Impact of Non-dysentery Shigella Infection on Growth and Health of children over time (INSIGHT) – a prospective case-control study protocol

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Study protocol

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Abstract

Background: *Shigella* spp. (Shigella) is a primary cause of moderate-to-severe diarrhea in children living in impoverished areas of the world. Shigella is known for causing dysentery with the presence of blood in the stool. However, majority of the children infected with Shigella present with non-dysentery Shigella-associated diarrhea (NDSD). According to the World Health Organization guidelines for the management of acute diarrhea, routine use of antibiotics is not recommended except when diarrhea is bloody and most NDSD cases are not treated with antibiotics. The absence of dysentery may not necessarily indicate a low risk of death and severe morbidity and does not exclude Shigella as a cause of diarrhea. In particularly vulnerable young children with malnutrition, diagnosis, and treatment of Shigella infection might be beneficial or even lifesaving. Identification of NDSD cases requires a rapid test to document these infections so that treatment can be initiated promptly. We thus designed the INSIGHT study to determine the potential benefit of identifying NDSD cases and determine their outcome compared to patients with Shigella dysentery (DS) and non-Shigella watery diarrhea (WD).

Methods: Children seeking care at the hospital in Bangladesh with DS (n=148) and NDSD (n=296), who are positive for Shigella will be enrolled and prospectively followed for one year. A third group of children with WD (n=148) will be enrolled and followed for 90 days. We will determine the impact of NDSD in comparison to DS and WD on morbidity, mortality, gut barrier function, systemic and gut inflammation, nutritional status, and cognitive development of the children. We will evaluate whether the simple and rapid test “Rapid LAMP based Diagnostic Test (RLDT)” could accelerate case detection and treatment of shigellosis in the clinical settings of rural hospitals in an endemic country like Bangladesh.

Discussion: The INSIGHT study will determine the impact of NDSD in children to determine if the treatment guidelines of shigellosis need to be revisited to improve clinical outcomes and development of the children diagnosed with NDSD. This study will also validate a rapid test capable of identifying the patients with Shigella who may benefit from early detection and treatment with antibiotics.

Background

Shigella spp (Shigella) isa bacterial pathogen which is frequently associated with diarrheal disease and is a significant cause of mortality and morbidity worldwide. Although the mortality associated with shigellosis has decreased in current years to ~70,000 [1, 2] in children,there areat least 80 million cases of shigellosis each year with estimated disability-adjusted life years (DALYS) at 7 million and year loss due to disability (YLD) at 744,000 [1-4]. Shigella invades the mucosa, causing inflammatory destruction of large intestinal epithelium which can result in environmental enteropathy, chronic malnutrition, cognitive and developmental impairment.

Shigella is known primarily as a cause of bacillary dysentery (blood/mucus in stool which can be associated with fever and abdominal cramps). However, a large proportion (~ 40% to 89%) of Shigella-associated diarrhea cases are non-dysentery or secretory diarrhea in nature [5-9].The clinical syndrome of dysentery caused by Shigella is readily identified clinically and warrants treatment with antibiotics, but Shigella infections causing watery diarrhea are not easily clinically recognized, and the clinical identification of these patients is difficult. [7]. The current World Health Organization (WHO) guidelines for the treatment of diarrhea recommend antibiotics when there is the presence of visible blood in diarrhea stool [10, 11]. Therefore, the non-dysentery Shigella-associated diarrhea (NDSD) cases are generally not treated with antibiotics.

Oral ciprofloxacin and azithromycin are considered first-line therapy for dysentery shigellosis (DS) in adults and children [12]. With effective antibiotic therapy for DS, clinical improvement occurs within 24 to 48 hours, resulting in a decreased risk of serious complications and death, shorter duration of symptoms and reducing fecal carriage from ~ 4 weeks to 3 days, while also reducing transmission [12-16]. Thus, the current WHO guidelines appear to manage dysentery effectively but might miss opportunities to reduce mortality and long-term growth potential among children infected with Shigella who present with watery diarrhea.

