Principal Investigator: Last, first, middle: Khan, Wasif Ali

98-021

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: Date:

RRC Approval: Yes/ No Date:

ERC Approval: Yes/ No Date:

1. **Title of Project (Do not exceed 60 characters including spaces and punctuations):**
Randomized, double-blind, controlled clinical trial to compare the efficacy of a single-dose of azithromycin versus a 3-day, muliple dose of erythromycin in the treatment of childhood cholera due to *V. cholerae* O1 or O139.

2a. Name of the Principal Investigator(s) (Last, Middle, First).	2b. Position / Title	2c. Qualifications
Khan, Ali, Wasif	Medical Officer, CRSC	M.B.B.S.

3. **Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.**
Clinical Sciences Division

4. **Contact Address of the Principal Investigator**

4a. **Office Location:** Medical Officer, CRSC
Clinical Sciences Division
ICDDR,B
Mohakhali, Dhaka 1212

4b. **Fax No:** 880-2-883116
4c. **E-mail:** wakhan@icddrb.org
4d. **Phone / Ext:** 880-2-871751-60/2314

5. **Use of Human Subjects** Yes [x] No [ ]

5a. **Use of Live Animal** Yes [ ] No [x]

5b. **If Yes, Specify Animal Species**
Not applicable

6. **Dates of Proposed Period of Support**
(Day, Month, Year - DD/MM/YY)

01-10-98 to 30-09-2000

7. **Cost Required for the Budget Period**
7a. Ist Year ($): *2nd Year ($):* *3rd Year:*

7b. **Direct Cost ($)** 128,475 **Total Cost ($)** 160,594

8. **Approval of the Project by the Division Director of the Applicant**

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

George Fuchs, M.D.
Name of the Division Director

Signature

August 05, 1998
Date of Approval

9. **Certification by the Principal Investigator**

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

10. **Signature of PI**

Date: August 05, 1998

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[√] Check here if appendix is included

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

Principal Investigator: Wasif Ali Khan

Project Name: Randomized, double-blind, controlled clinical trial to compare the efficacy of a single-dose of azithromycin versus a 3-day, muliple dose of erythromycin in the treatment of childhood cholera due to *V. cholerae* O1 or O139.

Total Budget: US $ 160,594 Beginning Date: October, 1998 Ending Date: September, 2000

Cholera remains a major public health problem in Asia, Africa, and Latin America. In regions where cholera is endemic, children are disproportionately affected in comparison to adults, and mortality remains high in the absence of adequate treatment. Antimicrobial therapy facilitates the treatment of cholera by reducing the duration and volume of diarrhea by approximately half. Options for antimicrobial treatment of childhood cholera is currently limited by the increasing prevalence of strains of *V. cholerae* resistant to the agents that have been used to treat this infection in children - furazolidone and trimethoprim-sulfamethoxazole - and the absence of a proven effective single dose therapy.

In this proposed study to be conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), we will compare clinical and bacteriologic efficacy of a single, 20 mg/kg dose (maximum of 1.0 gram) of oral suspension of a newer macrolide, azithromycin, and a 3-day course of the oral suspension of the older macrolide, erythromycin, administered in a dose of 12.5 mg/kg (maximum individual dose of 500 mg) every 6 hours in the treatment of children with clinically severe cholera due to *V. cholerae* O1 or O139 infection. The latter drug is the current treatment of choice for children with cholera at the ICDDR,B. Azithromycin has a longer half-life than erythromycin, attains very high stool concentrations, and has less adverse events than erythromycin. Treatment will be random, and will be blinded to investigators and patients.

Patients eligible for study will be male (1-15 years of age) or female (5-15 years of age), who have watery diarrhea of ≤ 24 hours and signs of severe dehydration (WHO guidelines), and who have cholera vibrios identified on dark-field microscopic screening of stools. Only those patients who have ≥ 20 ml/kg body weight of stool during a 4 hour observation period following initial rehydration period will be entered into the study; only patients from whom *V. cholerae* O1 or O139 is isolated, and who stay in-hospital for three or more days will be eligible for analysis. Written informed consent will be required from the parents/guardians of all children for their inclusion in the study. Patients will be asked to remain in hospital for full five days from the initiation of drug therapy, and to return for a follow-up visit ten days after discharge. Cultures of stool will be obtained on admission to the study, and daily during hospitalization. Stool will be collected, and the volume of watery stool measured for every six hour period of the study. Major outcomes to be compared in the two treatment groups will be clinical success of therapy, defined as resolution of diarrhoea within 96 hours of initiation of study drugs, and bacteriologic success of therapy, defined as the inability to isolate the infecting strain of *Vibrio cholerae* O1 or O139 after 48 hours of therapy. Other outcomes measures examined will be the incidence of adverse reactions to drugs in the two treatment groups, the duration of watery diarrhea and positive stool cultures, and the total volume of watery/liquid stools during study.

Azithromycin is active against multiply-resistant strains of *V. cholerae* O1 or O139, it is safe for use in children, and it is widely available. If single-dose of azithromycin is found to be effective in the treatment of cholera in this study, it will be a considerable advance in treatment of cholera in children; effective single-dose therapy of childhood cholera has not been previously identified with any drug.

Principal Investigator: Last, first, middle: Khan, Wasif Ali

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Wasif Ali Khan	Internal Medicine	Principal investigator
2. Mohammed Abdus Salam	Internal Medicine	Co-investigator
3. Michael L. Bennish	Pediatrics and Infectious Diseases	Co-investigator
4. Mrs. Monira Begum	Biochemistry	Research Officer

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

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

The hypothesis of this study is that in the treatment of cholera due to *V. cholerae* O1 or O139 in children aged 1-15 years, a single, 20 mg/kg dose (maximum of 1.0 gram) of azithromycin suspension will be as effective as the conventional, 3-day, multiple dose therapy with erythromycin suspension administered in a dose of 12.5 mg/kg (maximum individual dose of 500 mg) every 6 hours.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

The specific aims of the protocol are as follows:

1. Primary Aim

The primary aim of this study is to compare the clinical as well as bacteriologic efficacy of a single, 20 mg/kg dose (maximum of 1.0 gram) of azithromycin oral suspension with the conventional 3-day, 12.5 mg/kg (maximum individual dose of 500 mg) 6 hourly dose of erythromycin oral suspension in the treatment of clinically severe cholera in children due to *V. cholerae* O1 or O139.

