ject 1.
ACT SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 26/9/93
26/10/93

cipal Investigator Dr. Carlos Seas
lication No. 93-031 Dr. M. A. Salam
le of Study "Randomized, double-blind study of efficacy of Azithromycin in the treatment of adults with Shigellosis".

Trainee Investigator (if any) to be recruited
Supporting Agency (if Non-ICDDR,B)
Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

cle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — (Yes) No
(b) Non-ill subjects — Yes (No)
(c) Minors or persons under guardianship — Yes (No)

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — (Yes) No
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — (Yes) No
(c) Physical risks — (Yes) No
(d) Sensitive questions — (Yes) No
(e) Benefits to be derived — (Yes) No
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will signed consent form be required:
(a) From subjects — (Yes) No
(b) From parent or guardian (if subjects are minors) — Yes (No)
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
✓ Informed consent form for subjects
___ Informed consent form for parent or guardian
✓ Procedure for maintaining confidentiality
✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

ree to obtain approval of the Ethical Review Committee for any changes ving the rights and welfare of subjects before making such change.

Seas
Principal Investigator

Trainee

SECTION I-RESEARCH PROTOCOL

1. TITLE : RANDOMIZED, DOUBLE-BLIND STUDY OF EFFICACY OF AZITHROMYCIN IN THE TREATMENT OF ADULTS WITH SHIGELLOSIS

2. PRINCIPAL INVESTIGATOR : CARLOS SEAS M.B.B.S.
CO-PRINCIPAL INVESTIGATOR : MOHAMMED A. SALAM M.B.B.S.

CO-INVESTIGATORS : WASIF A. KHAN M.B.B.S.
MAHBUBUR RAHAMAN Ph.D.

CONSULTANT : MICHAEL BENNISH M.D.

TRAINEE RESEARCH MEDICAL OFFICER: TO BE RECRUITED

3. ANTICIPATED STARTING DATE : 01 JANUARY 1994

4. STUDY DURATION : 2 YEARS FROM INITIATION

5. TOTAL DIRECT COST : US$ 114,934

6. SCIENTIFIC PROGRAM HEAD : CLINICAL SCIENCES DIVISION

This protocol has been approved
by the Clinical Sciences Division

Signature of the Associate Director, CSD

Date: _____

7. ABSTRACT SUMMARY:

With the increasing frequency of multi-resistant *Shigella* isolates in Bangladesh and other developing countries it is necessary to look for alternative effective antimicrobial agents. Azithromycin is a new macrolide which is given orally, has good bioavailability, achieves unique intracellular concentrations, has a long half-life, a broad spectrum of activity, and is well tolerated. The usefulness of this antimicrobial agent against enteric bacteria, especially intracellular pathogens, including *Shigella*, has been suggested based on its *in vitro* activity. An evaluation of 30 strains of *Shigella* isolated from patients who attended the Clinical Research and Service Centre (CRSC) of ICDDR,B found an MIC_{90} in the range of high activity for *Shigella flexneri* and moderate *in vitro* activity for the rest of *Shigella* strains.

The objective of this randomized, double-blind study, is to evaluate the clinical and microbiological efficacy of a five day course of azithromycin compared to ciprofloxacin in the treatment of shigellosis in adults. A total of 70 patients with culture confirmed shigellosis who attend the CRSC of the ICDDR,B with acute dysentery will be enrolled after obtaining written informed consent. A history, physical, and laboratory examinations will be performed on admission and daily thereafter.

Patients will be randomly assigned to receive either ciprofloxacin 500 mg every 12 hours for five days or azithromycin 500 mg as a single dose on the first day, and 250 mg once a day

thereafter for a total of 5 days, using a double-dummy technique. The bacteriological and clinical response to therapy will be evaluated following pre-defined criteria previously used in clinical trials conducted at ICDDR,B. The incidence of adverse events will also be evaluated. Patients who develop serious adverse events will be treated with pivmecillinam, after opening their treatment codes. Patients considered to have had clinical or microbiological failures also will be treated with pivmecillinam without opening their treatment codes.

If azithromycin shows good clinical and microbiological efficacy in this trial will increase the current alternatives against shigellosis. Advantages as: once-a-day dosing regimens, low profile of side effects, and potential utility in children should be emphasized.

SECTION II-RESEARCH PLAN

A. INTRODUCTION

A.1 OBJECTIVE

The objectives of the study are:

A.1.1. To compare the effectiveness of azithromycin with that of ciprofloxacin in the treatment of adults with shigellosis.

A.1.2. To measure the peak and trough serum concentrations of both the study drugs, and also to measure their concentrations in stools.