It may be hypothesized that NDSD cases if identified quickly, should be treated with antibiotics or other therapeutics to improve survival and long-term developmental potential in the children. Shigellosis cannot be distinguished reliably from other causes of bloody diarrhea or other causes of watery diarrhea based on clinical features alone. Documenting the NDSD infections and rationally guiding treatment will require a point of care (POC) test for Shigella so that the treatment can be initiated promptly. Chakraborty *et al* previously developed and successfully evaluated in the Asian and African countries, a simple molecular diagnostic test for Shigella, the Rapid LAMP-based Diagnostic Test (RLDT), which detects Shigella from a fecal sample within one hour; thus has the potential to be used for rapid detection and treatment of NDSD [17-19].

We are conducting a prospective case-control longitudinal study (INSIGHT) in Mirzapur, Tangail, Bangladesh, to determine if there is a need to change the current guidelines of shigellosis treatment for better survival and development of children. We will determine the impact of NDSD on immediate and long-term (1-year) morbidity and development in children under 5 years old, compared with DS and non-Shigellawatery diarrhea (WD). We will also assess the potential for implementation of RLDT for Shigella in a rural primary healthcare Government facility in Bangladesh.

METHODS AND ANALYSIS

The protocol for the INSIGHT study was developed through a collaborative effort among the researchers from Johns Hopkins University and the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

INSIGHT is a prospective case-control study including 592 children under 5 years of age who are residents of Mirzapur seeking care in the Kumudini Hospital (KH) for diarrhea with NDSD, DS, or WD. Children will be given standard of care by the hospital physicians, as per the WHO and Bangladesh treatment guidelines. All children will be rehydrated with either intravenous therapy or ORS depending on the degree of dehydration and zinc will be provided. Children with DS will be treated with Azithromycin or other antibiotics as recommended by the hospital attending physician.

Aims

The objectives of this study are to (1) Determine morbidity, risk of hospitalization, and mortality associated with NDSD cases (2) Study the impact of NDSD on nutritional status and cognitive development of children; (3) Understand the impact of NDSD on gut barrier function, systemic and gut inflammation in children; (4) Determine resistance to commonly used antibiotics of the Shigella isolates using disc diffusion test and E-strips; and (4) Evaluate if the RLDT test could be applicable for case detection and treatment of shigellosis in the low resource clinical settings of a rural primary health care facility. The data from aims 1 to 3 will be compared between the NDSD and the other two groups, DS and WD.

Study setting and population

The study children will be enrolled in the KH and followed through twice weekly home visits. The KH is a nonprofit hospital located in the central urban union of Mirzapur, providing health services to the surrounding poor rural population. Mirzapur is a rural sub-district (Upazila) of Bangladesh that covers 374 Square km in Tangail district. It is located 60 km northwest of the capital city, Dhaka.

Study Design

Children between 1 and 59 months with acute watery diarrhea, presenting to the KH, with a stool sample that is positive for Shigella by the RLDT, will be enrolled in the study (NDSD group). For comparison, a second group of children with dysentery (presence of visible blood in stool) and positive for Shigella by RLDT (DS group) will be included (see enrollment flow chart, Figure 1). The DS group will be treated with antibiotics as a standard treatment. A third group of children with watery diarrhea negative for Shigella by the RLDT (WD group) will also be enrolled. All RLDT-positive stool samples and 10% of the randomly selected RLDT-negative stool samples will be confirmed by culture for Shigella.

Following enrollment, the children in all three groups will be evaluated and compared for morbidity – requiring of hospitalization (inpatient) and the severity of diarrhea (number of loose stools per day, sunken eyes, loss of skin turgor, intravenous hydration required), and other clinical symptoms (fever, rectal straining, abdominal pain and cramp, anorexia, convulsion, vomiting, etc.), length of illness and diarrheal severity score [20]. The length of the shedding of Shigella bacteria in stool following the index diarrhea episode will be evaluated using stool samples collected on every other day till day 14 and then twice per week tested by the RLDT and culture till two consecutive stool samples are negative by the RLDT for Shigella.