2. Secondary Aims

The secondary aims of this study are:

2.1 To determine the serum and stool concentrations of both study drugs at specified times, and also to determine their serum half-lives.

2.2 To determine the minimum inhibitory concentration of *V. cholerae* O1 and O139 to the study drugs.

2.3 To monitor and compare the incidence, type and severity of adverse events in association with the study drugs.

Background of the Project including Preliminary Observations

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

Cholera remains a major public health problem in Asia, Africa and Latin America. Its continued importance is reflected in the hundreds of thousands of cases, and tens of thousands of deaths that occurred when the seventh pandemic of cholera reached South America in 1991, the tens of thousands of deaths that have occurred among Rwandan refugees in the last two years, and the massive epidemics of cholera that occurred following the appearance of a new serogroup of cholera, *V. cholerae* O139, in Asia in 1993.

In countries where cholera is endemic, rather than epidemic, the majority of cholera morbidity and mortality occurs in children. Infection with *Vibrio cholerae* O1 or O139 is far more likely to cause dehydration than any of the other watery diarrheas of childhood, including those caused by enterotoxigenic *E. coli* and rotavirus [1]. If not effectively treated, cholera can lead to severe dehydration, hypovolemic shock, metabolic acidosis, and death within a few hours of the onset of disease. The explosive epidemic form of cholera is associated with much higher case-fatality rate, as was demonstrated during the 1994 cholera epidemic among Rwandan refugees in Goma, Zaire when an estimated 20,000 persons died from cholera in a 4-week period [2].

Fluid replacement and antimicrobial therapy are the mainstays of therapy of moderate to severe cholera. Antimicrobials reduce the volume and the duration of diarrhea by half. This is turn reduces the need for rehydration fluid and shortens the duration of hospitalization. Moreover, they shorten the duration of fecal excretion of *V. cholerae* and reduce transmission of infection to others [3-5]. All of these effects make antimicrobial therapy of cholera a cost-effective intervention.

Tetracycline, chloramphenicol, doxycycline, furazolidone, trimethoprim-sulfamethoxazole and erythromycin are all effective in the treatment of cholera when given for 3-5 days [3,6-8]. Single dose tetracycline and doxycycline therapy are also effective in the treatment of cholera, although bacterial eradication is slower than with multiple dose therapy. Ciprofloxacin is also effective in the treatment of cholera in multiple doses, and we have recently demonstrated that a single dose of ciprofloxacin is more effective than single dose doxycycline therapy of cholera in adults [9]. Tetracycline and doxycycline, however, are not recommended for use in children <8 years of age, and in pregnant and lactating women; chloramphenicol is a relatively toxic drug; and newer quinolones including ciprofloxacin are not recommended for routine use in children. Although of toxicity with any of these drugs is small when used for short courses of therapy, such as with cholera, there remains a reluctance to use these drugs in children, and they often are not available in a liquid formulation that would assist in administration to children.

Treatment of cholera in children has been further complicated by the emergence of multiply-resistant strains of *V. cholerae*. Strains resistant to tetracycline (which also implies resistance to doxycycline), furazolidone (the drug that has been most commonly used to treat childhood cholera), and trimethoprim-sulfamethoxazole have been reported from Asia (including Bangladesh), Africa and Latin America [10-17]. Currently, over 90% of the *V. cholerae* O1 isolates at the treatment centres of ICDDR,B are resistant to trimethoprim-sulfamethoxazole and furazolidone, and approximately 30% are resistant to tetracycline.

Because of the high prevalence of multiply-resistant strains of *V. cholerae* and the lack of alternative agents, erythromycin has become the first-line therapy for cholera in children at the ICDDR,B. Erythromycin is administered in multiple doses for 3 days, and it has a high incidence of gastrointestinal adverse events. Because of the problem of resistance, and the lack of a drug safe for use in children that has been demonstrated to be effective when given as single-dose, we propose to study the efficacy of single-dose azithromycin therapy in childhood cholera. Based on a number of attributes of azithromycin, as summarized in the following section, it is reasonable to think that this drug, known to be safe for use in children, would be effective as single dose therapy for cholera in children.

Activity, Pharmacokinetics, and Safety Profile of Azithromycin

The good *in vitro* and *in vivo* activity of erythromycin against intracellular pathogens has been a stimulus for development of the new generation of macrolides. Four new generation macrolides including azithromycin have been synthetically derived from erythromycin through modification of its chemical structure [18,19]. These alterations resulted in better resistance to

the range of pH found in the stomach, and thus better oral bioavailability. They also have better *in-vitro* activity against many pathogens, achieve higher intracellular concentrations, have longer half-lives allowing infrequent dosing, and better tolerability than erythromycin [19-22].

Azithromycin inhibits bacterial protein synthesis by binding to the 50S component of 70S ribosomal subunit. It is 300 times more stable in the range of stomach pH than erythromycin, and has higher oral bioavailability (37% versus 25%). Its absorption, however, is reduced by about 50% in presence of food and antacids [23-25]. After a 500 mg oral dose, a peak serum concentration of 0.4 mg/l is reached in 2-3 hours, and its trough serum concentration 24 hours after a dose is 0.04 mg/l [24,26]. The agent has a low affinity for serum proteins, and protein affinity decreases with increasing serum concentrations of the drug (protein binding of 50% and 12% at drug concentrations of 0.05 mg/l and 0.5 mg/l respectively) [26]. Once the serum peak is reached, concentrations then sharply decline, indicating a rapid distribution phase and a high (23 l/kg) volume of distribution [23,24,26].