A.1.3. To monitor adverse events related to therapy with both the study drugs

A.2 STUDY BACKGROUND

A.2.1 Importance of shigellosis and need for newer antimicrobials in its treatment

Acute dysentery caused by *Shigella* remains as a major public health problem in developing countries (1). The disease is associated with a high incidence of complications and also high mortality, especially in children below 1 year (2). Shigellosis is one of the few diarrhoeal diseases for which treatment with antimicrobial agents is recommended (3), and several studies have shown that when an appropriate antimicrobial agent is used, the duration of the disease as well as the excretion of the bacteria is reduced (4,5,6). Furthermore, providing early and effective antimicrobial

agents the complications of the disease as well as the mortality may be prevented (7).

The sensitivity pattern of *Shigella* in Bangladesh has dramatically changed since 1986, and at the present time, ampicillin and cotrimoxazole, once considered the standard antimicrobial therapy, are no longer the drugs of choice (7,8). The main reason for that change is the improper use of antibiotics (8). The antibiotic treatment for patients infected with these multiple resistant strains is complicated as resistance is appearing against antimicrobials currently in use (7,8,9). Data from the CRSC of ICDDR,B (Figure) shows that in 1992, ampicillin, cotrimoxazole and nalidixic acid were ineffective against *S. dysenteriae* type 1, while pivmecillinam and ciprofloxacin were the only alternatives available. *S. flexneri, S. sonnei* and *S. boydii* are still sensitive to nalidixic acid.

Nowadays, the current practice in the CRSC is to use nalidixic acid if the suspected *Shigella* agent is other than *S. dysenteriae* type 1, and pivmecillinam if the clinical features favour a diagnosis of this strain, both are given for 5 days (9). Strains resistant to pivmecillinam have, however, been identified (10). While pivmecillinam is still active in Dhaka, in Matlab, a field station of ICDDR,B, a sudden increase in pivmecillinam resistant strains has been recently observed (*Chowdhury H. et al. drug-resistant Shigellae: a serious problem for the clinician in choosing the proper antibiotic for treatment. Abstract 14. Programme and Abstracts. Second Annual Scientific Conference,*

1993). It is probable that the frequency of pivmecillinam resistant strains in Dhaka also will increase in the near future. Clearly, new alternatives are necessary. The quinolones have shown good efficacy in the treatment of shigellosis in both outpatients (11) and inpatients (12,13). Their relatively high cost and their restricted use in children limit their utilization, however (9). Newer alternatives such as oral cephalosporines are currently under evaluation. Among the drugs that have good *in vitro* activity against *Shigella* and other enteric pathogens, the new macrolide azithromycin, a synthetic derived of erythromycin, deserves evaluation in controlled clinical trials (14).

A.2.2 Renewed interest on macrolides

Recently a renewed interest in developing new macrolides have occurred, mainly due to the recognition of good *in vitro* and *in vivo* activity of erythromycin against intracellular pathogens, specially in immunosupressed hosts (15).

Three new generations of macrolides have been synthetically obtained from erythromycin (16). All of them show better properties than erythromycin, i.e, higher bioavailability due to their resistance to stomach pH, longer half-lives, higher intracellular concentrations, better antimicrobial spectrum of activity, and better tolerability and safety (16).

The ability of these antimicrobial agents to concentrate several times more in the intracellular compartment than in the serum; the fact that they are actively carried and stored by the

inflammatory cells and fibroblasts, assures their higher concentrations in the injured tissues than the commonly used antimicrobial agents attain (16,17). The concentration achieved in tissues are several times higher the required MIC for the pathogens (16,17). This is the main property of all the new macrolides but especially of azithromycin (18).

A.2.3 Rationale for use of azithromycin

Azithromycin, an azalide, has clear advantages over the other macrolides. Four differences deserve mention: better pharmacological properties, wider antibacterial spectrum, better tolerability, and better safety profile (19). Azithromycin was synthetically obtained from erythromycin by replacing the 9a carbonyl in the aglycone ring with a methyl substituted nitrogen (20). This substitution confers unique pharmacological and microbiological properties, as well as avoids the formation of the hemiketal product, responsible of the common side effects seen with erythromycin (21). The antibacterial mechanism of action involves interference with bacterial protein synthesis by binding to the 50S component of the 70S ribosomal subunit (22).

The most innovative property of the macrolides and specially of azithromycin is the ability to concentrate several times more in the intracellular space than in the serum (16,17,18,23). Additionally, azithromycin is concentrated in human phagocytes several times more than in the extracellular space without altering the natural killing mechanisms of the inflammatory cells, and the

cells continue uptaking the drug for a period of 24 hours after the first dose (24). High concentrations are maintained even when the serum level is very low (24). Azithromycin is rapidly released by phagocites and fibroblasts, this assures high concentrations of the active drug at the site of interest (24). The high tissue concentrations achieved with azithromycin in the infected organs explains its activity against intracellular pathogens. This property may have special relevance in the treatment of shigellosis and typhoid fever, where intracellular concentrations are thought to be more important than luminal ones (25).

