller than 1 intercostal space.

artifacts that are associated with anatomic changes and pathology and not with acute anatomic changes (Table 10). We defined pneumonia on lung ultrasound as a consolidation  $\geq 1$  cm in size or greater than one intercostal space, or a pleural effusion with any of the following: consolidation  $< 1$  cm in size,  $\geq 3$  B-lines or presence of air bronchograms. Interstitial abnormalities were defined as  $\geq 3$  B-lines or presence of pleural abnormalities. Atelectasis was defined as a consolidation  $< 1$  cm in size or smaller than one intercostal space.

**Ultrasound procedures.** The use of lung ultrasound began after all study pediatricians had at least one month of practice using the ultrasound machine for pneumonia diagnosis. At the onset of the study, all ultrasounds were conducted and interpreted by a study physician and re-assessed remotely by one of three expert physicians. However, as the volume of patients increased a two reader process was implemented with an expert reader only reviewing discrepancies. The study physician who conducted the ultrasound was the first reader. A second study physician randomly selected from the team would provide an independent second read. If there was disagreement in the interpretation of the lung ultrasound image between two physicians, then the image is reviewed by an expert sonographer who would then act as the ombudsman and provide a final diagnosis. We initially developed a standardized case-report form that was used by both the study physicians and expert readers. However, seven months into the study the case volume of child participants became too high, and we had to develop a shorter CRF that was easier to complete by the study physicians. The content of the CRF remained consistent throughout the course of the study, and the case report form for the expert readers remained the same.

Procedures for the case-control and incidence monitoring studies are the same as those described under Objective 3. Follow-up visits for children with ultrasound-confirmed pneumonia may include a repeat ultrasound.

#### Objective 5: Assessment on the impact on NP carriage

An assessment of the baseline rates of NP pneumococcal colonization among participants with radiograph-confirmed pneumonia began following the start of the chest radiograph study in September 2015. Physicians collect nasopharyngeal swabs on all consenting clinical pneumonia cases. Participants are requested to sign a separate consent for NP swab collection. NP specimens are collected using nylon flocked swabs in accordance with updated WHO core methods<sup>28,29</sup>. A single NP specimen is collected from the posterior nasopharynx of each subject by inserting the swab, rotating it 180 degrees and removing it. The swab is inserted immediately into one mL of liquid STGG transport medium and transported on ice to the clinical laboratory in Sylhet. Swabs are frozen at -20°C and stored for up to seven days. All swabs of confirmed radiographic pneumonia cases are cultured for pneumococcus; the remaining swabs are discarded. In the lab, the specimen are vortexed, aliquoted into two separate specimens (one with the

swab retained), and a sample inoculated into broth for enrichment. Following broth enrichment, the sample is plated onto a gentamicin-blood agar plate and incubated in a CO<sub>2</sub> incubator for isolation of pneumococcus. An aliquot of the broth enrichment as well as the remaining STGG NP sample are frozen (-80°C). Pneumococci are detected based on morphology, optochin susceptibility and bile solubility.

To determine the density of pneumococcal colonization, a second sample from the STGG vial is subjected to 10-fold dilutions up to 10<sup>-5</sup>. An amount of 100 µl of each dilution is plated onto gentamicin blood agar plates and the semiquantitative results are documented, based on the numbers on the plates with countable number of pneumococcal and dilution factors. Pneumococcal strains are serotyped by the capsular swelling procedure (quellung reaction) with type-specific anti-pneumococcal omni, pool, type or group, and factor sera (Statens Serum Institut, Copenhagen, Denmark).

Data on PCV10 vaccination status are collected from parents of all participants enrolled in the NP carriage study at home visits if vaccination card is available. If the child's parents fail to produce a card at enrollment or at their home but state a history of the child having received vaccines, the registry at the local immunization clinic registry is consulted. Demographic and socio-economic characteristics are captured once during the study on all participants via a household survey.

#### Coverage survey and incidence trend analysis

We have conducted two PCV coverage surveys, one in August-September 2016 and the other in August-September 2017. The surveys included simple random samples of 3,753 and 5,421 vaccine age eligible children stratified by age in months and union (with an average population of 25,000; there are 30 unions in the study area), respectively. This assures that every union's coverage of <24 month-old children can be estimated to within ten percentage points (or less) with 95% confidence during any study month. The estimated vaccine coverage for the entire area for any given month will be more precise. These data will allow us to relate vaccine coverage to disease incidence via a Poisson regression model with one observation for each union-month.

#### Training and quality control

**Community health workers.** Before initiating data collection, CHWs are provided with training on surveillance activities, screening and identification of suspected IPD cases including pneumonia and meningitis in the community. The training includes theoretical discussions as well as real-life assessment of children in Sylhet Medical College Hospital. CHWs are also provided training on how to supervise VHWs. Field supervisors supervise and oversee CHW work at home. Supervisors meet with CHWs every two weeks and updates and continued training is provided. Supervisors accompany the CHWs twice a month to observe CHW activities and carry out independent home visit and data collection in a 5% random sample of

households. Physicians conduct regular validation of CHW screening procedures and a refresher training is arranged when necessary.

**Physicians.** All physicians receive standard integrated management of childhood illness (IMCI) training before starting screening and enrolment. Every 1–3 months throughout the project all study physicians receive intensive individual supervision of respiratory screening of children by the study pediatric pulmonologist (EDM), including immediate individual feedback as well as study reports in order to track individual study physician performance to identify those in need of remediation. In addition, study physicians receive twice annual refresher trainings facilitated by the study pediatric pulmonologist (EDM). Lumbar puncture is done by physicians who are trained on the technique at Dhaka Children Hospital or Sylhet Medical College Hospital. Physicians who carry out ultrasound are trained with a seven-day training course followed by on-site supervision and remote quality assurance by a pulmonologist with significant expertise in lung ultrasound for diagnosis of pediatric pneumonia.

A group of expert sonographers supervised the study physicians in performing the ultrasounds onsite for two weeks following the training course, and for a period of one month all ultrasound clips were transmitted to one of the two expert sonographers for confirmation of physician interpretations, prior to use of the study physician interpretations. We also conducted re-training and re-standardization throughout the study period, either as refreshers for current study physicians or to standardize new study physicians. We also had quarterly quizzes to review interpretation of lung ultrasound images. A total of 31 physicians underwent lung ultrasound training and standardization as part of this study. Moreover, to build local capacity in lung ultrasound, two study physicians were trained as local experts to conduct quality assessments and continued training. Separately, an expert sonographer over-read 20% of images for quality control purposes. All ultrasound clips were digitally and securely stored on a portable hard drive, and uploaded to a cloud server for interpretation (Ultraling New York, NY).