Azithromycin is eliminated without significant metabolism; <5% of the drug is demethylated in the liver to inactive metabolites [24]. Its primary route of excretion is biliary and transintestinal. This ensures high stool concentrations of the drug [24]. In our recently completed study of azithromycin in adults with shigellosis, the median stool concentrations on study days 2 and 4 (after 500 mg on admission followed by 250 mg at 24-hour intervals) were 196 micro gram/gram of stool and 387 micro gram /gram of stool [38]. With the exception of antacids, no major interactions have been noted between azithromycin and commonly used drugs [25]. Dose adjustment is only required for patients with severe renal insufficiency (creatinine clearance <40 ml/min.) or hepatic failure [25]. The pharmacokinetic properties of azithromycin are similar in adults and children [25,27].

The better *in-vitro* activity of azithromycin compared to erythromycin and other newer macrolides is due in part to its better penetration of the cell wall of gram-negative bacteria [18,28-32]. Several *in vitro* studies have found azithromycin to be active against *H. influenzae* [29] as well as common enteric pathogens including *Escherichia coli, Salmonella, Campylobacter, Shigella* and *Vibrio cholerae* [30]. The MIC of azithromycin against *Enterobacteriaceae* is influenced by the pH of the media. Values are lower in alkaline pH [33,34] - the usual environment for *V. cholerae*. While erythromycin is generally considered as bacteriostatic, azithromycin is considered bactericidal due to its sustained high concentrations in infected tissues [35].

Data on 3,995 patients treated with azithromycin in the United States and Europe in phase II and III trials indicate that its tolerability is comparable to commonly used drugs such as penicillin, amoxicillin and oral cephalosporins, and is markedly better than erythromycin [25,27]. These studies were done on patients with respiratory, sexually transmitted and skin infections, and the total dose used was 1.5 g (500 mg on the first day and 250 mg daily for 4 days). Gastrointestinal disturbances were the most common adverse effects noted; diarrhoea was reported to occur in about 4%, nausea in 3%, and abdominal pain in 3% of the patients. Sixty percent of these adverse events were characterized as mild [25]. This rate of adverse events is much lower than that observed with erythromycin. Severe adverse events were noted in 7% of patients treated with azithromycin compared to 14% of patients treated with other antimicrobial agents, and serious adverse events requiring withdrawal of drug were reported in only 0.7% of the patients taking azithromycin [25]. Interestingly, unlike most antimicrobial agents, adverse events in association with azithromycin were both less common and less serious in children than in adults [25,27]. Azithromycin was not associated with major or consistent effect on laboratory measures of hematological, hepatic or urinary function [25].

Research Design and Methods

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

1. STUDY DESIGN

Prospecive, randomized, double-blind, controlled clinical trial

2. SELECTION OF PATIENTS FOR THE STUDY

A total of 116 evaluable children required to be enrolled for this study will be selected from those who attend the Dhaka Hospital of ICDDR,B according to the following selection criteria:

2.1 Inclusion criteria

- Age / Gender: 1-15 years (male) or 5-15 years (female)
- Duration of illness: 24 hours or less
- Written informed consent for participation in the study from either parent/ guardian
- Severe dehydration according to WHO guidelines [32]
- Positive stool dark-field microscopic examination for *V. cholerae* (pre-enrollment), and isolation of *V. cholerae* O1 or O139 (post-enrollment) from a pre-therapy stool and/or rectal swab culture.

2.2 Exclusion criteria

- History of receiving > 1 dose of an antimicrobial agent effective in the treatment of the isolated strain of *V. cholerae*, or even a single dose of azithromycin.
- Concomitant infection requiring antimicrobial therapy which may interfere evaluation of efficacy and/or safety of the study drugs.
- A concomitant illness that may interfere with the evaluation of outcome.
- Patients with chronic renal, cardiac, or hepatic problems.

Note: Patients will be enrolled at any time of the day, and on any day of the year, subject to fulfillment of enrollment criteria.

3. OBSERVATIONS AND MEASUREMENTS

3.1 Base-line observations and management

Male children, aged 1-15 years, who are brought to the Dhaka Hospital of ICDDR, B for their watery diarrhoea of ≤ 24 hours and signs of severe dehydration, and those who have not received any antimicrobial therapy for their diarrhoea will be initially screened for the study. They will be admitted into the Clinical Study Ward of the Dhaka Hospital and weighed (dehydration weight). Their vital signs and dehydration status will be recorded, and then they will be rehydrated using "Rice ORS" (3.5, 2.9, 1.5 and 50 grams of sodium chloride, trisodium citrate, potassium chloride and dried rice powder respectively for preparation of 1.0 litre of solution) or "Dhaka Solution:, an intravenous polyelectrolyte solution (133,

13, 98 and 48 mmol/l of sodium, potassium, chloride andf bicarbonate/1.0 litre of solution respectively) in accordance with the WHO guidelines [32]. This period will be named as "Rehydration Period" and will be about 3-4 hours in duration. During this period, a stool specimen will be sampled for dark field microscopic examination for demonstration of *V. cholerae*. Patients will be reexamined to ensure that all clinical signs of dehydration has disapperaed.

3.2 4-hour, "Observation Period"

After "Rehydration Period" is over, children will be observed for another four-hour period, to be called "Observation Period". This is the maintenance phase, and the period will also be used to determine severity of illness; a stool output of ≥ 5.0 ml/kg.hour during this period will be considered as indicative of a severe disease. Rice ORS will be the maintenance fluid for all patients, however, patients with severe, persistent vomiting (usually ≥4/hour) or a very high rate of purging (usually ≥10 ml/kg.hour) may require intravenous fluid for maintenance of hydration. Patients will be allowed to drink plain water *ad libitum* during this period, and intake and output records will be carefully maintained.

3.3 Final inclusion in the study

Patients who have a stool output of ≥ 5.0 ml/kg.hour during the observation period and have evidence for infection with *V. cholerae*, as demonstrated by dark field microscopic examination of their freshly passed stool specimen, will be eligible for final enrollement in the study, and their parents/guardians will be requested for their consent.

3.4 Admission history and physical examination

Upon final inclusion into the study, an elaborate medical history will be obtained and a thorough physical examination, including assessment of dehydration, will be performed. All findings will be recorded in pre-designed data collection forms.