An important limitation of erythromycin and other new macrolides than azithromycin is their narrow antibacterial spectrum, that does not include *Hemophilus influenzae* and *Enterobacteriaceae* (26). In several *in vitro* studies azithromycin has showed to be active against *Hemophilus influenzae* (27) and also against *Enterobacteriaceae* such as *E. coli, Salmonella spp., Campylobacter spp., Shigella spp. and Vibrio spp.*, the common bacterias associated with diarrhoea (28).

Because of good results experienced in *in vitro* and in animal studies, it is speculated that azithromycin may have great potential for the treatment of intestinal infections including *Shigella* (14,29). However, well designed, controlled clinical trials are necessary to determine its role in the management of such infections.

A.2.4 Pharmacokinetics of azithromycin

Azithromycin has good stability at low pH; over the stomach pH range it is 300 times more stable than erythromycin (30). The oral bioavailability (37%) is higher than that of erythromycin (25%), (30). However, absorption is reduced by about 50% with foods or antacids (31). The serum kinetics of the drug shows that the decline in plasma levels, which occurs rapidly after peak concentrations are reached, is indicative of a rapid distribution phase with a high (23L/Kg) volume of distribution (22,30,32). The peak serum level is 0.4mg/L, which occurs 2-3 hours after an oral dose, and the trough level is 0.04mg/L (30,32). Azithromycin shows a low affinity for serum proteins. As the serum concentrations of azithromycin increase the affinity decrease, i.e, at 0.05mg/L the affinity is 50% but at 0.5mg/L it is only 12% (32). The serum half-life is 40 hours, after a 500 mg oral dose. The tissue half-life is even more prolonged (2-3 days) allowing a once-a-day dosing regimen (30). Additionally this property may make a shorter duration of therapy possible.

As was mentioned briefly above, the cellular pharmacokinetics of this drug are quite remarkable. Azithromycin is rapidly and highly concentrated into inflammatory cells and fibroblasts due of its dibasic amphiphilic nature,, and both of them act as a tissue reservoir for the drug (30,32). Experimental studies have shown that when an infection is induced, the drug is rapidly delivered from the reservoir to the tissues infected (33). The measured intracellular/extracellular (I/E) concentration ratio for

azithromycin in the human polymorphonuclear has been determined to be 79 in one study, which is nearly 10 times the ratio attained by other drugs that have high intracellular concentrations (30). This ratio is nearly 100 in many tissues after a 500 mg oral dose (34). A recent study, using a more sensitive technique than that have been used before to evaluate the intracellular concentrations of the drug in human phagocytic cells, found an I/E ratio of 300. This study also found that the uptake and release of the drug from the polymorphonuclear is sustained over the time and is not saturable (35). The tissue kinetics of the drug shows that the concentrations in tissues such as lung, tonsils, gastric mucosa, paranasal sinuses, skin, and other organs varies between 2 and 9mg/Kg, measured 12-48 hours after an oral dose, and that the mean concentrations were usually greater than 4mg/L, higher than the MIC for the majority of the pathogens for which it is active (30,32). Based on these data, the usual dosing regimen is 500mg given once the first day, and 250mg once daily thereafter for a total of 5 days (30,32,33).

The drug is eliminated without significant metabolism. Only a small proportion (< 5%) is demethylated in the liver to an inactive product (30). The primary route of excretion is transintestinal and biliary thus resulting in high concentrations of the drug in the stools (30). No important interactions with commonly used drugs have been described; the effect of antacids on availability has been mentioned (31). The pharmacokinetic properties are similar in children and adults, although information on pharmacokinetics in

children is meagre (31). There is no concern, however, on its use in children (31,36). For patients with renal insufficiency correction of the dosage is recommend only if the creatinine clearance is less than 40 ml/min (31). Patients with hepatic failure also need a correction in the dose (31).

A.2.5 Antibacterial activity of azithromycin

Azithromycin can be used against four groups of organisms: (1) against rapidly growing pyogenic bacteria commonly susceptible to beta lactam drugs; (2) against bacteria resistant to benzylpenicillin and erythromycin; (3) against organisms for which antimicrobial agents such as erythromycin and tetracyclines are indicated; and (4) against organisms for which antimicrobial therapy is limited (37).