During the follow-up period of 1 year for the NDSD and DS groups and 3 months for the WD group, the field workers will visit the households twice per week to collect morbidity data. Data on future episodes of diarrhea including shigellosis (DS and NDSD), hospitalizations, and antibiotics use will be collected during these visits. Stool samples will be collected every month in addition to diarrhea episodes and tested by the RLDT and culture for Shigella. Parents or primary caregivers of the enrolled children will undergo standardized interviews to solicit demographic, socioeconomic, sanitation, hygiene, epidemiological, and clinical information. Diarrhea will be defined as three or more loose or liquid stools during a 24-hour period, and dysentery as one or more loose stools with visible blood. A diarrheal episode will be defined as “new” if the diarrhea definition is met after at least three or more days free of diarrhea or

dysentery. Anthropometry (weight, length, mid upper arm circumference, and head circumference) will be measured at the enrollment in the KH and every month during home visits by the field workers. Cognitive, language and motor development of the children will be measured using the Bayley IV for 1 to 42 months of age and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV) and Ages and Stages Questionnaire for the older children [21]. Ages and Stages Questionnaire (ASQ-3) is a developmental screening tool will be used for measuring motor development of older children. Children's behavior will be measured during cognitive assessment through five Wolke scales: response to the assessor in the first 10 minutes which is approach, emotional tone, cooperation with the assessor, vocalization, and activity level throughout the test. These ratings have been used on Bangladeshi children [22, 23]. Home Observation for Measurement of the Environment (HOME) will be monitored once at the first visit after enrollment. Children's behavioral assessment will be assessed using Wolke's Behavior Rating scale [24] by observing their behavior during the Bayley or WPPSI test. This assessment includes responsiveness to the examiner, activity level, emotional tone, cooperation with test procedure, and vocalization on a 9-point scale during the test. The scale has been sensitive to intervention effects and picked up group differences in Bangladesh [23, 25-26]. Two trained female assessors at the field office will administer the Bayley-IV, WPPSI, ASQi (motor component), and behavior rating at the field office through direct assessment, observations, and maternal interviews two days after the index diarrhea episode is resolved and at 3 and 12 month follow up visits. The assessors will receive a month-long extensive training on all the developmental measures by senior psychologist of the study and will be allowed for data collection after achieving >85% inter-rater agreement with the trainer. To ensure data quality throughout the study period, the senior psychologist will conduct ongoing reliability-checks of 10% of the total sample and ensure refresher training quarterly. Two female enumerators will collect HOME data at household-level along with other demographic information.

For detection of Shigella by culture, stool samples will be cultured on MacConkey agar and Salmonella-Shigella agar followed by biochemical tests, and serotyped using commercially available antisera (Denka Seiken, Tokyo, Japan). The Shigella isolates will be tested for susceptibility to ciprofloxacin, azithromycin, cefixime, ceftriaxone, trimethoprim-sulfamethoxazole, nalidixic acid, ampicillin, and pivmecillinam by the Kirby–Bauer disc diffusion method and determining the minimum inhibitory concentrations using E strips. The isolates will be stored in glycerol stock.

Inclusion and Exclusion Criteria. The inclusion criteria are (1) children between >1 month and <60 months of age seeking care at the KH for diarrhea; (2) residing in the catchment area of KH and willing to be available for sample and data collection during the scheduled visits. In addition, for NDSD and DS groups stool samples positive for Shigella tested by the RLDT will be included. The exclusion criteria are (1) diarrhea episode starting more than 72 hours before enrollment; (2) antibiotics taken within the last 3 days; (3) children with severe acute malnutrition (below -3z-score of the median WHO growth standards), and (iv) another significant disease process requiring specific therapy is present. In addition, for NDSD and WD groups, children with the presence of visible blood in stool will be excluded.

Collection, preparation, and archiving of biological samples. Stool and blood specimens will be collected at the baseline during enrollment at the hospital by the study staff and during follow-up home visits by the field staff following the schedule (Table) to evaluate inflammatory and immune markers. Gut barrier will be assessed using the lactulose rhamnose (LR) permeability test in which urine is collected before and at the 2 hours and 5 hours after feeding LR solution to determine the ratio of L:R of the study children. Using blood serum we will measure C reactive protein, intestinal fatty acid binding protein, lipopolysaccharide, and flagellar (FliC) IgA and IgG to measure systemic inflammation, enterocyte death, and translocation of pathogens. We will monitor gut inflammation by measuring levels of myeloperoxidase (MPO) and lactoferrin
(LF) in stool samples. The panel of 10 inflammatory cytokines (IL-2, -4, -6, -8, -10, -13, -1ß, TNF-α, IFN-γ, IL-17) will be tested both on serum and stool. Other major enteric pathogens will be detected in stool using quantitative PCR. All samples will be collected, processed, and stored following the standard operating procedures developed in the study protocol and following the schedule of events (Table 1).