3.5 Treatment regimen and randomization

After informed consent has been obtained, patients will be randomly assigned in equal numbers to receive either a single 20 mg/kg (maximum 1.0 g) dose of azithromycin oral suspension, or 12.5 mg/kg (maximum individual dose of 500 mg) of erythromycin oral suspension every 6 hours for 3 days (12 doses). Upon final enrollment in the study, patients will be assigned consecutive study numbers which will have been randomly pre-assigned to one of the two treatment regimen using a computer generated set of random numbers and a block randomization method using a fixed block length of 4.

The time of administration of drug will be considered as the beginning of the study, and each consecutive 24-hour period from this time will constitute a study day. For patients who vomit within 10 minutes of ingestion of the study drug, the dose will be repeated and the event will be recorded; incidence of repeat dosing will be compared between the two study groups at the time of analysis.

A double dummy technique will be used in order to blind treatment regimen,; study drugs will be made available in two colour coded bottles "A" and "B" for each study children. For children in the azithromycin treatment group, bottle "A" will contain active drug and bottle "B" will contain a placebo for erythromycin. For children in the erythromycin treatment group, bottle "A" will contain placebo for azithromycin and bottle "B" will contain active erythromycin. For all study patients, the first dose of study medication will be dispensed from both of the bottles "A" and "B" and the remainder of the doses will be dispensed from bottle "B". Active and placebo for azithromcyin and erythromycin will be identical in

appearance, and their concentration will be adjusted to allow dispensing drug as ml/kg of body weight basis (identical in volume/kg). Random numbers will be generated by Pfizer, USA, who will also provide coded drugs for this study.

In the event of occurrence of vomiting within 10 minutes of administration of the study drug, the dose will be repeated. In the event this leads to short-fall in the amount of drug required, which will be a rare event, extra sets of both study drugs (same colour coading) will be made available. These extra bottles will be retained by the same person withholding randomization code, to be broken in the event of emergency, however, s/he will not be involved with the study in any way; on assessing the need s/he will dispense the right set of bottles in response to the investigators requests.

3.6 Determination of sample size

Sample size will be based on the proportion of clinical success of therapy in the two groups. Two previous studies conducted at ICDDR,B evaluated efficacy of erythromycin in the management of cholera in children. In one of them, the rate of occurrence of clinical success (resolution of diarrhoea) within 96 hours of initiation of therapy was 95% of the children who received 12.5 mg/kg of erythromycin suspension every 6 hours for 3 days (the dose that will also be used in this study) [39]. We expect that the rate of clinical success with a single dose of azithromycin will be similar to that of erythromycin. Thus, using the methods of Makuch and Johnson [37] for equivalence studies, we calculated the sample size as follows:

$$n = 2 \times P \times (1-P) \times (Z_{\alpha} + Z_{\beta})^2/d^2$$

where: P is the overall proportion of success (95%)

Z_{α} is the upper tail point of the standard normal distribution (1.645)

d (0.10) is the maximum difference in the upper 95% confidence limits (100 x (1- α)% for the difference in success in two treatment groups with the probability of (1- β).

Based on the above equation we estimate a sample size of 58 patients in each treatment arm, or a total of 116 patients, with a type I error of 5% and a type II error of 20%. To account for patients whose admission stool cultures are negative for *V. cholerae* O1 or O139, or who prematurely withdraw from the study, we estimate that about 160 patients will be required to be initially enrolled in order to obtain 116 patients evaluable for analysis, however, enrollment will continue until 116 patients evalauable for analysis are obtained.

3.7 Clinical evaluation and laboratory studies

Study patients will be hospitalized for full five days beginning from the time of initiation of study drug therapy. The following clinical and laboratory evaluations will be performed on the study patients at various time period of the study:

- **Weighing**: Before rehydration, after rehydration, just before initiation of the study drug (after the 4-hour observation period), every 24 hours during hospitalization, and at follow-up.
- **History and physical examination**: Before rehydration, after rehydration, at the time of initiation of study drug therapy, daily during hospitalization, and at follow up. In addition to evaluation of hydration status, the history and physical examinations will also focus on adverse events including vomiting, gastrointestinal distress, and rash.
- **Vital signs**: Rectal or oral temperature, pulse and respiratory rates, and blood pressure will be recorded before rehydration, after rehydration, just before administration of study drug, and then every 6 hours during hospitalization.
- **Quantitation of stool volume and/or weight**: This will be done for the rehydration and the observation periods, and for each 6 hour period of hospitalization.

- **Stool character:** Stool will be characterized as follows:

  Watery: Stool that can be poured like water

  Soft: Stool that cannot be poured, but that takes the shape of the container.

  Formed: Stool that retains its shape.

- **Stool culture for *V. cholerae* O1, O139 and non-O1; and *Shigella* and *Salmonella*:** Will be done before initiation of drug therapy on the day of admission, on study day 3, and at follow up 7 days after discharge from the hospital.
- **Rectal swab culture for *V. cholerae***: Will be done before initiation of the study drug on the day of admission, and daily during hospitalization.
- **Hematologic studies**: Serum electrolytes, creatinine, hematocrit, and serum Sp.gr. will be measured before and after rehydration, and at 24 hours from the time of initiation of study drug therapy. Complete blood count and platelet count will be performed only once, after initial rehydration, however, may also be done at other time(s) if clinically indicated. A total of 2.5 ml + 3.0 ml + 2.5 ml of venous blood will be collected at above specified times.
- **Pharmacokinetics studies**: For determining serum concentration of the study drug, 1.0 ml of blood will be sampled from the first 40 patients enrolled in the study only once- 3, 12, 24 or 48 hours after initiation of study drug therapy drugs. To reduce the number of venipunctures, an indwelling catheter with two way stop cock will be introduced at the time of drawing first blood sample for the study which will be kept in place for a maximum of upto 3 hours, i.e. the earliest time blood will be sampled for pharmacokinetic studies; sampling of blood subsequent to this time will be done by individual pricks. Serum will be separated and stored at - 70°C until assayed. For determination of stool concentration of the study drugs, aliquots of stool (about 5.0 ml) will be sampled from stool collected for each 6 hour period of first 2 days of the study from the same patients, and frozen at - 70°C until assayed.