The major advantage of azithromycin over the other macrolides is its recognized activity against *Hemophilus influenzae* and *Enterobacteriaceae* (15,27,28,29), due in part to its better penetration through the cell wall of gram negative bacteria (38). Values of MIC lower than 4 ug/ml (in the range of activity) against *E. coli*, *Salmonella typhi* and *non typhi* and *Shigella spp*, including strains resistant to ampicillin, has been shown in several *in vitro* studies (14,29,39,40,41,42). A more recent study evaluated the *in vitro* activity of several enteropathogens at two different pH concentrations. The results indicate that azithromycin is active against *Shigella spp.*, *Salmonella spp.*, *Salmonella typhi*, *E. coli*

and *Campylobacter spp.* with MIC_{90} of less than 4 ug/ml (43). Moreover, the MIC of azithromycin to *Enterobacteriaceae* depends on the pH of the media. Lower values have been seen at alkaline pH (44,45), the usual pH of the colonic environment infected by *Shigella*. These MICs, however, are well below the intracellular level of azithromycin attained in macrophages and tissues (46), and indicate a potential role in the treatment of infections caused by this kind of bacterias (14,29,39,41,43). While erythromycin is generally considered bacteriostatic, azithromycin is bactericidal, due basically to high and sustained concentrations achieved in tissues (33).

In an experimental study, the efficacy of azithromycin in mice challenged with *Salmonella enteritidis* (a tissue associated infection model), correlated directly with liver levels of azithromycin, but not with serum concentrations (47). When the potency of azithromycin was compared against ciprofloxacin, azithromycin was more effective in reducing the number of bacteria in the liver despite its higher MIC_{90} (0.006 ug/ml for cipro vs, 1.56 ug/ml for azithromycin) and lower serum concentrations. This was thought to be due to its higher and more sustained intracellular concentrations (47). Studies evaluating its activity against *Legionella* and *Chlamydia* confirmed the effectiveness of this drug against intracellular pathogens (48).

The good *in vitro* activity against *Salmonella* has recently been confirmed *in vivo*. Clinical and bacteriological efficacy of two once-a-day oral regimens of azithromycin: 500 mg daily for 7

days and 500 mg daily for 14 days were similar that of chloramphenicol, in an open and randomized trial conducted in patients with culture proven typhoid fever in Chile (*Butler T. et al. Efficacy of azithromycin for treatment of typhoid fever. Abstract No 1579. Abstract book of the 1992 ICAAC*). This results suggest that azithromycin may reduce the duration of treatment for typhoid fever achieving same efficacy that standard regimens, and also that the drug has good clinical efficacy against enteric invasive pathogens.

An evaluation of 30 strains of *Shigella* (10 *S. dysenteriae* type 1, 10 *S. flexneri*, 5 *S. boydi* and 5 *S. sonnei*) isolated from patients who attended the CRSC of ICDDR,B showed an MIC_{90} of 1.875 ug/ml for *S. flexneri*, in the range of high in-vitro activity; and 3.75 ug/ml for *S. dysenteriae*, *S. boydii* and *S. sonnei*, respectively, indicative of moderate activity. No one resistant strain was observed. The current recommendations for interpretation of *in vitro* tests against *Enterobacteriaceae* are as follows: highly susceptible; MIC $\leq$ 2ug/ml, resistant; MIC $\geq$ 8ug/ml (43,45).

A change in the current accepted pharmacokinetic rules to evaluate antimicrobial agents, specially those which attain good intracellular concentrations, has been suggested (19). According with recognized rules, the concentration of the active drug at the place where the infection occurs should be in excess than the measured MIC to be considered as an effective drug (19). Due to difficulties in the methodology for measuring intracellular concentrations, clinicians have used serum or plasma concentrations

of antimicrobial agents as a surrogate for tissue concentrations. To consider a drug as effective they require that the serum level should be at least 4 or 5 times in excess the MIC measured (19). All this propositions may be valid for drugs that does not attain good intracellular levels, such as beta lactams or amynoglycosides, but are not valid for drugs as azithromycin which clearly attains intracellular concentrations higher than the MICs for the pathogens studied (19,30,32,34,46).

In summary, good clinical and microbiological results recently attained in typhoid fever, an intestinal disease as severe as shigellosis in adults, the acceptable MIC values seen with the *Shigella* tested in our laboratory, the possibility of even better activity at the alkaline pH in the colon than at other body sites, the good pharmacological properties, the high concentrations in the stools, and the even higher concentrations achievable in the tissues suggest that azithromycin may have advantages over the standard therapies for invasive enteric pathogens. These properties, plus the safety of azithromycin in children, represent the rationale to evaluate its efficacy against multi-resistant *Shigella* strains in Bangladesh.