#### Data management, analysis plan and description of the nature of the variables to be derived

Data generated in the field reach the data management unit within three weeks and are entered in computers using a custom relational data entry system developed by our team (PCV v5.9). The system has built-in checks for range, consistency and validity of data to minimize errors. Data required for field monitoring and patient management (e.g., lab results) are entered in our field offices immediately after collection using an online web-based data entry system. Laboratory Management System Software is used to maintain Good Laboratory Practices. Most equipment is computerized. Data cleaning is performed periodically to ensure availability of cleaned data tables within five weeks of data collection. Data analysis is performed using STATA 13.

**Objective 1.** Overall, vaccine type and non-vaccine type IPD rates will be estimated from the data generated in Objective 1 period.

**Objective 2.** For descriptive purposes, the relative frequencies of the demographic, socioeconomic, matching, clinical, and other study variables will be compared for the cases and controls. We will conduct two separate case-control analyses, one using hospital controls and one using community controls. To adjust for multiple confounding variables as well as to evaluate effect modification, conditional logistic regression method designed for matched studies will be used<sup>30</sup>. This analysis will provide adjusted odds ratios and its 95% confidence intervals (CIs). Since perfect age-matching may be difficult, age may remain a potential residual confounder because it is related to both disease and likelihood of vaccination. To handle this potential problem, vaccine efficacy (VE) estimates will be age adjusted by including a linear term for age in months in all models. Estimates of vaccine effectiveness will be calculated using the formula:

$$VE = (1 - \text{matched OR}) \times 100,$$

where OR indicates the odds ratio for receiving PCV in cases and controls<sup>31</sup>. Case-control analyses for each group of controls (hospital controls and community controls) will be conducted separately and the results compared and discussed. Data from the surveillance component of the study will be analyzed to quantify the magnitude of the incidence of IPD and to determine the clinical and epidemiologic characteristics of IPD.

**Objective 3.** Similar to IPD case-control study, we will conduct two separate case-control analyses for radiographically-confirmed pneumonia, one using hospital controls and one using community controls. To adjust for multiple confounding variables as well as to evaluate effect modification, the conditional logistic regression method designed for matched studies will be used in this analysis as well<sup>30</sup>. Similar to the IPD analysis, we will also generate the adjusted OR and its 95% CI and since residual confounding by age may be an issue with this analysis as well, we will likely age adjust vaccine efficacy estimates by including a linear term for age in month in all models, as described for Objective 2. We will define VE as done in Objective 2, but in this analysis OR will indicate the odds ratio of radiologically confirmed pneumonia to PCV vaccination status<sup>31</sup>. In the absence of any selection bias, the OR will estimate the incidence rate ratio.

The incidence rate of chest radiograph-confirmed pneumonia will be computed by dividing the number of radiograph-confirmed pneumonia cases by child years of observation. The 95% CI will be calculated using the Poisson distribution. Separate point and interval estimations for different types of endpoints as defined by the classifications of chest radiograph results will be computed.

To estimate the impact of the vaccine, the incidence rate ratio will be calculated by dividing the incidence rate in study months 13–24 by the incidence rate in study months 1–12; standard errors and 95% CIs will be reported. Poisson regression will be used to assess trends in the incidence rate over time. We will also investigate whether any clustering by study hospital or evaluating pediatrician is evident.

**Objective 4:** Our analytic methodology for Objective 4 will be similar to that for Objectives 2 and 3, except that the endpoint will be ultrasound-confirmed pneumonia rather than radiographically-confirmed pneumonia.

**Objective 5:** The primary study outcome will be prevalence of vaccine-type pneumococci (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) among children with radiographically-confirmed pneumonia, stratified by vaccination status. We will calculate this prevalence rate for all children with X-ray confirmed pneumonia during a rolling calendar period, beginning the first week of NP swab collection. Standard deviations and 95% confidence intervals for the prevalence rates will be calculated. Rates will be calculated both unadjusted and adjusted for participant demographic characteristics. Rates will be plotted against time separately for vaccinated and unvaccinated participants using the midpoint of the interval as the time point shown on the x-axis, and with population prevalence of immunization superimposed, as in Loughlin *et al.* (2014)<sup>32</sup>.

In a secondary analysis, we will use a multivariate logistic regression model to test the effect of time trend, vaccination status and the interaction of time and vaccination status on carriage of VT pneumococci in this population of pediatric Bangladeshi pneumonia patients. Other analyses may explore the joint effect of vaccination status and time on carriage of NVT pneumococci.

### Ethical approvals

The study protocol was approved by the Institutional Review Board (IRB) of the Johns Hopkins Bloomberg School of Public Health (IRB 00005421), and the Ethical Review Committee of the International Centre for Diarrhoeal Diseases Research, Bangladesh (PR-13095).

### Study status

At the time of writing this manuscript, fieldwork for all objectives has been completed with the exception of the IPD case-control and incident trend studies, which will be ongoing through June 2018. Data cleaning and preliminary analysis are currently underway.

### Dissemination of results

At this point, some preliminary data analysis has been completed. Five abstracts were submitted and accepted for presentation at the ISPPD-2018 conference in April 2018 in Melbourne, Australia. Data analysis will continue throughout 2018, and a dissemination seminar in Bangladesh will be held in late 2018 in collaboration with our main stakeholder, the Bangladesh

Ministry of Health and Family Welfare. We anticipate that at least one dozen peer-reviewed publications will be produced from this study.

### Discussion

The GoB MOHFW introduced PCV10 on March 2015, making it the second South Asian country to add PCV to their national vaccine schedule. While developed countries have seen significant decreases in pneumococcal disease after the introduction of PCV vaccines, there are a number of challenges that should be considered when introducing a new vaccine in resource poor settings such as in Bangladesh<sup>33–37</sup>. First, pneumococcus has over 90 different serotypes, and the currently licensed vaccines contain only either 10 or 13 of the different serotypes. Additionally, the serotype distribution of the pneumococci that cause disease varies by location, and some countries have observed serotype replacement<sup>38–40</sup>. GAVI currently provides co-funding for the introduction of vaccines such as PCV. Countries such as Bangladesh will be required to bear the financial burden for the implementation of all the vaccines including PCV, which is very expensive. Because of these issues, the WHO recommends that all countries have an assessment of the introduction of PCV10 to determine its impact in each setting.

The Pneumococcal Conjugate Vaccine Impact Study, expected to be completed in mid-2018, is a prospective community- and facility-based observational cohort study with a variety of nested studies including case-control and pre-post studies to evaluate the impact of PCV10 in Bangladesh. We designed a comprehensive, multiple outcome impact assessment because each outcome has its own strengths and drawbacks. Classic impact assessments generally measure and compare rates of IPD in vaccinated and unvaccinated children. However, there are several limitations to such a design. For example, not all IPD is associated with bacteraemia, and the majority of cases of pneumococcal pneumonia are blood culture negative<sup>41</sup>. Therefore, estimating the impact of vaccine on IPD alone will underestimate the burden of pneumococcal disease and will underestimate the true value of the vaccine. Additionally, many children arrive at health facilities with recent or current antibiotic use, increasing the difficulty to isolate pneumococcus in the lab from their specimen<sup>42</sup>. To account for the attenuated estimate expected when measuring IPD rates and to get a more complete assessment of the impact of PCV10 in our population, we have considered 3 additional outcomes: 1) chest radiograph-confirmed pneumonia; 2) lung ultrasound-confirmed pneumonia; and 3) NP carriage.