3.8 Handling of Treatment Failures

If the study treatment is judged to have failed clinically patients will be treated for 3 days with an agent other than azithromycin or erythromycin to which the isolate of *V. cholerae* will be found to be susceptible to. The treatment code will not be broken for these patients. Patients with microbiological failure will be treated with a suitable drug as determined by antimicrobial susceptibility of such isolates.

4. OUTCOME MEASURES

As measures of effectiveness, the following outcome measures will be compared:

4.1 Primary outcome measures

- Rates of clinical success
- Rates of bacteriologic success

4.2 Secondary outcome variables

- Duration of diarrhoea
- Rates of clinical relapse
- Rates of bacteriologic relapse
- Duration of fecal excretion of *V. cholerae*-O1 or *V. cholerae* O139
- Six-hourly, daily and total volume of watery stool during the study period
- Proportion of patients requiring unscheduled intravenous fluids
- Frequency of vomiting and its volume
- Resolution of diarrhoea among patients in two treatment groups on each study day

5. HANDLING OF SERIOUS ADVERSE EVENTS

Patients developing serious or life-threatening adverse events will have the study drug stopped, the adverse event appropriately treated, and an adverse event form completed. The investigators must notify the Ethical Review Committee on the event within 24 hours of its occurrence.

6. RATIONALE

In this study we propose to compare the efficacy of a single 20 mg/kg (maximum 1.0 g) oral dose of azithromycin oral suspension with 12.5 mg/kg (maximum individual dose of 500 mg) 6 houly dose of erythromycin oral suspension administered for 3 days (12 doses) in the treatment of children with cholera due to *V. cholerae* O1 or *V. cholerae* O139. If azithromycin is found effective in this study, it will be the first single dose therapy demonstrated to be effective in children. Thus, the findings of this study may have important implications for treatment of endemic cholera, and more importantly in the management and control of large epidemics, particularly when the infections are caused by strains of *V. cholerae* O1 and O139 resistant to conventional drugs used in children- trimethoprim-sulfamethoxazole and furazolidone.

7. DEFINITIONS

7.1 **Clinical success**: Cessation of watery stool within 96 hours of administration of the study drug without relapse

7.2 **Clinical failure**: Continuation of watery stool for > 96 hours

7.3 **Clinical relapse**: Cessation of watery stool within 96 hours of administration of the study drugs, its absence for 24 hours of more, and its recurrence anytime during study or between discharge and follow up 7 days from the time of discharge.

7.4 **Bacteriological success:** Eradication of the infecting *V. cholerae* organism from fecal specimens within 48 hours of initiation of study drugs without its subsequent isolation

7.5 **Bacteriological failure**: Isolation on study day 3 or thereafter from fecal samples of the study patients the same biotype and serotype of *V. cholerae* as was isolated on admission.

7.6 **Bacteriological relapse**: Failure to isolate the infecting *V. cholerae* from stool specimen on two consecutive study days, followed by its isolation any time after this, including at the follow-up visit.

7.7 **Reinfection**: Isolation of a different biotype or serotype of *V. cholerae* as was isolated on admission, or of the same biotype and serotype with a different antimicrobial susceptibility pattern.

7.8 **Duration of diarrhea**: The interval between administration of study drugs to the end of the last 6-hour period the patient had a watery stool.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

Site: The Research Ward of the Dhaka Hospital of ICDDR,B will be used for hospitalization of the study children. This facility is being used for similar clinical trials for decades.

Study population: The Dhaka Hospital provides treatment to over 110,000 diarrheal patients each year, about 70% of whom are children <15 years of age. Cholera is endemic in Bangladesh, and thousands of children with cholera are treated at this hospital each year; study children will be selected from among them.

Laboratory facilities: The clinical laboratory services of the Laboratory Sciences Division of ICDDR,B will be used for all study related laboratory investigations (tests). These laboratories are regularly used for all protocols of the Centre.

Equipments: No equipment other than those required for routine clinical examination of children will be required for this study.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

1. Comparability of study groups

1.1 Before administration of the study drug: Age, dehydrated body weight, degree of dehydration, diarrhoea duration, vomiting frequency, and stool output during the rehydration and observation periods as well as other admission characteristics will be compared between the groups.

1.2 Comparison of primary outcome measures: Rates of clinical and bacteriologic success in the two treatment groups will be compared.

1.3 Comparison of the secondary outcome measures

- Rates of clinical and bacteriologic relapse.
- Duration of diarrhoea in hours, and duration of fecal excretion of *V. cholerae* O1 or O139 in days.
- Volume of watery/liquid stool for each 6 and 24 hour of the study, and also the total amount of watery/liquid stools during the study period.
- Frequency of vomiting and the amount of vomitus, and proportion of patients with vomiting on each study day.
- Intake of oral and intravenous fluids for each 24 hour as well as the entire duration of the study.
- Proportion of patients with resolution of diarrhoea on each study day.
- Proportion of patients with a positive culture for infecting *V. cholerae* O1 or O139 on each study day.

2. Statistical methods

Data will be entered onto computer using SPSS for Windows (Version 6.0). Range check and double-entry system will be employed to facilitate data editing and cleaning. The significance of differences in continuous variables will be assessed by Student's t test for data that are normally distributed, or by Mann-Whitney U test for data which are not normally distrubuted. Significance of differences in the proportions will be assessed by the chi-square test, and Fisher's exact test will be employed when the expected number in any cell in the comparison is 5 or less. Survival analysis (Log Rank Test) will be done for the duration variables such as the proportions of patients with diarrhea, and a positive stool culture for *V. cholerae* O1 or O139 on each day of the study.

Ethical Assurance for Protection of Human Rights

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

Justifications for conducting this research in human subjects

This objective of this study is to compare clinical and bacteriologic efficacy as well as adverse events in association with therapy for children with clinically severe cholera with a single dose of azithromycin oral suspension with a 3-day course of erythromycin. Both of the study drugs are registered with the Drug Administration, Peoples' Republic of Bangladesh for use in the country.

Cholera is a human disease, and thus the efficacy of antimicrobials can only be determined in humans. Although treatment of cholera in adults is possible with a single dose of tetracycilne, doxycycline and ciprofloxacin, no single-dose therapy for cholera in children has yet been possible. A single-dose therapy will not only be the most cost-effective, but will also have the advantage of being the simplest form of therapy which will eliminate the possibility of non-compliance by the patients- an important factor for emergence of resistant strains.