A.2.6 Safety of azithromycin

The data of 3,995 patients treated with azithromycin in the United States and Europe, in phase II and III trials, comparing it with commonly used antimicrobial agents (penicillin, amoxicillin, and oral cephalosporins) indicate that tolerability was comparable to

majority of the drugs but markedly superior to that of erythromycin (31,36). In these studies the majority of patients had respiratory, sexually transmitted and skin infections, and the total dose was 1.5 g (500 mg on the first day and 250 mg daily for 4 days). Gastrointestinal adverse events were the most common; diarrhoea in 3.6%, nausea in 2.6% and abdominal pain in 2.5% (31). In 60% of instances adverse events were mild, and only in 7% they were severe, compared to 14% severe side effects with the other antimicrobial agents (31). Only 0.7% of the patients required withdrawal from drug treatment due to severe adverse events. Surprisingly, the adverse events were less common and less serious in patients above 65 years and in children (31,36). When the laboratory values were evaluated, the drug did not show a major or consistent effect on them, including haematological, hepatic and urinary function tests (31).

In general, azithromycin is safer than erythromycin and other common antimicrobial agents used in adults and children (31,36).

B. METHODOLOGY

The methodology of this study will be similar than that of previous clinical trials conducted in the Clinical Sciences Division of the ICDDR,B (12,13).

B.1 Sample size calculation

We used the following formula:

$$n = \frac{p_1(100-p_1) + p_2(100-p_2)}{(p_1-p_2)^2} \times f(\alpha,\beta)$$

where:

- p_1: 100% for ciprofloxacin, as was shown in a recently conducted study at the CRSC of ICDDR,B (13)
- p_2: 80% for azithromycin, as we consider that to be an alternative, azithromycin should be clinically and bacteriologically effective in at least 80% of the cases
- type I error 0.05, type II error 20%

a sample size of 32 in each arm of therapy has been calculated using the above equation, we assume that 35 patients in each group will be adequate. According with previous studies on shigellosis (6,12,13), an estimate of 10-20% of negatives cases should be expected.

B.2 Patient selection criteria

* Age: 18-60 years
* Sex: only males. For cultural reasons it is difficult to take rectal swabs from females in Bangladesh and also it is difficult to keep them in the hospital for full 6 days
* Duration of diarrhoea before attending the hospital ≤ 72 hours
* Stool characteristics: Frank bloody, or bloody mucoid on inspection

B.3 Exclusion criteria

* Patients who do not give written consent to participate in the study
* Prior therapy with an antimicrobial agent active against *Shigella*, such as nalidixic acid, pivmecillinam or ciprofloxacin
* Demonstration of erythro-phagocytic trophozoites of *Entamoeba histolytica* on stool microscopic examination
* Coexistence of any other infectious diseases that may require antimicrobial agents in addition to the drug under study, such as pneumonia, meningitis, septicemia, etc
* Patients with known history of allergy to macrolides or quinolones

* Patients who were initially enrolled into the study, but subsequently both of their admission stool and rectal swab cultures fail to grow *Shigella*
* Females

B.4 Requirement of informed consent

Written informed consent will be required from the patients

B.5 Study site

Patients will be admitted into the Clinical Study ward of the CRSC. We will use the facilities existing in the centre for this research.

B.6 Screening of the patients for study

The screening will began after 06:00 hours in the morning and continue until 15:30 hours to permit history taking, physical examination, stool microscopic examination, and first sampling of blood for serum concentration of the drugs before 17:00 hours.

B.7 Clinical evaluation and laboratory procedures

Patients will be hospitalized for six days after the initiation of therapy, and will be requested to return 7 days after discharge. The following will be the schedule for the routine clinical and microbiological evaluation (annexure A):

a. History, physical examination and weight: On admission and daily thereafter during the whole study period, and at follow-up 7 days after discharge

b. Determination of vital signs: Pulse/heart rate, oral temperature, respiratory rate, and blood pressure every 6 hours throughout the study period

c. Enumeration and characterization of stools: Daily, throughout the study period. Patients will be required to collect individual stool irrespective of volume onto plastic lined, non-absorbable diapers. This will enable characterization of individual stools, and determination of stool frequency by counting the number of diapers. The staff member of the study ward are accustomed with this procedure as was used in 2 previous clinical trials (12,13) and is currently used in a study with an oral third generation cephalosporine in shigellosis

d. Rectal swab cultures for identification of *Shigella*: Will be performed on admission (prior to start of study drugs), on all subsequent days of hospitalization, and at follow-up visit 7 days after discharge

e. Stool culture for identification of *Shigella* and *Salmonella*: Will be done on admission before starting study drugs and on study day-3

f. Stool microscopic examination: On admission, on day 3 and on day 5

g. *In vitro* susceptibility testing of the admission *Shigella* isolates to azithromycin, ampicillin, cotrimoxazole, nalidixic acid, pivmecillinam and ciprofloxacin by Kirby-Bauer method. Only our lab staff officer, not involved in the management of the patients, will know about the sensitivity pattern of the *Shigella* isolates during the study. She will keep the confidentiality of these results until the end of the trial, when the codes will be broken.