We have measured NP carriage because a precursor to disease is carriage of the bacteria in the nasopharynx. If the vaccine reduces VT NP carriage, then we would potentially expect the transmission of disease to be lower and that there will be less disease. However, while up to 70% of children may be colonized with the pneumococcus in the nasopharynx, a very small proportion of these children will become ill with this disease<sup>43</sup>. Nevertheless, the reduction of NP carriage is one measure of the impact of the vaccine. Moreover, reduction of NP carriage also

reduces the circulation of the organism in the community and thus confers protection to unimmunized children who live in the same community – “herd immunity”<sup>38</sup>.

Although chest radiographs and lung ultrasound imaging outcomes are non-specific to pneumococcus, radiographically- and sonographically-confirmed pneumonia could be markers for bacterial pneumonia. Two placebo randomized controlled trials (RCTs) in The Gambia and South Africa evaluated VE of PCV against WHO-defined radiographic pneumonia in children<sup>44,45</sup>. These studies validated the use of the WHO-defined radiographic pneumonia definition in PCV impact studies by observing a VE of 20% (95% confidence interval (CI), 2%, 35%) in South African children without HIV-infection, and a VE of 37% (95% CI, 25%, 48%) among Gambian children against this endpoint. These RCT findings support our decision to include the WHO-defined radiographic pneumonia endpoint (i.e., primary endpoint pneumonia) in this study.

Given PCV VE is now well established against radiographic pneumonia in settings outside of South Asia, a placebo RCT designed study is no longer ethical. We are therefore utilizing both case-control and time-series designs in this PCV impact study to assess VE against radiographic pneumonia. One recent case-control study from The Gambia reported mixed results using WHO-defined radiographic pneumonia among children 3–59 months old<sup>46</sup>. In this study the authors used conditional logistic regression, as we also plan to do, but found that they were overall underpowered to show VE, largely due to the high percentage of controls receiving PCV. Their best estimate for VE was an adjusted OR of 0.57 (95% CI, 0.30, 1.08) among children 3–11 months old. One important strength of this study from The Gambia is that the authors also conducted a time series analysis on the same dataset and found a statistically significant reduction in the incidence of WHO-defined radiographic pneumonia across all pediatric age ranges in children below five years of age. Utilizing multiple analytic strategies allowed the authors to better estimate the impact of PCV.

Another recent case-control study from South Africa did find statistically significant VE among vaccine eligible children utilizing a modified WHO-defined radiographic pneumonia definition that included children as cases if they were found to have the WHO radiographic classification of ‘other infiltrate’ along with an elevated C-reactive protein inflammatory biomarker >40 mg/L<sup>47</sup>. WHO-defined radiographic ‘other infiltrate,’ however, has been shown to be a notoriously unreliable imaging endpoint as multiple studies have reported low published kappa levels between image readers<sup>21,48</sup>. Most recently, the Pneumonia Etiology Research for Child Health study unsuccessfully attempted to standardize and adjudicate imaging readers to this ‘other infiltrate’ endpoint, finding a Cohen’s kappa of only 0.15 between the first two radiographic readers<sup>48</sup>. The authors

concluded that despite a rigorous standardization process imaging reader agreement remained poor for the classification of WHO ‘other infiltrate.’ Based on this literature, we will use WHO-defined radiographic pneumonia as our radiographic case definition and will not consider ‘other infiltrate.’ Instead, we plan to balance the potential limitation of being insufficiently powered to detect VE of radiographic pneumonia using the case-control design by conducting a complementary time series analysis using this same imaging endpoint.

Including both chest radiograph and lung ultrasound imaging, compared to IPD only, will likely provide a better understanding of true disease burden estimates and impact of the vaccine<sup>49</sup>. While the chest radiograph is a useful tool to diagnose pneumonia the machines are not easily mobile, expensive, expose children to ionizing radiation, and they provide only a one-dimensional image for assessment. Comparatively, the lung ultrasound method of diagnosis is potentially easier to perform, can be done at the patient’s bedside, and it produces video images of multiple locations of the chest without ionizing radiation exposure, permitting the reader additional perspectives of the child’s lungs for interpretation. Additionally, physicians can be trained to use the lung ultrasound machines and diagnose the patient, and treatment does not need to be delayed while waiting for a radiologist to read radiographic images. Using lung ultrasound for diagnosing pneumonia, however, is still in investigational stages, and its efficacy for measuring pneumonia is still to be determined<sup>50,51</sup>.

## Data availability

No data is associated with this article.

## Competing interests

No competing interests were disclosed.

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## Open Peer Review

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### Version 1

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**Marie R. Griffin**

Department of Health Policy, Vanderbilt University Medical Center, Nashville, TN, USA

This is a very ambitious study of impact of PCV10 on serious morbidity in children which should yield important information. The case control methodology is appropriate when pneumococcal serotype can be ascertained. However, I agree with Dr. Dagan that performing a case control study for all cause pneumonia may underestimate vaccine impact. For the ecologic analyses of impact, it would be good to have some assurance that surveillance was similar and as complete as possible, before and after vaccine introduction. If uptake of vaccine was sufficiently high over a short period of time, then use of a time series analysis rather than strict before – after may strengthen causal inference. Other analytic techniques could include difference in difference approaches; that is, before-after in regions with high vs low vaccine uptake.

I applaud incorporating ultrasound into this study and wonder if it might also be the appropriate technology for resource rich as well as resource poor settings.

Given that the NP carriage data (objective 5) will only be of pneumonia cases, and not of the source population, it is not clear how these data should be interpreted. This is different from the study cited, which looks at carriage in a population sample.

It is unclear from the protocol if any changes that might influence detection of cases has occurred over the study period. Some background on antibiotic use in this population would provide context. For example, how available are antibiotics and how frequently administered outside traditional health care system. It would seem important to document whether there were changes in antibiotic use due to either vaccine effectiveness or to the educational efforts that accompanied this vaccine intervention and evaluation. It would be important to document that efforts to provide a definitive diagnosis of pneumonia would also result in timely antibiotic administration for children with danger signs.

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Partly

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

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Ron Dagan

The Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

This represents an ambitious, generally well-thought and well-designed study. There are some difficulties in obtaining comprehensive data and high quality data for the population studied, but I believe this is addressed by the investigators. The very ambitious approach to pneumonia impact is to be commended. We definitely need prospective studies on the topic of PCV impact on respiratory infections. I have a few minor points to raise, and one major point as remarks. I believe that the major point (herebelow) in regard to the analysis of pneumonia should be taken seriously to prevent mistakes previously made by other investigators.