The objectives of the study can only be met by conducting the study in children.

Protection of human rights

The intent of the research program, the study protocol, and the informed consent form to be used in the study will be submitted to the Ethical review Committee of ICDDR,B, and the study can be initiated only after receiving a favorable response from the Committee.

Informed Consent

Since the study will be conducted in children, informed consent must be obtained from the parents or guardians of each child in accordance with the Declaration of Helsinki. It will be the responsibility of the investigator to obtain written informed consent from the them after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. The investigator must also explain that the parents/guardins are completely free to refuse to enter their children in the study, and also to withdraw them from the study at any time. This informed consent must be obtained in the presence of a witness along with their signature, and also of signature or thumb impression (for those who can't write) of respective parents/guardians along with the date.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Animals will not be involved in this study in any way

Literature Cited

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

1. Khan MU, Eeckels R, Alam AN, Rahman N. Cholera, rotavirus and ETEC diarrhea: some clinico-epidemiological features. Trans R Soc Trop Med Hyg 1988;82:485-8.
2. Goma epidemiology group. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire in July 1994? Lancet 1995;345-44.
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4. Wallace CK, Anderson PN, Brown TC, Khanra SR, Lewis GW, Pierce NF, Sanyal SN, Segre CV, Waldman RH. Optimal antibiotic therapy in cholera. Bull WHO 1968;39:239-45.
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6. Rabbani GH, Islam MR, Butler T, Shahrier M, Alam K. Single dose treatment of cholera with furazolidone or tetracycline in a double-blind randomized trail. Antimicrob Agents Chemother 1989;33:1447-50.
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9. Khan WA, Bennish ML, Seas C, Khan EH, Ronan A, Dhar U, Busch W, Salam MA. Randomised controlled comparison of single-dose ciprofloxacin and doxycycline for cholera caused by V. cholerae O1 or O139. Lancet 1996;348:296-300.
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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The findings of the study will disseminated as follows:

1. Presentation(s) at Scientific Forums, ICDDR,B for dissemination amongst scientists of the Centre.
2. Presentation at the Annual Scientific Conference (ASCON) of ICDDR,B for dissemination amongst scientists and health officials of the Govt. of Bangladesh and of the Non-Govt. Organizations.
3. Presentation at Regional and International Scientific Conferences.
4. Publication in peer-reviewed international medical journal.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This study will be done in collaboration with Dr. Michael L. Bennish of the New England Medical Center, Boston, MA, an a coinvestigator.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
1. Wasif Ali Khan	Medical Officer, CSD, ICDDR,B.	19 January, 1963
2. Mohammed Abdus Salam	Chief Physician, CRSC, ICDDR,B.	01 January, 1952
3. Michael L. Bennish	Associate Professor, NEMC, Boston.	01 May, 1951

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
1. Chittagong Medical College	M.B.B.S.	1987	Medicine & Surgery
2. Dhaka Medical College	M.B.B.S.	1976	Medicine & Surgery
3. Michigan State University	Medicine	1977	Medicine

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

NOTE: THIS IS THE COMMON PUBLICATION LIST FOR ALL INVESTIGATORS OF THE STUDY

1. Khan WA, Begum M, Salam MA, Bardhan PK, MR, Mahalanabis D. Comparative trial of five antimicrobial compounds in the treatment of cholera in adults. Trans Royal Soc Trop Med Hyg. 1995;89:103-6.
2. Khan WA, Dhar U, Salam MA, Seas C. Bacterial meningitis in a diarrhoeal disease treatment centre in Bangladesh. Acta Paediatr 1995;84:693-4.
3. Khan WA, Dhar U, Begum M, Salam MA, Bardhan PK, Mahalanabis D. Antimicrobial treatment of cholera due to *Vibrio cholerae* O139 (Synonym Bengal). Drugs (Extended abstract) 1995;40 (Suppl.2):460-2.
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15. Siddique AK, Salam A, Islam MS, Akram K, Majumder RN, Zaman K, Fronczak N, Laston S. Why treatment centres failed to prevent cholera deaths among Rwandan refugees in Goma, Zaire. Lancet 1995;345:359-61.
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Detailed Budget for New Proposal

Project Title: Single-dose Azithromycin Therapy for Childhood Cholera

Name of PI: Wasif Ali Khan

Protocol Number: Name of Division: Clinical Sciences Division (CSD)

Funding Source: NEMC Amount Funded (direct): 128,475 Total: 160,594 Overhead (25%) 32,119

Starting Date: October, 1998 Closing Date: August, 2000

Strategic Plan Priority Code(s): 11

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary (m)**	**1st Yr (3 m)**	**2nd Yr**	**3rd Yr (9 m)**
	Dr. Wasif Ali Khan	PI	40%	837	1,004	4,218	3,322
	Dr. Mohammed A. Salam	Co-investigator	25%	1,673	1,255	5,270	4,150
	Medical Officer, Matlab	Co-investigator	20%	819	491	2,064	1,625
	Mrs. Monira Begum	Research Officer	40%	450	540	2,268	1,786
	Mr. Humayun Kabir	Data Entry Tech	40%	391	469	1,971	1,552
	Secretary		15%	482	217	911	717
	Research M.O., (50% x2)	Dhaka, Matlab	100%	708	2,124	8,921	7,025
	Ward Attendants x 2		100%	65	390	2,457	1,953
	Sub Total				**6,490**	**28,080**	**22,130**
	Consultants						
	Local Travel				300	800	400
	International Travel				2,000	2,000	4,000
	Sub Total				**2,300**	**2,800**	**4,400**
	Supplies and Materials (Description of Items)						
	Office supplies				200	500	300
	Hospital supplies				200	500	300
	Laboratory supplies				200	500	300
	Non-stock supplies				400	400	200
	Sub Totals				**1,000**	**1,900**	**1,100**