h. Determination of the minimum inhibitory concentration (MIC) of the two study drugs to all the *Shigella* isolates using standard techniques at the end of the study period

i. Complete blood count, platelet count, serum electrolytes (Na^+, Cl^-, K^+ and HCO_3^-), serum total protein, and serum creatinine will be done on admission before initiation of study drugs. The rationale is to have a baseline laboratorial information of the patients. This tests may be performed on any other day if considered necessary for the management of the patient

j. Serum peak concentrations of study drugs at 90 minutes and 3 hours after the first dose, and at 1 minute before the subsequent dose of the second study day. Serum samples will be stored at -70°C until be tested; at the end of the study period and outside Bangladesh

k. Stool concentrations of the study drugs: to be determined from an aliquot of first stool of the study day collected on study day 1, 3 and 5. Stool samples will be stored at -70°C

until be tested; at the end of the study period and outside Bangladesh

l. The cultures, haematological and biochemical examinations will be done using routine methods available in our laboratories.

m. Urinalysis: Will be performed only if indicated for the management of the patient

n. Radiological studies: Will be done only when indicated for management of the patient

B.8 Treatment regimens

Patients will be randomized in equal numbers to receive either: azithromycin 500 mg in a single dose at the beginning of the study and 250 mg once a day thereafter for another 4 days, or ciprofloxacin 500 mg twice daily for 5 days using a double-dummy technique. Azithromycin comes in capsules of 250 mg and Ciprofloxacin comes in tablets of 500 mg. The procedure for the double-dummy is as follows:

- first study day:
  - first dose: 2 capsules + 1 tablet
  - second dose: 1 tablet
- subsequent study days (2-5):
  - first dose: 1 capsule + 1 tablet
  - second dose: 1 tablet

Each dose is giving 12 hourly. In case of receiving azithromycin: the capsules will be the active drug, and the tablets the placebo,

with the same presentation than that of ciprofloxacin. In case of receiving ciprofloxacin: the tablets will be the active drug, and the capsules the placebo, with the same presentation than that of azithromycin. Each patient will receive 10 tablets and 6 capsules irrespective of the active drug assigned.

B.9 Concomitant therapy

No other antimicrobial agents will be permitted during the study. Patients who require such therapy from the beginning will not be enrolled. Those patients who will develop other diseases that require antimicrobial agents or other drugs after enrolment in the study will be withdrawn from the study without opening the codes, and will be treated with pivmecillinam. The reason for exclude patients with concomitant infections or other process that require antimicrobial agents or other drugs is that it is difficult to properly evaluate the clinical response and side effects of the treatments when 2 diseases coexist and different drugs are given. For example, concomitant infections or other process may produce fever, diarrhoea or other gastrointestinal symptoms that will confuse the proper evaluation of those due to shigellosis. Otherwise, not knowing what is the drug the patient is receiving it is not possible to prevent possible interactions among the study drug and other medications.

Patients who have fever with an oral temperature $\geq 38.5^{0}C$ will receive oral acetaminophen as concomitant therapy. Such cases will be taken into account during analysis.

B.10 Diets

The patients in this study will receive the usual diet of the CRSC

B.11 Rehydration

Severe dehydration among adult patients with shigellosis is uncommon. The patients will receive rice ORS for rehydration and maintenance of hydration. If oral rehydration it is not possible, adequate intravenous solutions will be administered. Dehydration status on admission, proportion of patients rehydrated with oral and intravenous solutions, and the amount of such fluids required will be registered. The patients will receive the study drug after being complete rehydrated. The amount of fluids that they will require, if any, after receiving the study drug will also be registered and analyzed.

B.12 Randomization

Patients will be assigned a study number once enrolled. Study numbers will be assigned consecutively, each study number will have previously randomly assigned to one of the study medication. Randomization will be done using a block randomization method with a variable-length block size.