1. Page 6, 1<sup>st</sup> paragraph, right column, line 1-2: Clinical pneumonia is not considered usually as IPD, since in most cases it is a result of aspiration. It is important not to analyze this as IPD unless *S. pneumoniae* was isolated from blood or pleural effusion.
2. Table 2: Why exclusion criteria for obtaining blood or CSF culture? Previous enrollment or previous recent antibiotics will not always prevent positive cultures in many circumstances.
3. Pneumonia: In general, I commend the X-ray approach for the Dg of consolidated pneumonia. I also am happy with the intention to test for incidence dynamics as this represent real-life impact of PCV10. However, case-control studies for pneumonia (excluding pneumonia with positive pneumococcal culture and serotypes) are inappropriate. Although, case-control studies were conducted by several investigators, the method is inappropriate for the following reasons:

- a. Consolidated pneumonia is often caused by *S. pneumoniae* albeit not always.
- b. Even if caused by *S. pneumoniae*, it can be caused by non-vaccine serotypes *S. pneumoniae*.
- c. Introduction of PCV will deplete cases of consolidated pneumonia for caused by vaccine-serotypes, if the vaccine is efficacious.
- d. The more the vaccine is efficacious, and the more the vaccination program achieves high vaccination coverage, the less likely will the remaining consolidated pneumonia be seen covered by vaccine serotypes.
- e. Therefore, in other words, the higher the impact of PCVs, the less likely is the remaining consolidated pneumonia to be caused by vaccine serotype. Therefore, the more the vaccine impacts on pneumonia, the less the vaccine is effective against the remaining pneumonia.
- f. Thus, immediately after the vaccine introduction, case-controls studies for effectiveness of the vaccine, when the pathogen cannot be identified will show lower-than-real effectiveness, and the trend will be more severe as more children are vaccinated.
- g. Of course, this is different when cases for whom vaccine serotype *S. pneumoniae* is isolated, since we only count those that are not supposed to be decreased by PCVs.

I therefore strongly suggest not to conduct case-control studies for symptomatic pneumonia, but rather make all efforts to study incidence dynamics, thus determine **impact** rather than **effectiveness**.

4. Similar remarks are for ultrasound confirmed pneumonia cases

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Partly

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 25 June 2018

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Peter Waiswa 

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Thanks for the opportunity for me to review this important protocol. The protocol is well written. However, the main challenge is that of assessing the vaccine impact especially surveillance by using CHWs and the fact that there are multiple providers some information can be a challenge to collect with precision. I know these CHWs and providers are being engaged through training and supervision, but ensuring that all incident cases of disease are captured can be a daunting task. In addition, I find the ultrasound part of the study to be just an opportunistic one - not necessarily part of the PCV impact assessment but maybe it is good as a bonus in the study. Finally, the authors are publishing this protocol almost at the end of the study - can they discuss a little more of what they had initially planned/designed and what changes they have so far made and why?

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

# Safety and Efficacy of Simplified Antibiotic Regimens for Outpatient Treatment of Serious Infection in Neonates and Young Infants 0–59 Days of Age in Bangladesh

## *Design of a Randomized Controlled Trial*

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 Dipak Mitra, PhD, \* A. K. M. Shamsuzzaman, DCH,¶ Wazir Ahmed, DCH,|| Luke C. Mullany, PhD, \*  
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**Background:** Because access to care is limited in settings with high mortality, exclusive reliance on the current recommendation of 7–10 days of parenteral antibiotic treatment is a barrier to provision of adequate treatment of newborn infections.

**Methods:** We are conducting a trial to determine if simplified antibiotic regimens with fewer injections are as efficacious as the standard course of parenteral antibiotics for empiric treatment of young infants with clinical signs suggestive of severe infection in 4 urban hospitals and in a rural surveillance site in Bangladesh. The reference regimen of intramuscular procaine-benzyl penicillin and gentamicin given once daily for 7 days is being compared with (1) intramuscular gentamicin once daily and oral amoxicillin twice daily for 7 days and (2) intramuscular penicillin and gentamicin once daily for 2 days followed by oral amoxicillin twice daily for additional 5 days. All regimens are provided in the infant's home. The primary outcome is treatment failure (death or lack of clinical improvement) within 7 days of enrolment. The sample size is 750 evaluable infants enrolled per treatment group, and results will be reported at the end of 2013.

**Discussion:** The trial builds upon previous studies of community case management of clinical severe infections in young infants conducted by our research team in Bangladesh. The approach although effective was not

widely accepted in part because of feasibility concerns about the large number of injections. The proposed research that includes fewer doses of parenteral antibiotics if shown efficacious will address this concern.

**Key Words:** safety, efficacy, simplified antibiotic regimens, young infants, clinical severe infection

(*Pediatr Infect Dis J* 2013;32:S12–S18)

**A**n estimated 3.0 million annual neonatal deaths occur globally; 99% of these deaths occur in developing countries.<sup>1–4</sup> In many settings, neonatal and infant mortality now make up the vast majority of under-5 child deaths. For example, in Bangladesh, 57% of under-5 deaths occur within the first 28 days after birth and another 23% take place in the postneonatal period.<sup>5</sup> Approximately 10–20% of newborns develop life-threatening infections<sup>6</sup> and one-third to one-half of all neonatal deaths are due to infection,<sup>1,7–9</sup> including sepsis, pneumonia, tetanus, meningitis and diarrhea. Timely and appropriate treatment can avert most of these deaths.<sup>6,10</sup>

The World Health Organization (WHO) recommends that all cases of clinical severe infection in neonates and young infants (0–59 days old) be treated in hospitals with a 7- to 10-day course of injectable antibiotics—penicillin or ampicillin and gentamicin. In low-resource settings, however, reliance on a strategy of hospitalization of young infants with clinical severe infections has a number of inherent disadvantages. Parents/caregivers often consider care-seeking outside the home to be unacceptable in the early postpartum period, or they may not be able to travel to a health facility; upon reaching the facility, infants may receive inadequate treatment because of barriers due to cost, under-staffing or lack of available beds and supplies.<sup>11–15</sup> Hospitalization increases the risk of exposure to multidrug-resistant nosocomial pathogens that are increasingly difficult to treat<sup>16,17</sup> and raises the cost of health care.<sup>18</sup>

Strategies for community-based management of severe infections in young infants have been developed and evaluated in several research settings.<sup>8,19,20</sup> A cluster-randomized controlled trial of a package of maternal and neonatal interventions, which included assessment and management of newborns by village-based community health workers, was conducted by our group in Sylhet district, Bangladesh. Neonates with signs of severe infection were referred to a qualified provider or treated in the home with intramuscular procaine penicillin and gentamicin (Treatment regimen included a total of 10 days of gentamicin and procaine penicillin). Dosage of gentamicin was adjusted based on neonate's weight as follows: 10 mg every other day if <2.0 kg, 10 mg/day if 2.0–2.5 kg and 13.5 mg/day if >2.5 kg. Dosage for penicillin was

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The trial registration number was ClinicalTrials.gov NCT00844337. The views and opinions expressed in this article are those of the author(s) and not necessarily the views and opinions of the United States Agency for International Development.