Table 1. Schedule of events

Study Days/month (M)	1	3	5	9	14	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Enrollment	X																
*Morbidity	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Stool for microbiology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sociodemographic, sanitation and hygiene questionaries		X									X						
Urine L:R ratio		X			X			X			X						X
Stool collection for inflammatory/immune markers	X	X	X	X	X	X		X			X			X			X
Blood collection for Inflammatory/immune markers	X	X		X		X		X			X			X			X
Anthropometry	X				X	X	X	X	X	X	X	X	X	X	X	X	X
Bayley or WPPSI score		X						X									X
HOME		X															

Note: *Morbidity data and stool will be collected every other day following the baseline visit till day 14 and then two days in a week till the participant with DS or NDSD are negative for Shigella. Following the stool is negative for Shigella, morbidity data will be collected at home visits twice a week and stool will be collected as per schedule.

To evaluate if the RLDT could be implemented in a rural primary care hospital in Bangladesh, we will train the hospital staff in the RLDT and certify them. During the screening for enrollment in the INSIGHT study, randomely selected 225 stool samples will be sent to the rural hospital lab to rescreen with the RLDT by the trained hospital staff, independently. The results will be compared with the RLDT that were performed by the INSIGHT study staff for enrollment purpose. We will determine the ease of use, level of technical support needed and acceptability of the RLDT among the hospital lab staff using a questionnaire.

Statistical considerations

Sample Size and power

The study hypothesis is that the rates of hospitalization (inpatient) will be similar among the NDSD and DS cases. This is a matched set of cases (DS) and controls (NDSD) with 2 matched control(s) per case. The second comparison group is WD (1:1 matched with case). Based on the previous data on hospitalizations among shigellosis in Mirzapur (personal communication Faruque *et al*), we assume that the probability of hospitalization among the controls will be 20%. Since it is not known, as a general practice, the correlation coefficient for exposure between the matched cases and controls is set to 0.2. If the true odds ratio for hospitalization in the exposed subjects relative to the unexposed subjects is 0.5, we will need to study 123 cases of shigellosis with dysentery with 2 matched control(s) per case to be able to reject the null hypothesis that this odds ratio equals 1 with probability (power) 0.8. If the alternative hypothesis is not satisfied, then we will accept the null hypothesis of no difference in hospitalization rates between cases and controls. The Type I error probability associated with the test of this null hypothesis is 0.05. With estimating 20% lost to follow up we will enroll 148 children each in DS and WD groups and 296 in the NDSD group (total 592).

For the RLDT evaluation in the primary health care facility, a sample size of 225 (45 subjects with Shigella) achieves 80% power to detect a change in sensitivity from 0.60 to 0.80 using a two-sided binomial test and 100% power to detect a change in specificity from 0.60 to 0.80 using a two-sided binomial test with the target significance level of 0.05. The actual significance level achieved by the sensitivity test is 0.0336 and achieved by the specificity test is 0.0397. The prevalence of the disease is ~20%.

Statistical analysis

The indicators of severity and symptoms determined by the morbidity indicators, hospitalization, and diarrheal disease score between cases and controls at baseline (enrollment) will be evaluated using conditional logistic regression adjusting for sex and other confounding factors. The impact of the initial shigellosis or WD episode on the future morbidity, hospitalization, and mortality of the children, during the follow-up period of 12 months for NDSD and DS groups or 3 months for WD group, will be analyzed accounting for the confounding factors. The antibiotic use during the follow-up period will be recorded and considered during analysis. Initially, bivariate conditional logistic regression models will be developed considering each one of the socio-demographic factors for the risk of shigellosis to select the variables for adjustment in the multivariable model.