B.13 Evaluation of outcome

Primary outcomes:

a. Bacteriologic cure

b. Clinical cure

Secondary outcomes:

a. Adverse events: Incidence, nature and severity of them

b. Daily and total stool frequency

c. Stool characteristics: Time to first watery, soft and formed stools, last bloody-mucoid, watery and soft stools

d. Presence of blood and mucus in stools: Time to last blood, and last mucus in stools

e. Fever: Time to last temperature >37.8°C

f. Straining/tenesmus: Presence of straining/tenesmus on every study day, and time to last straining/tenesmus

g. Abdominal pain and tenderness: Presence of pain/tenderness, and time to last abdominal pain and tenderness

h. Number of WBC and RBCs in stool on microscopic examination on study day-3 and on study day-5.

i. Number of cases with negative culture for *Shigella* on each study days

C. DEFINITIONS

C.1 Bacteriologic cure

Bacteriologic cure will occur if *Shigella* can not be isolated from a stool or rectal swab sample on study day-3 and on any subsequent study days.

C.2 Bacteriologic relapse

If stool/rectal swab culture after being negative for *Shigella* on two consecutive days become positive again.

C.3 Reinfection

Infection due to a different strain of *Shigella* than the strain identified from the beginning of the study.

C.4 Clinical cure

Will be evaluated on the basis of the clinical status on day 5. We will consider as clinical cure: patients under the categories; ***Resolution of illness*** or ***Marked improvement***, and clinical failure: patients under the categories ***Slight improvement*** or *Failure*. The definitions of the categories are as follows:

Resolution of illness

- No frank dysentery stool on naked eye examination
- No watery stool
- ≤ 3 stools within 24 hours
- Afebrile

Marked Improvement

- No frank dysenteric stool on naked eye examination
- ≤ 1 watery stool
- ≤ 6 stools within 24 hours
- Afebrile

Slight improvement

- Up to 1 dysenteric stool on naked eye examination
- ≤ 3 watery stools
- ≤ 9 total stools
- Afebrile

Failure

- > 1 dysenteric stools on naked eye examination, or
- > 3 watery stools, or
- > 9 total stools, or
- Febrile

Clinical failures will also be evaluated on study day 3. If no improvement of the patient's condition is observed, i.e.; if the patient remains febrile, continues to have the same stool characteristics as on admission, continues to have frank blood in stools, stool frequency is either not decreased or increased;

patients continue to have moderate or severe abdominal pain, and/or tenderness on light palpation; patients found to be toxic etc, will be considered to have clinical failure and they will be analyzed together with the clinical failures as described above.

C.5 Fever

Oral temperature of > 37.8°C.

C.6 Frank dysentery stool

Stool that contains only mucus and blood; no faecal material.

C.7 Watery stool

That can be poured easily from one container to another like water, either without, or with insignificant adherence to the surface of the container.

C.8 Soft stool

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

C.9 Formed stool

That retains its shape

C.10 Withdrawal from study

There are two major reasons for withdrawal:

- For failure to respond: Will be determined at the end of study day-3, as has been described above. Such patients will have

their study drugs discontinued. Their drug codes will not be opened, and they will be treated with pivmecillinam for 5 days. Such cases will not be replaced by fresh cases.

- For major adverse effects: Such cases will have their codes opened, and they will be treated with pivmecillinam. Such cases too, will not be replaced by fresh cases.

C.11 Handling of Treatment failures

If the study treatment is judged to have failed by either clinical or bacteriologic criteria, the study code will not be opened and the patients will be treated with pivmecillinam. The development at any time during the study of an adverse events (skin rash, anaphylaxis, etc.) that is possibly or probable related to drug therapy, will be an indication for stopping of the study drug and starting an alternative drug, pivmecillinam, after opening the codes. Failures rates will be compared in both groups during analysis.

C.11 Statistical Methods

Difference in proportions will be evaluated using Chi square, or Fisher's exact test when appropriate. Difference in means will be evaluated using Student's t test if the data is normally distributed or Mann Whitney U test if the data is not normally distributed. In more detail:

(a) We will use Chi square or Fisher's exact test (if the expected number in some cell is less than 5) for comparison of the next proportions by study group:

- admission characteristics: frequency of; fever, some degree of dehydration, tenesmus, abdominal pain, *Shigella* strains isolated
- post randomization characteristics: clinical cure, bacteriological cure, presence of: strain/tenesmus, abdominal pain and tenderness on every study day, frequency of cases with negative culture for *Shigella* on each study day, and frequency of adverse events.

b) We will use student's t test if the data is normally distributed, or Mann Whitney U test if otherwise the data is non normally distributed to compare the following means by study group:

- admission characteristics: age, duration of illness and number of stools passed previous to admission
- post randomization characteristics: daily and total stool frequency, time to last; fever, bloody/mucoid stool, abdominal pain, tenderness, strain-tenesmus and time to first; soft and formed stool, and required volume for rehydration.

Statpac Gold will be used for data entry and both Statpac Gold and SPSS-PC+ will be used for data analysis.