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80,000 units if <2.0 kg and 160,000 units if ≥2.0 kg.) if the caretaker declined referral but consented to home treatment. The rate of referral compliance was 34% among those diagnosed as being in the severe disease category, and another 43% accepted home treatment; the case fatality rate for neonates treated by community health workers was statistically not different from that of neonates treated by doctors and other medically qualified providers and was 78% lower than for those who received no treatment or were treated by untrained providers (adjusted hazard ratio 0.22, 95% confidence interval [CI]: 0.07–0.71).<sup>21</sup> In a trial providing home-based treatment with a regimen of oral co-trimoxazole and intramuscular gentamicin, Bang et al<sup>22</sup> reported a 60% reduction in neonatal infection case fatality. One barrier to scaling up findings from these trials is that major challenges are associated with providing parenteral antibiotic therapy in the community. It is resource intensive to train community-based health workers to provide injections and to ensure supply of antibiotics and safe administration of parenteral antibiotics daily for 7 to 10 days.

Evidence suggests that oral antibiotic therapy also reduces mortality in neonates and young infants with suspected infections.<sup>10</sup> A meta-analysis of trials of community-based case management of pneumonia found a 27% reduction in neonatal mortality and a 20% reduction in infant mortality.<sup>23</sup> Five of the 7 studies included in the meta-analysis used oral antibiotic regimens. In an open-label trial in Pakistan, 3- to 59-month-old children (n = 2037) with severe pneumonia were randomly allocated to either (1) hospitalization and parenteral ampicillin (100 mg/kg per day in 4 doses) for 48 hours, followed by 3 days of oral amoxicillin (80–90 mg/kg per day in 2 doses), or (2) home-based treatment for 5 days with oral amoxicillin (80–90 mg/kg per day in 2 doses).<sup>24</sup> At 7 days after the start of treatment, no difference in treatment failure rates was observed between the hospitalized group (8.6%) and the ambulatory group (7.5%; risk difference 1.1%; 95% CI: –1.3 to 3.5). High failure rates, however, were associated with age 3–5 months, very fast breathing (>70 breaths per minute for children <12 months old) and low weight for age, suggesting that providing oral antibiotics alone may be inadequate for young infants with clinical severe infection. Alternatives for these infants include restricting the number of injectable antibiotics either by combining a initial short course of parenteral antibiotics with a switch to oral antibiotics or a combination of injectable and oral antibiotics.<sup>10</sup>

In 2007, a global consultation convened by the Saving Newborn Lives Initiative of Save the Children, the United States Agency for International Development and the WHO concluded that there was insufficient evidence on infection management in young infants in community-based settings to make policy recommendations for global programs. The consultation highlighted the need for research to test combinations of low cost existing oral and intramuscular antibiotic regimens that might feasibly be implemented in first-level facilities and the community, and that would be acceptable in settings characterized by weak health systems.

## METHODS

### Study Design

We are conducting a trial in Bangladesh to determine if 2 home-based antibiotic regimens are as efficacious as the standard regimen of intramuscular procaine-benzyl penicillin and gentamicin given once daily each for 7 days for the empiric treatment of young infants (age 0–59 days) with clinical signs suggestive of severe infection (clinical severe infection). The 2 alternative regimens are (1) intramuscular gentamicin once daily and oral amoxicillin twice daily for 7 days and (2) intramuscular penicillin and gentamicin once daily for 2 days followed by oral amoxicillin twice

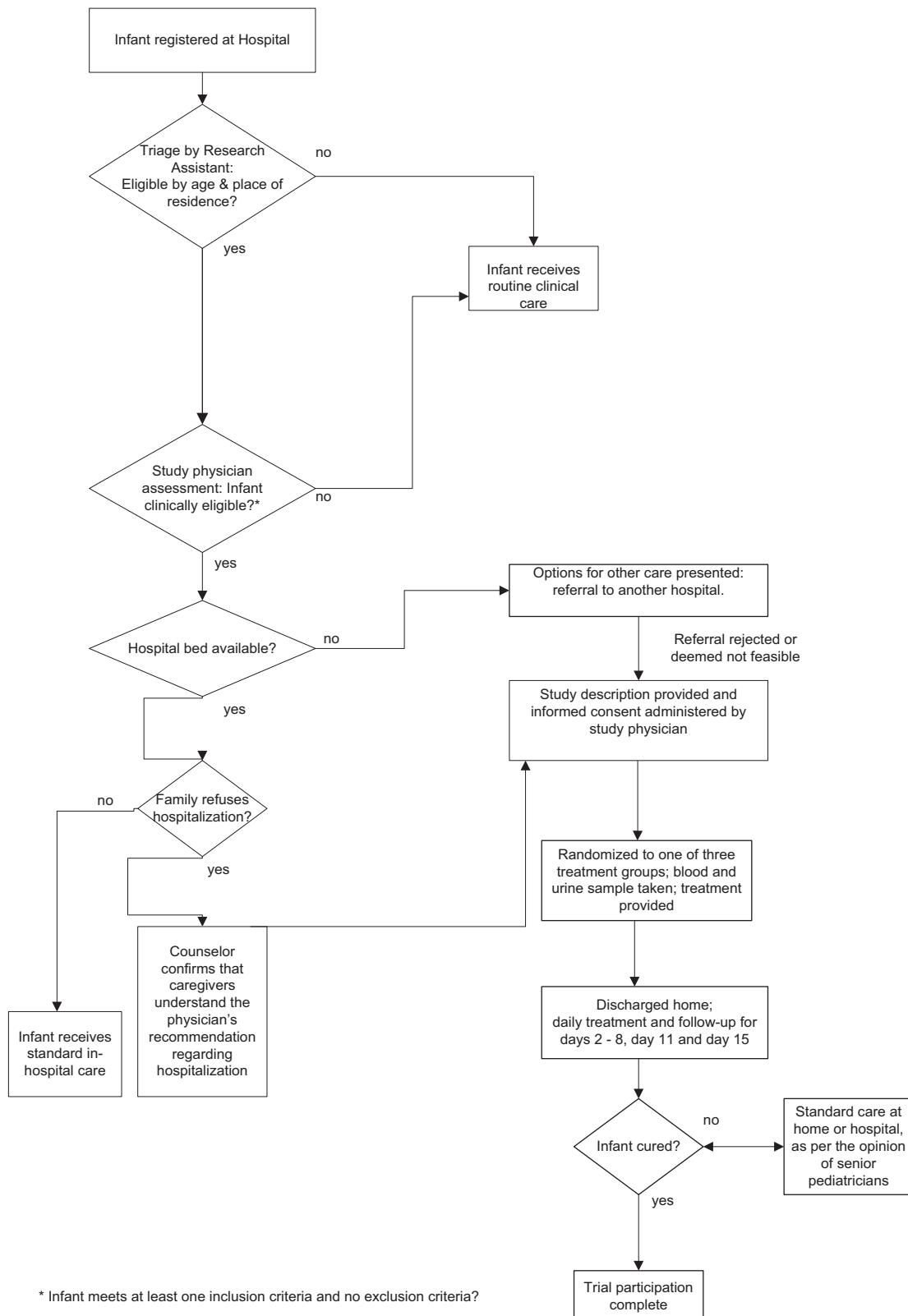
daily for 5 days. The primary hypothesis is that the proportion of infants who fail treatment will be 10% in the reference group and each of the alternative treatment groups. The null hypothesis is that any one of the alternative therapies is inferior and will yield a treatment failure proportion that is at least 5% points higher than that of the standard therapy group. The null hypothesis will be rejected if the upper bound of a 95% CI around the absolute difference (alternative minus standard) is less than 5.0%. The trial's secondary objectives are (1) to identify baseline clinical predictors of treatment failure in severe infections in young infants and (2) to determine the proportion of infants with relapse, defined as young infants who were considered cured by day 7 but developed any of the signs of clinical severe infection by day 14.