	Other Contractual Services			
	Repair and Maintenance	100	200	100
	Rent, Communications, Utilities	1,000	2,500	1,500
	Training Workshop, Seminars			
	Printing and Publication			500
	Staff Development			
	Sub Total = 5,900	**1,100**	**2,700**	**2,100**

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests	570	1,000	675
	Microbiological tests	1,500	6,000	4,480
	Biochemistry Tests	500	2,500	1,200
	X-Rays	50	50	50
	Patients Study	4,000	10,000	7,600
	Research Animals			
	Biochemistry and Nutrition			
	Transportation		1,000	
	Staff Clinic Subsidy	50	100	50
	Matlab water and land transport	300	700	500
	Xerox, Mimeographs etc.	200	200	100
	Sub Totals	**7,170**	**21,550**	**14,655**
	Other Operating Costs	**500**	**1,500**	**1,000**
	Capital Expenditure	**6,000**		

Total Direct Cost : 128,475 **Overhead Cost (25%) : 32,119**

TOTAL PROJECT COST = 160,594

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

1. **Personnel**

This study will enroll an estimated 160 children fulfilling the study eligibility criteria to get 116 evaluable patients over a two year period. The clinical care to these children must be provided by the investigators of this study, namely Drs. Salam and Wasif. In view of the fact that the incidence of cholera vary quite significantly from year to year, it will be important to be very aggressive in the patient enrollment. The investigators have other committments and responsibilities, and thus they will not be able to remain engazed only with this study. Thus, for providing care to the study children on all seven days of the week, assistance of 50% time of a medical doctor will be required. Because of limited time for completion, it is anticipated that the study may be required to be extended to the Matlab Hospital of the Centre. Although a coinvestigator has not yet been identified, 50% time of a medical officer will also be required to work at Matlab Hospital. The attendants will be required to perform initial screening of the patients, and also to assist in other study works.

The research officer will be responsible to maintain the study drugs and dispense them upon request from the investigators, maintain drug inventory, and store all unused and used drugs. Additionally, the person will be responsible to ensure timely performance of all laboratory test, and to compile and maintain results of all laboratory tests. These will require 100% time of a research officer.

Data will be entered twice as soon as they become available. The person will also help in cleaning of data as well as performing some of the basic statistics, and assist the research officer in performing her tasks. All these activities will require 100% time of a data entry technician.

Ward attendants will be required to screen potential study patients at least at study two locations and possibly also at other hospitals/clinics, and also for motivating parents/legal guardians to bring their children for follow-up visits and to help bringing the patients for follow-up evaluation in the event they fail to return to the hospital in time on their own. For all these activities, it will be essential to recruit two Ward Attendants.

2. **Local travel**

This will be required for covering the cost for frequent movement of the study medical officer and the investigators to and from Matlab.

3. **Supply and material, Other contractual and Interdepartmental**

These reflect exact cost of supplies and materials which will be required for the study.

4. **Capital cost**

This will be required to buy a photocopier which will be extensively used for production of data collection forms, and will remain useful for the office of the investigators.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

1. One study, short titled "Short-course ciprofloxacin therapy for childhood shigellosis due to *S. dysenteriae* type I is being conducted. The study is sponsored by Bayer (Pte) Ltd. South Africa through New England Medical Center, Boston, MA. and has a budget of about US $ 100,000.

2. Another protocol is being developed to determine efficacy and safety of a 7-day course of ciprofloxacin in the treatment of typhoid fever. Anticipated budget for this two-year study will be about 125,000, and it is hoped that the study will be supported by Bayer AG.

3. One research protocol, short titled "Diagnosis of pneumonia in children with dehydrating diarrhoea" is currently being reviewed by USAID under the competitive grant agreement (Tentative budget = US $ 50,000)

4. Another 3-4 protocols are being developed to test efficacy of antimicrobials in the treatment of shigellosis and cholera, particularly short course therapies. At least two of the protocols are likely to be funded by Pfizer Inc., USA and another by Bayer AG.

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included	√
2. Approval of the Division Director on Face Sheet	√
3. Certification and Signature of PI on Face Sheet, #9 and #10	√
4. Table on Contents	√
5. Project Summary	√
6. Literature Cited	√
7. Biography of Investigators	√
8. Ethical Assurance	√
9. Consent Forms	√
10. Detailed Budget	√

APPENDIX -1

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Single-dose Azithromycin Therapy for Childhood Cholera.

Principal Investigator: Wasif Ali Khan

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Your child is suffering from diarrhoeal disease which we suspect to be cholera caused by a germ called *Vibrio cholerae*. Management of severe cholera not only require correction of fluid-and salt-deficits but also management of ongoing losses which are done using appropriate oral or intravenous fluids. This also require treatment with an appropriate antimicrobial drug to kill the germ which causes this infection. However, a drug may become ineffective after using for some years due to development of resistance to them by the germs. Currently, such germs have become resistant to furazolidine and the combination drug, trimethoprim-sulphamethoxazole, two safe drugs for use in children. Thus, erythromcin is the only safe drug left for treatment of cholera in children. This drug has to be administered to the children every 6 hours for 3 days; a single-dose treatment of chlolera, possible in adults, has not yet been possible with the currently available drugs.

Azithromycin is a newer drug which belong to the same class of drug as erythromycin. Among others, the advanatage of the drug over erythromcin include it's better tolerability and less frequent, usually once daily administration. We hope that this drug will be effective in a single dose in the treatment of cholera in children, however, a trial is necessary to determine if that indeed is the case. If azithromycin is found to be effective, an easier and safer antibiotic drug will be available for the first time for single-dose treatment of cholera in children.

We request your permission for participation of your child in this study. If your child participates, he will receive the usual good care and treatment of this hospital, and will also be able to contribute to society by enhancing our understanding of cholera, which remains an important disease in Bangladesh. If you allow your child to participate in the study you can expect the following:

1. Admission of your child in hospital for five full days or until resolution of his diarrhoea, the one that is longer, and he will also be required to return for his follow up evaluation, 10 days after his discharge from the hospital.

2. Your child will receive the standard medical care of this hospital for cholera with the exceptions noted below.

3. A detailed medical history and physical examination will be performed, several times on the day of admission, daily thereafter until his discharge from the hospital, and also at the time of follow up visit; your child and/or you will be asked about his condition on each of these occasions.

