ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 28th June 1984

Principal Investigator Dr. A.K.Mitra

Trainee Investigator (if any) 20

Application No. 84-030

Supporting Agency (if Non-ICDDR,B)

Title of Study Double-blind controlled Clinical Trial with BIOFLORIN (Streptococcus ...cium) in management of Acute Diarrhoea in Bangladesh.

Project status:
- (✓) New Study
- ( ) Continuation with change
- ( ) No change (do not fill out rest of form)

Circle 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 Ettee. for review.

3 JUL 1984

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

Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

1. Title : Double-blind Controlled Clinical Trial with BIOFLORIN (Streptococcus faecium) in Management of Acute Diarrhoea in Bangladesh.

2. Principal Investigator : Dr. A. K. Mitra

   Co-Investigators : Drs. M.Yunus, K. Zaman M.Giashuddin, Mr. B.Hossain.

   Consultant/Adviser : Dr. W.B. Greenough III

3. Starting Date : September '84

4. Completion Date : August '85

5. Total Incremental Cost : US $ 6022

6. Scientific Program Head :

This protocol has been approved by the Pathogenesis Therapy Working Group.

Signature of Scientific Program Head: _____

Date: 25-6-84

7. Abstract Summary

The widespread use of antibiotics has some negative consequences; the most serious one is the increased antibiotic resistance of bacteria. In addition some oral antibiotics may destroy the intestinal flora, and cause diarrhea by themselves. These are the reasons why alternatives to antibiotics have to be examined in order to provide a useful first choice treatment of enteritis.

This study of biological approach will try to document a valid alternative to antibiotics in enteritis, rather than a supplement to them.

Hospitalised patients having history of diarrhoea not exceeding 48 hrs will be taken into study. Patients having watery diarrhoea, divided into four age groups, each with 50 cases, will be selected by a systematic sampling. Another 50 cases of adults only, having bloody mucoid diarrhoea, will be taken into this study. The study will be conducted over one-year-period.

Thorough physical examination will be done immediately after admission by a physician collaborator. Routine stool microscopy, complete blood count and urine analysis will be done on admission and repeated as necessary. Fresh stool specimen will be collected daily from each patient for culture of vibrios, shigella, salmonella, campylobactor, ETEC and ROTA virus. It will be continued until culture becomes negative for three consequtive days, but not exceeding total five days from initiation of treatment.

Treatment will be given with the study drug 'Bioflorin' and a placebo which will be dispensed as capsules for patients above 9 yrs and as syrup for those under 9 yrs and supplied in 3 dose schedule. This will be a double blind controlled clinical trial.

Clinical and bacteriological progress will be recorded each day for each patient. Any complication will be carefully recorded.

8. Reviews:

a. Ethical Review Committee

b. Research Review Committee

c. Director

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

a) To document the effectiveness of Bioflorin against different etiologic agents of diarrhea in different age group.

2. Background:

Acute diarrhoeal diseases have long been recognised as a major public health problem. Nearly 500 million children suffer from acute diarrhoea annually world wide[1]. Diarrhoeal diseases are a major cause of mortality and morbidity in developing countries[2-4]. In Guatemala, India and Indonesia careful prospective field studies were done to accurately determine the annual incidence of paediatric diarrhoea. They showed a mean of one to two attacks per child per year, during the first three years of life. In Bangladesh, during 1966 diarrhoea ranged from 20% to 45% of deaths from all causes[2]. More than two episodes of serious diarrhoea each year are the rule for children under the age of four [5].

Having such a problem, knowledge of the cause of disease is crucial to its control, both clinical and epidemiological. Despite persistent attention to aetiology, a specific agent has been found in only 10-25% of most investigated series of patients. Recent recognition of additional viral agents and of toxigenic E.coli extends our ability to assign cause, in one study to as many as 78% of patients (Guerrant et.al., 1975).

Since October 1979, a surveillance system is operating at ICDDR,B Dhaka hospital. A recognised pathogenic organism was identified for 66% of patients. Enterotoxigenic E.Coli was the most common enteropathogen detected in all age groups (20%), followed by rotavirus (19%), Campylobacter jejuni (14%), and Shigella (12%). Watery diarrhoea was the most common chief complaint (65%) and was significantly more frequent in patients with V.cholerae O:1 (91%), ETEC (78%), rotavirus (77%) and C . jejuni (71%)[6]. This finding is in agreement with other studies in both developed and developing countries[7-10].