### Setting, Enrollment and Randomization

The study recruits young infants from the outpatient departments of Dhaka Shishu (children) Hospital, Shishu Sasthya Hospital in Dhaka, Institute of Child and Mother Health Hospital in Dhaka, Chittagong Ma O Shishu Hospital in Chittagong and a rural surveillance sites in Sylhet, Bangladesh. All 4 hospitals are in the urban areas of 2 major cities of Bangladesh, receive young infants with very similar complaints and use similar approaches to clinical management of severe infections in young infants. In the rural site, all pregnant women in the study area are identified by female community health workers (CHW) through established pregnancy surveillance and are offered a standard package of antenatal counseling. Families and birth attendants notify the CHW as promptly as possible after a birth has occurred. After birth, CHWs aim to visit all newborns in the home within 6 hours of birth and not later than 24 hours. This early postnatal visit is important to capture early-onset infections. The general health status of the infant is assessed by the CHW using criteria from the WHO Young Infant Study Group.<sup>25</sup> The CHW returns on days 2, 6, 13, 20, 27, 34, 41, 48 and 59 after birth to inquire about any illness of the infant in the intervening period and to reassess the status of the infant. CHWs refer infants meeting the criteria for clinical severe infection to 1 of 2 designated hospitals for further evaluation and care.

Recruitment and participation procedures are summarized in Figure 1. Research assistants who work for the study screen young infants presenting to the outpatient departments of the participating hospitals to determine if age and place of residence meet initial eligibility criteria. Potentially eligible infants are then screened by the study physician for signs of clinical severe infection according to the inclusion and exclusion criteria described below. Infants with ≥1 inclusion criteria and no exclusion criteria are considered clinically eligible; however, study physicians first recommend hospitalization before enrolment in the trial. If no hospital bed is available, the infant is referred to another hospital. If the infant's family refuses hospitalization or referral, the study physician presents the option for home treatment through study participation.

To be eligible for inclusion, infants must be 0–59 days of age, residents of a predefined geographical area based on accessibility for follow-up visits, have at least 1 sign in the 5-sign algorithm for severe infection and none of the 12 signs of critically severe infection or disease and caregivers must refuse hospitalization or referral to another hospital as well as indicate that they plan to remain in the study area for at least 2 weeks. The study aims to exclude infants with signs of either very mild or very severe infections. Thus, clinical inclusion criteria are based on a 5-sign algorithm that is a modified version of the WHO Young Infant Clinical Signs Study Group's algorithm<sup>25</sup>; a comparison of the 2 algorithms is presented in Table 1. Signs in this study's algorithm include: (1) severe lower chest wall indrawing, (2) axillary temperature ≥100.4°F (≥38.0°C) confirmed by second

**FIGURE 1.** Trial profile.

**TABLE 1.** Comparison of WHO Young Infant Study Algorithm and the Trial Algorithm Used to Identify Infants With Clinical Severe Infection

WHO Young Infant Study-II Algorithm	Algorithm in Use in This Trial
History of convulsion	—
Respiratory rate ≥60/min	—
Severe chest indrawing present	Severe chest indrawing present
Fever: axillary temperature >99.5°F (>37.5°C)	Fever: axillary temperature ≥100.4°F (≥38.0°C)
Hypothermia: axillary temperature <95.9°F (<35.5°C)	Hypothermia: axillary temperature ≤95.9°F (≤35.5°C)
Lethargic or less than normal movement	Lethargy (movement only with stimulation)
History of feeding problems	Feeding difficulty (confirmed by poor suck on examination)

— indicates not included in the algorithm used in this trial.

reading, (3) axillary temperature ≤95.9°F (≤35.5°C) confirmed by second reading, (4) lethargy (defined operationally as movement only upon stimulation by the examining physician) and (5) history of feeding problems, confirmed by poor suck on examination. The criterion of respiratory rate ≥60 breaths per minute is excluded because data from previous studies in Bangladesh suggests that this sign alone is not predictive of severe illness.<sup>21</sup> Infants with signs of very severe infection or disease are excluded because home-based treatment is felt to be potentially unsafe for this group. Very severe infection or disease is defined as the presence of any of the following signs: (1) unconsciousness, (2) history of or presence of convulsions present at assessment, (3) inability to feed, (4) apnea, (5) inability to cry, (6) cyanosis, (7) bulging fontanel, (8) major congenital malformations, (9) major bleeding, (10) surgical conditions needing hospital referral, (11) persistent vomiting after 3 attempts to feed the baby within half an hour or (12) physician's suspicion of meningitis. Infants are also excluded from the study if they weigh <1500 g, have been hospitalized for illness in the last 2 weeks or were previously included in the study. Legal guardians of infants meeting the study's eligibility requirements are offered participation in the study through a written informed consent process. There are quality assurance teams in all study sites to monitor activities monthly with respect to quality and consistency of study procedures.

Infants are randomized to 1 of the 3 home treatment regimens using site- and age-specific (<7 days or 7–59 days) computer-generated randomization sequences with varying random block sizes of 3, 6 and 12. The allocation sequence for each site and age groups is placed in serially numbered, sealed and opaque envelopes and delivered to each site. After consent and enrollment, the study physician selects the next envelope, and the treatment corresponding to the allocation code printed within the envelope is assigned to the infant.