The Z-scores of all anthropometric measurements of the cases and their matched controls at different time intervals will be compared using paired t-tests. We will perform linear regression analyses comparing baseline Z scores and changes in Z scores from the enrolment to 3- or 12-month follow-up, adjusting for enrolment Z score, duration of follow-up, sociodemographic covariates, antibiotics use, future diarrhea episodes, and any hospitalization in the follow-up period, using jack-knife estimates of standard error [27]

The same analytic techniques will be used for evaluating the data from Bayley and WPPSI scales by comparing both the Z-scores and age-adjusted composite scores of all the developmental domains in the unadjusted model. In the multivariable-adjusted liner regression model, we will adjust specific baseline test scores for each domain and other related socio-demographic variables that are significantly different by groups at baseline, between lost to follow up and tested children, and are significantly associated with developmental outcome measures e.g. socio-economic, sex, mother's education, HOME, etc. Five behavioral outcomes will be summed up as total scores and will be used in a similar analysis. Sensitivity and mediation analysis will also be done using Structural Equation Model (SEM) [28, 29] to explore the underlying mechanism or process influencing the outcome.

The magnitudes and kinetics of the inflammatory markers will be compared at enrollment and over the follow-up time between DS cases and NDSD cases. Analyses for comparisons of dichotomous outcomes such as fold increase/decrease of the markers from baseline will be performed with the chi-square test or Fisher's exact test if cell counts are sparse. For comparisons of the levels at a day between the two groups, a Student's t-test will be performed. MPO and LF data will be adjusted for breastfeeding. The data from the cases and their matched controls will be evaluated using paired *t-tests*. Linear regression analyses will be compared between cases and controls adjusting for anthropometry and cognitive developmental changes, future diarrhea episodes, and antibiotic use in the follow-up period, and sociodemographic risk factors for inflammation.

For the RLDT evaluation at the rural primary health care facility, the RLDT test results from the hospital staff will be compared with the results from the INSIGHT study staff (gold standard), analyzing sensitivity, specificity, positive and negative predictive values. We will also use pairwise comparisons with Cohen's kappa (agreement between binary outcomes +/- of tests without gold standard).

Discussion

INSIGT is a case-control, prospective, longitudinal study to understand if the currently recommended therapy of rehydration along with zinc, without antibiotics, is adequate for NDSD cases. In a systemic review, five (71%) of seven studies examining Shigella mortality relative to other causes of diarrhea found that the odds of death to be significantly higher in children with Shigella infection than in those without Shigella infection (pooled OR 2.8, 95% CI 1.6–4.8; p=0.000), however, dysentery was not associated with mortality (pooled OR 1·3, 0·7–2·3; p=0·37) [7, 30]. In a case-control study in severely malnourished children in Bangladesh, Shigella mortality stratified by the presence of dysentery, found no significant difference between inpatients with DS and those with NDSD in the association between Shigella infection and death. These findings of lower mortality among DS children are likely the consequence of effective management strategies, including the administration of antibiotics for dysentery cases. Although, variability in populations, study designs, diagnostic tools, clinical management strategies, enrolment periods, and small sample size, resulted in marked heterogeneity in the magnitude of association across the studies, overall, the systematic reviews found *Shigella* infection to be associated with mortality in children presenting with diarrhea and that dysentery (presence of blood in stool) did not adequately identify children with *Shigella* infections in many settings. The implication is that the exclusion of *NDSD* from treatment recommendations might be leaving many children vulnerable to acute and subacute adverse clinical manifestations and developmental delays. The INSIGHT study will determine the impact of NDSD following the rehydration therapy (recommended for watery diarrhea), on morbidity, hospitalization, gut health, in children compared to DS cases who are treated with antibiotics. This study will also evaluate the consequences of NDSD, SD and WD on children's physical and mental developmental domains as there are limited and inconsistent data available in this area [31-34].

For the management of diarrhea, although, WHO recommends antibiotics only for children with bloody diarrhea, over 40% of children with non-bloody watery diarrhea currently receive antibiotics as part of non-standard treatment in low- and middle-income countries [35]. This overuse of antibiotics contributes to the development and spread of antimicrobial resistance, both at individual and population levels. If the INSIGHT study determines that NDSD cases should be treated with antibiotics to improve survival and long-term developmental potential in children, identification of such cases will require a rapid test to document these infections so that treatment can be evidence-based and initiated promptly.