D. SIGNIFICANCE

Azithromycin has better pharmacological properties than other intracellular drugs and has been shown to have acceptable *in vitro* activity against *Shigella* strains at the CRSC of ICDDR,B. If azithromycin shows good *in vivo* efficacy would increase the current available alternatives against shigellosis, with the advantages of its potential use in children, once a day schemes, and low frequency of side effects.

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SECTION IV- STUDY BUDGET

1.	**PERSONNEL**		US$	40,200
1.1	**Charge-in**			
	Dr. Carlos Seas	50% x 24 m		14,000
	Dr. Mohammed A. Salam	25% x 24 m		7,000
	Dr. Wasif A. Khan	25% x 24 m		4,500
	Dr. Mahbubur Rahman	15% x 24 m		4,200
	Mr. Humayun Kabir	25% x 24 m		2,500
	TOTAL			32,200
1.2	**New Recruitment**			
	Trainee Physician	100% x 2 x 24 m		8,000
	TOTAL			8,000
2.	**PATIENT HOSPITALIZATION**			14,400
2.1	70 study patients x 6 days x US$ 30/d			12,600
2.2	20 study patients x 3 days x US$ 30/d			1,800
	TOTAL			14,400
3.	**CLINICAL PATHOLOGY INVESTIGATIONS**			1,175
3.1	Stool microscopy			
	70 study patients x 3 test/pt x US$ 1.65/test			347
	20 culture negative patients x 1 test/pt x US$ 1.65			33

3.2	Urine analysis	
	20 patients x 1 test/pt x US$ 4.4/test	88
3.3	Peripheral blood T/C, D/C, Hct%	
	70 patients x 1 tests/pt x US$ 3.85/test	270
	20 culture negative patients x 1test/pt x US$ 3.85	77
3.4	Peripheral blood platelet count	
	70 patients x 1 test/pt x US$ 4/test	280
	20 culture negative patients x 1 test/pt x US$ 4	80
	TOTAL	1,175
4.	BIOCHEMISTRY INVESTIGATIONS	900
4.1	Serum electrolytes, protein, and creatinine	
	70 patients x 1 tests/pt x US$ 10/test	700
	20 culture negative pts x 1 test/pt x US$10	200
	TOTAL	900
5.	MICROBIOLOGICAL TESTS	8,361
5.1	Rectal swab culture for Shigella and Salmonella	
	70 study pts x 8 tests/pt x US$ 6/test	3,360
	20 culture negative pts x 2 tests/pt x US$ 6	240
5.2	Stool culture for Shigella and Salmonella	
	70 study pts x 2 tests/pt x US$ 6/test	840
	20 culture negative pts x 1 test/pt x US$ 6	120

5.3	Antibiotic susceptibility by Kirby-Bauer	
	70 patients x 1 tests/pt x US$ 4.58/test	321
5.4	Stock culture and retrieval	
	70 patients x 2 stocks/pt x US$ 2.18	305
5.5	Determination of MIC (broth dilution)	
	70 isolates x 2 antibiotic x US$ 10	1,400
5.6	Determination of MIC in serum	
	70 isolates x 2 antibiotic x US$ 10	1,400
5.6	Blood culture and sensitivity	
	25 total patients x 1 test/pt x US$ 15	375
	TOTAL	8,361
6.	**PHARMACOKINETICS**	8,400
6.1	Serum level of antibiotics	
	70 study patients x 3 samples/pt x US$ 20	4,200
6.2	Stool levels of antibiotics	
	70 study patients x 3 tests/pt x US$ 20	4,200
	TOTAL	8,400
7.	**FOLLOW-UP TRANSPORT COST REIMBURSEMENT X 70 PTS**	400
8.	**OFFICE SUPPLIES**	1,000
9.	**HOSPITAL SUPPLIES**	500
10.	**LABORATORY SUPPLIES**	500
11.	**NON-STOCK SUPPLIES**	1,500

12.	XEROX/MIMEOGRAPH	200
13.	MEDICAL ILLUSTRATION	250
14.	LOCAL TRAVEL	500
15.	TRANSPORTATION	1,000
16.	RENT/COMM/UTILITY	1,500
17.	STAFF CLINIC SUBSIDY	1,000
18.	REPRINT	500
19.	MAINTENANCE	250
20.	BIOENGINEERING	200
21.	TRAVEL	5,000

NET OPERATING COST: US$ 87,736

OVERHEAD COST (31%): US$ 27,199

TOTAL PROJECT COST: US$ 114,934

RESISTANT PATTERN OF *SHIGELLA* ISOLATES CRSC-ICDDR,B AT DHAKA. 1992