## Drug Dosages and Treatment Provision

Dosages (presented in Table 2) were selected to optimize efficacy, safety and feasibility. Extended-interval (24-hourly) gentamicin regimens using doses of 4–5 mg/kg/day have been shown to be effective<sup>26,27</sup> and remain within the range commonly used in the United States.<sup>28</sup> For procaine penicillin, daily intramuscular doses of 25–50 mg/kg are recommended for neonatal infections,<sup>28</sup> and we have set a target range of 40–50 mg/kg per 24-hour dose. Amoxicillin is structurally almost identical to ampicillin, an antibiotic commonly used intravenously to treat invasive neonatal infections; however, amoxicillin is more commonly used in the oral form because of its high oral bioavailability. Although typically used at doses of 40–90 mg/kg/day (divided into 2 doses per day in the newborn period), oral amoxicillin doses of 200–300 mg/kg/day have been shown to be safe in newborns treated for group B streptococcal sepsis.<sup>29</sup> For this study, a target dose ranging from 90 to 115 mg/kg/day divided into 2 doses per day was chosen as this is similar to the dose of intravenous ampicillin recommended for the treatment of neonatal infections that ranges from 75–200 mg/kg/day,<sup>28</sup> yet cautiously remains close to a standard “high-dose” amoxicillin regimen (90 mg/kg/day).

All enrolled infants are given the first doses of the assigned antibiotics and discharged home after counseling about home management. Study physicians provide intramuscular injections at home and assess infants daily for the next 7 days to assess for treatment failure; clinical assessments are conducted on day 11 and day 15 to determine if a relapse has occurred. Caregivers are taught to give the oral antibiotics. If an infant vomits within 20 minutes of oral dosing, the caregiver is instructed to readminister a complete dose; this is a safe approach, even if both of the doses were to be fully absorbed, because the total maximum daily dose of amoxicillin would be <200 mg/kg/day. If the infant vomits within

**TABLE 2.** Antibiotic Dosage by Weight

Infant Weight Range (kg)	Gentamicin Concentration: 40 mg/mL		Procaine Penicillin Concentration: 200,000 IU/mL		Amoxicillin Concentration: 100 mg/mL	
	Volume (mL)	Daily Dose (mg)	Volume (mL)	Daily Dose (IU)	Volume (mL)	Daily Dose (mg)
1.500–1.749	0.18	7.20	0.35	70,000	1.6	160
1.750–1.999	0.20	8.00	0.40	80,000	1.9	190
2.000–2.499	0.25	10.00	0.50	100,000	2.3	230
2.500–2.999	0.30	12.00	0.60	120,000	2.8	280
3.000–3.499	0.35	14.00	0.70	140,000	3.3	330
3.500–3.999	0.40	16.00	0.85	170,000	3.8	380
4.000–4.499	0.45	18.00	0.95	190,000	4.3	430
4.500–4.999	0.50	20.00	1.00	200,000	4.8	480
5.000–5.499	0.55	22.00	1.10	220,000	5.3	530
5.500–5.999	0.60	24.00	1.20	240,000	5.8	580
6.000–6.499	0.65	26.00	1.35	270,000	6.3	630

20 minutes of the second oral dose, the caregiver is instructed to seek medical attention. It is not feasible to blind study participants or study physicians to treatment group allocation.

### **Definition of Treatment Failure**

The trial's primary outcome is treatment failure in the 7 days after enrolment defined as 1 or more of the following 9 criteria:

1. Any time before the day 8 assessment: Death.
2. On or before the day 8 assessment: Clinical deterioration based on the presence of at least 1 of the following 8 danger signs documented by the study physician based on physical examination findings: unconscious, convulsions (may also be diagnosed based on convincing history), unable to feed, apnea, cyanosis, bulging fontanelle, major bleeding, persistent vomiting (defined as vomiting after 3 attempts to feed the baby within half an hour as assessed by study physician).
3. On or before the day 8 assessment: Decision by a study physician to change the antibiotic regimen or add another antibiotic for either of the following reasons:
  - a. New-onset infectious comorbidity (ie, severe omphalitis, bone or joint infection, or severe skin or soft tissue infection), or
  - b. Serious nonfatal antibiotic-associated adverse event (ie, severe diarrhea associated with dehydration, Stevens-Johnson syndrome, anaphylaxis or acute renal failure).
4. On or before the day 8 assessment: Hospitalization for any reason.
5. On or after day 3: Occurrence of new signs of clinical severe infection (any of the 5 signs). A "new" sign is one that was not present at the time of enrolment. Signs are defined in the same manner as for initial eligibility.
6. On day 4, for infants with multiple signs at enrollment: Presence of at least 2 of the signs that were present on enrolment;
7. On day 4, for infants with a single sign on enrolment: Presence of the same sign that was present on enrolment.
8. On or after day 5: Recurrence of at least 1 of the following signs: temperature  $\geq 38^{\circ}\text{C}$  or  $\leq 35.5^{\circ}\text{C}$ , severe chest indrawing, lethargy or poor suck on any follow-up visit. Recurrence implies the presence of the sign on enrolment and documented resolution of the sign on at least 1 follow-up visit with subsequent reappearance of the same sign on at least 1 follow-up visit on or after day 5.
9. On day 8: Persistence of any of the 5 signs of severe infection that was present on enrolment.

All surviving infants meeting clinical treatment failure criteria by study physicians on routine follow-ups are designated as provisional treatment failures and transported to the hospital accompanied by study personnel. At the hospital, the infant undergoes a repeat examination without history-taking by a second study physician. To the extent possible, the second physician assessor is blinded to the treatment allocation and prior history of the infant. If the second assessment supports the ascertainment of treatment failure, the case is considered a confirmed treatment failure. If the second medical assessment disagrees with first assessment, the decision is referred to a supervising senior physician, whose decision is the final determination. Infants designated as treatment failures are referred for further hospital care according to standard hospital practices. Results of blood cultures may be used to guide specific therapy for treatment failures.

A random ~5% subsample of nontreatment failure visits are assessed in the home or facility by a second study physician for quality control purposes. To the extent that it is feasible, efforts are being made to blind the second physician assessor to the first physician's assessment (ie, he/she is not informed as to whether

an infant has been brought to the hospital because of provisional treatment failure or because of random selection). The assessment of the second physician does not routinely affect study procedures or outcome ascertainment. However, if danger sign(s) are deemed to be present by the second physician, the infant is brought to the hospital for management and adjudication of treatment failure by a third physician.

### **Data Analysis and Sample Size**

We hypothesize that each of the alternative therapies will not be inferior to standard therapy and that the treatment failure proportions among infants receiving both the standard therapy (A) and alternative therapies (B and C) will be 10%. The alternative therapies will be considered not inferior to the standard therapy if the upper bound of the CI for the difference in treatment failure proportions (alternative therapy minus standard therapy) is less than 5%. For each comparison (B vs. A; C vs. A), the point estimate of the failure rate difference between the 2 treatment arms will be calculated together with a 2-sided 95% CI. In order to take into account any between-group differences in any potentially confounding variables, the difference will also be expressed as a ratio of the rate in alternative therapies to failure rate in standard therapy and model using binomial regression models with a log link.