4. Stool specimens and/or rectal swabs will be collected from your child on admission, on each day he is in the hospital, and at the time of follow up visit. Other than momentary discomfort, the taking of a rectal swab is not associated with pain or any other risk.

Blood will be drawn from your child for various tests which will help us in evaluation of his condition at the time of admission, after rehydration and 24 hours after administration of the first dose of the study drug. Thus, a total of 7.5 ml (about 1.5 teaspoonful) of blood will be drawn from a vein of his/her elbow on a total of three occasions. In addition, to determine the concentration of drug in blood, 1.0 ml of blood will be drawn from vein of your child only once at variable time interval from the time of administration of the first dose of the study drug- 3, 12, 24 or 48 hours. To avoid repeated venipunctures on the admission day, we'll introduce a thin plastic catheter into his vein in the elbow which will remain in place until a maximum of 3 hours from the time of administration of the first dose of the study drugs, and we'll draw blood using the catheter. Your child will feel slight pain during

insertion of the catheter and needles, and there is a chance for local, temporary discoloration of skin at the site of insertion of catheter and also a very small risk of infection, however, we will take precaution to avoid them.

5. We will collect a small amount (5 ml; about one teaspoonful) amount of stool from your child at various times during his hospitalization.

6. We will keep all medical information and results of laboratory tests of your child confidential, and no one other than the investigators of this study, the monitors of this study, and the Ethical Review Committee of this Centre can have an access to this information after discharge from the hospital. All records will be locked in a safe cabinet. If you are interested to know results of any/all of the laboratory tests performed on your child we'll be happy to provide that to you those as and when they become available; we would, however, like to inform you that results of some of the tests will be only available long after the study is over and the follow up evaluation of your child is done. While your child is in the hospital, only those persons concerned with your child's care who normally have access to the record, will be able to access the record.

7. After entry into the study, your child will receive either a single, 20 mg/kg body weight (maximum 1.0 g) dose of azithromycin orally followed every 6 hours for 3 days by a pretend drug, or 12.5 mg/kg body weight (maximum individual dose of 500 mg) dose of erythromycin oral suspension every 6 hours for 3 days. Neither you or your child, nor we will know which of the two drugs he is receiving. Erythromycin is routinely used in this hospital for the treatment of children with cholera, and we hope that a single dose of azithromycin will also be as effective and perhaps be more effective than erythromycin. Almost all antimicrobials have undesirabale, side effects, and erythromycin and azithromycin are no exceptions. However, serious side effects are rare with either of the drugs, and minor side-effects such as stomach upset, is less common with azithromycin compared to erythromycin. Minor or moderate side effects of both drugs include vomiting,/feeling of vomiting and diarrhoea.

8. You are the only person to decide whether or not your child participates in this study. Your child will receive the standard care and treatment of this hospital for cholera even if she or he does not participate in the study, and even if you withdraw your consent to participate at any time during the study.

If you agree for participation of your child in the study, please put your signature, or your left thumb impression at the specified space below. Thank you for your cooperation.

_____ _____

Signature/LTI of the guardian Date

_____

Address of the guardian

_____ _____

Signature of Investigator Date

_____ _____

Witness's signature Date

_____

Address of the witness

APPENDIX - 2

STUDY FLOW CHART

Activity	Screening	Rehydration	Observation	Day 1	Day 2	Day 3	Day 4	Day 5	F-up
History	+		+	+	+	+	+	+	+
Physical Exam	+		+	+	+	+	+	+	+
Weight	+		+	+	+	+	+	+	+
Vital Signs (4 hourly)	+		+	+	+	+	+	+	+
Intake Output		+	+	+	+	+	+	+	+
Stool Dark-field		+							
Stool Microscopy		+							
CBC + platelet count			+						
Electrolyte, creatinine	+		+	+					
Serum Sp. gr.	+		+	+					
Stool culture for *Shigella*, *Salmonella*, and vibrios			+			+			
Rectal swab culture for vibrios			+	+	+	+	+	+	+
K-B antibiotic susceptibility*				+					
Drug therapy				+	+	+			
Serum drug conc				+	+				
Stool drug conc				+	+				

* will also be determined for *V. cholerae* isolated from cases of microbiological relapse.

ABSTRACT SUMMARY FOR THE ETHICAL REVIEW COMMITTEE

1. The aim of the study is to compare clinical and bacteriologic efficacy of a single dose of azithromycin oral suspension with a 3-day, multiple dose therapy with erythromcyin oral suspension in the treatment of clinically severe cholera in children caused by *V. cholerae* O1 or O139 infection. Children who have watery diarrhoea of 24 hours or less, signs of severe dehydration, and have a positive dark field microscopy for *V. cholerae* will be eligible for enrollment. Enrollment of children in the study will require consent of their parents/guardian.

2. The only risk to the patients is the rare possibility of infection consequent to venipunctures to be done for sampling blood on three occasions for determination of serum electrolytes, creatinine and complete blood count (CBC) which will be useful for clinical management of patient, and only once for determination of serum concentration of the study drugs which will not be useful for management of patients.

3. We believe that the very small risk of infection due to venipunctures, and their potential benefit in assessing study children justifies this minor risk.

4. Patient information will be kept under lock, and the computer database on the study children will not include the name of the patients or of the parents/guardians.

5. The parents/guardians will be told of the nature of the study at the time of initial screening by the health worker. The consent form will be given to them to read, or read to them by the investigators for those parents/guardians who are unable to read.

6. A brief one page history form will be filled in by the investigators which will require about 10 minutes of the parent's/guardian's time. The questions do not depart from the questions that would be asked to make a clinical diagnosis under the usual circumstances.

7. The benefits to the patient are that they will be examined by investigators well-trained in pediatrics, and have thorough investigation for their medical problems. The benefits to the general population are that important information may be derived for development of a simple form of antimicrobial treatment for cholera in children which is currently not available. If confirmed, this form of treatment will almost certainly be used in the management of children at the Dhaka Hospital of ICDDR,B.

8. The study will require use of blood, stool and urine samples.