Shigellosis cannot be distinguished reliably from other causes of bloody diarrhea or other causes of watery diarrhea based on clinical features alone. The most frequent and the gold standard diagnostic method used for Shigella is the direct culture method [5, 36] Recent studies have shown that culture method is not sufficiently sensitive [8, 37] Moreover, culture takes 2 - 3 days and is therefore not feasible as a POC diagnostic test. PCR or quantitative PCR (qPCR) are highly sensitive [8, 37] however, these assays are highly technology and equipment dependent, require improved laboratory facilities and trained personnel, and are time-consuming, and expensive. Therefore, the current diagnostic methods in practice are not practical for routine use and not applicable in most healthcare settings where Shigella infections are endemic. INSIGHT study will evaluate the performance and implementation of the simple, rapid, and sensitive RLDT assay for Shigella in a primary healthcare facility in Bangladesh to determine if the RLDT could be a potential diagnostic tool to rapidly detect and treat shigellosis. Identifying and targeting treatment for the children with shigellosis may potentially lower antimicrobial resistance.

Declarations

Study Status

Recruitment for the INSIGHT study began in November 2021 and is currently ongoing. The enrollment is expected to conclude in June 2025.

Authors' contributions.

All named authors adhere to the authorship guidelines. All authors have agreed to publication.

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Availability of data and materials. The datasets generated during the current study are available from the corresponding author upon reasonable request.

Consent for publication.

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

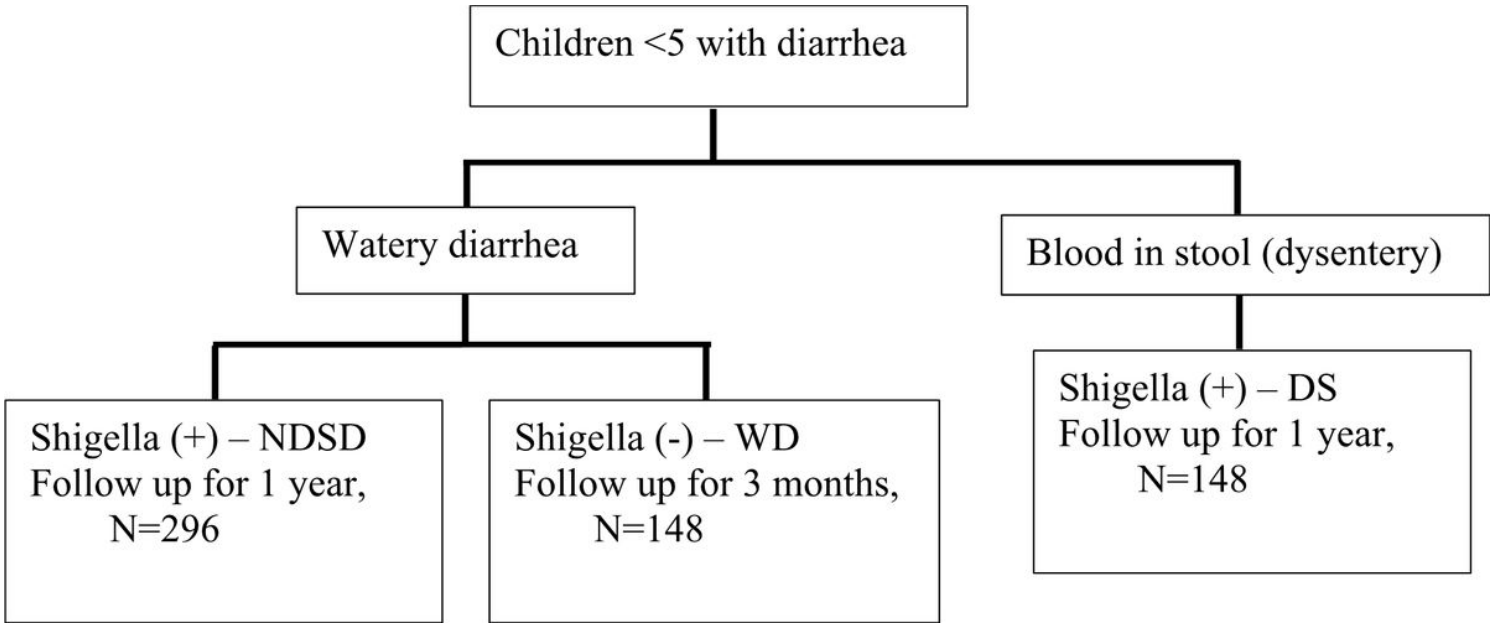

Figure 1