The samples size for this 3-armed study was estimated by the method of Blackwelder<sup>30</sup> and the formula

$$N = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(p_1 - p_2 - \Delta)^2}, \text{ where } p_1 \text{ and } p_2 \text{ are}$$

the true treatment failure rates in the standard and alterative regimens, respectively, and  $\Delta$  is the margin used to define similarity. Table 3 shows the expected power of different effective sample sizes to demonstrate the similarity of 2 treatments (with a similarity margin of +5%). It is assumed that the true failure rate in the standard treatment arm will be 10% and that the true failure rate in the experimental arm will either be identical or only slightly worse (11%). Enrolment of 750 evaluable children in each of 3 arms (2250 total) will yield 90% power to demonstrate similarity to within +5%, if the true failure rates are identical. If the difference between the true failure rates between the standard and alternative therapy is 1%, the power to demonstrate similarity to within +5% will be 71%. Some children may have missed visits, incomplete treatment compliance or may be withdrawn from the study before the completion of treatment, which will reduce the number of children who are eligible for inclusion in the primary per-protocol analysis. Infants who receive 100% of the doses of scheduled antibiotics on all 7 days or by the time of treatment failure if treatment failure occurs, and are not known to have received any other antibiotic by study or nonstudy physician, are considered "fully adherent" to study treatment. An infant who is not fully adherent is considered "partially adherent" if he/she received 100% of scheduled antibiotics on days 1–3 or by the time of treatment failure; received at least 50% of the scheduled doses of each antibiotic during days 4–7, or by the time of treatment failure; is not known to have received any nonstudy injectable antibiotic before day 8 assessment; and is not known to have received any nonstudy oral antibiotic on days 1–3. Infants who do not fulfill the criteria of either fully or partially adherent are considered nonadherent. Infants who receive scheduled follow-up on all 7 days or up-to the time of treatment failure if treatment failure occur are considered to have complete clinical follow-up. An infant is considered to have partial clinical follow-up if he/she has 1 or more days of follow-up missing, but follow-up was completed on assessment days 2–4 and on at least 1 of days 5–8, and vital status on day 8 was known. Infants who do not fulfill

**TABLE 3.** Power of Different Effective Sample Sizes to Demonstrate the Similarity of 2 Treatments (With a Similarity Margin of +5%) Under Different Assumptions About True Similarity of the 2 Treatments (Identical or Difference of 1% in True Failure Rates)

True Failure Rate in Group A (%)	True Failure Rate in Groups B or C (%)	Number of Per-protocol Children per Arm	Allowed Similarity Margin (%)	Probability That Study Will Demonstrate Similarity of the 2 Treatments Within the Allowable Margin (%)
10	10	550	+5	79
10	10	600	+5	82
10	10	650	+5	85
10	10	700	+5	88
10	10	750	+5	90
10	10	800	+5	92
10	10	850	+5	93
10	10	1500	+5	99
10	11	550	+5	58
10	11	600	+5	62
10	11	650	+5	65
10	11	700	+5	68
10	11	750	+5	71
10	11	800	+5	74
10	11	850	+5	77
10	11	1500	+5	95

the criteria of complete or partial clinical follow-up are considered to have incomplete clinical follow-up. The primary analysis will be a modified per-protocol analysis that includes infants with either complete or partial follow-up and who are either fully or partially adherent. Infants with either incomplete clinical follow-up or who are nonadherent are considered lost to follow-up and will be excluded from the primary analysis. Based on previous experience in similar settings, we have allowed for up to 15% loss to follow-up, and therefore will aim to enroll 866 children to each arm or 2598 total.

### Approvals

This study was reviewed and approved by the Johns Hopkins Bloomberg School of Public Health's Institutional Review Board, the Bangladesh Institute of Child Health's Ethical Review Committee and the WHO's Research Ethics Review Committee.

### DISCUSSION

Results from the Bangladesh simplified antibiotic regimens trial are expected to be made available in late 2013. The trial will determine whether the 2 antibiotic treatment regimens that include fewer doses of parenteral antibiotics are as efficacious as standard parenteral antibiotic treatment in young infants with signs of severe infection. Clinical equipoise exists because the relative efficacies of various antibiotic regimens for clinical severe infection in infants less than 2 months old are unknown in the outpatient setting. The findings are expected to inform decisions related to the scale-up of community-based care of young infant infections.

In this home-based trial in a highly vulnerable population, an obligation to protect the safety of participants exists alongside the duty to generate evidence that can guide practical policy decisions in the resource-poor context. A central ethical issue is that all arms of the trial involve the implementation of antibiotic therapy in the outpatient setting, a method of treatment delivery that is not standard of care for clinical severe infection in young infants but has been successfully implemented in resource-poor settings. This trial is designed not to challenge the appropriateness of hospitalization as a universal standard of care, but to develop an evidence base relevant to infants for whom hospitalization is not feasible or refused by parents/caregivers.

Hospitalization refusal is common in Bangladesh and many other similar settings. A pilot study for this trial found that caregivers refuse hospitalization because of perceived financial burdens and concern about potential disruptions to family life, such as provision of care for older children at home while a mother remains in hospital with her young infant. Bed shortages are also common at the participating hospitals. When no bed is available, the standard procedure is to recommend that the family take the baby to another hospital, but families rarely pursue this alternative because pediatric bed availability at other public hospitals is limited, travel costs may be prohibitive or other factors.

Therefore, in designing this trial, the study investigators, the trial steering committee and protocol reviewers were faced with a situation in which sick infants may go without care when hospitalization would be ideal, and thus conducting the trial would represent an expansion of clinical services for the community. However, we did not want the implementation of the trial to further discourage families from accepting hospitalization when that would be the preferred treatment option from a medical perspective. For this reason, families are not offered study participation unless: (1) caregivers refuse to accept hospitalization, or (2) no bed is available at the study hospital and referral to another hospital is refused or deemed impractical. We have instituted procedures designed to diminish the potential for unduly influencing caregivers to accept home-based treatment when a hospital bed is available. Hospital staff have been instructed to avoid any discussion of the study with parents of prospective participants until after a spontaneous refusal of admission after diagnosis and suggested treatment plan occurs, or it is determined that no beds are available. The study physician is required to document that attempts were made to convince the caregivers to consent to hospital admission. Referring or treating physicians are not rewarded, financially or otherwise, on the basis of the number of prospective participants referred, nor are study physicians coinvestigators, in which case they could have academic interests at stake. Before families enroll in the study, they meet separately with trained study personnel called counselors whose role is to discuss their decision to refuse hospitalization at the study hospital. The counselor's primary role is to ensure that the caregivers understand that the study physician has recommended that the infant be admitted to hospital based on current standard care of clinical severe infection and to confirm that the family has refused hospital admission despite counseling.

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