Several strategies have been prospesed to attack this global problem of diarrhoea. These include role of rural hospital,[11] oral rehydration therapy,[12-15] sanitary interventions,[16] health education of hand washing[17] and immunization.[18]

Besides fluid replacement, antibiotics are given in most cases of Shigellosis and Cholera. In Dhaka hospital, during the first three months of surveillance in 1979, 96% of patients were treated with antibiotics, primarily ampicillin and tetracycline. Due to improved guidelines for antibiotic use, it declined to 43% between March-November,1980. Survey of drug sales from local pharmacies in Matlab rural area of Bangladesh shows that in this area antibiotics are rarely appropriately prescribed and are quite often inappropriately dispensed.[19]

Because of indiscriminate use, there is a growing number of resistant cholera and Shigella species in our country. In December 1979, strains of V.cholera O:1 resistant to tetracycline as well as to ampicillin, Kenamycin, Streptomycin, and trimethoprim-sulfomethoxazole were recovered from patients with cholera at the Matlab field hospital.[20] Sh.flexneri strains tested were found to be highly resistant to tetracycline.[21] Ampicillin resistant Shiga bacillus was isolated in Bangladesh in 1973.[22] Most recently Trimethoprim sulfamethoxazole resistant Shigella dysenterie type I was found in 83% in 1983 in contrast to 5% in 1979 in Matlab rural treatment Centre.[23]

The widespread use of antibiotics brings about increased antibiotic resistance of bacteria.[24] In addition, some oral antibiotics may destroy the intestinal flora, and cause diarrhoea by themselves.[25] These are the reasons why alternatives to antibiotics have to be examined in order to provide a useful first-choice treatment of enteritis.

The results of recent studies suggest that the SF68 strain of streptococcus faecium may be useful for the treatment of diarrhoeal disorders both in man[26] and in animals.[27] Several controlled clinical observations proved that SF68 is very effective in treatment of acute enteritis in paediatric patients[28] and also in adults.[29] The elimination of diarrhoea and related clinical disturbances was significantly more rapid and complete in the S.faecium treated group with an earlier normalization of Stool cultures.

The biological approach appears to be particularly appropriate, because it is based on the use of micro-organisms which are able to normalize the intestinal microecology by their physiologic activities. Some microorganisms have already been used successfully as a supplement to antibiotic therapy, mainly to avoid the negative intestinal side-effects which occur in antibiotic treatment.[26]

Streptococcus faecium SF68 was first isolated in Sweden and is now patented in Switzerland and some other countries. Bioflorin countains 75 millions SF68 strain of streptococcus faecium in lyophilized form. This type of enterococcus is characterized by very short generation time, short lag-phase, production of lactic acid, lack of pathogenicity, high tolerability and no side-effects[26]. SF68 was suspended in solutions of low PH. These in vitro tests showed no loss of viability of SF68 after incubation at P^H 2.5 for 30 minutes.[26] The relative insensitivity of SF68 to low P^H is of importance, as orally administered organism have to pass through the stomach. These lactic acid producing bacteria are resistant to multiple chemo-antibiotics. These resistances are non-transferable and don't seem to be plasmid-mediated.

The colonizing ability of streptococcus faecium strain SF68 at different [illegible] of the gastrointeotinal tract was assessed. It suggested that the SF68 rapidly and transitorily grows in the intestinal human without a strict connection with the mucosa. The growth of SF appeared quite high at the different intestinal tract levels if compared to the counts of both aerobic and anaerobic germs.[30] The rapid growing of strain SF68 especially in the colon obviously increases the aerobes/anaerobes ratio. This modification and the biological properties of this enterococcus could change the activity of the intestinal flora towards a a fermentative metabolism with a reduction of the putrifactive one.[30]

The range of antimicrobial properties of SF68 has been observed. In vitro, this strain can antagonize some enteropathic micro-organisms, such as entero pathogenic E.Coli, Salmonellae, Clostridia and Shigellae[26,31]. In addition, it seems to act against some viruses[26]. The SF68 is believed to act by means of a) competition phenomenon, b) bacteriocins production[26,31], c) restoration of normal intestinal flora[25].

3. Rationale

Indiscriminate use of antibiotics in diarrhoea results in increasing resistance. Sometimes they are the cause of untoward reactions. Antibiotic-induced diarrhea is also reported. This are the reasons why alternative to antibiotics to be sought. Bioflorin, a biological product, will restore normal intestinal flora and is also a well tested agent with perhaps the least side effects in treating diarrhoea. We are proposing to look for its effectiveness in eliminating the symptoms of diarrhoea in patients in our country. This study is consistent with the research component of CDD programme which tries with drug development and management of acute diarrhoea. Similar studies are being done in other recognised hospitals of WHO in different parts of the world. The results will help physicians in looking for an useful alternative to antibiotics.

B. SPECIFIC AIMS:

1. To find out whether treatment with Bioflorin rapidly rids the patients' intestine of organisms causing diarrhea.

2. To see the effectiveness of the drug against different etiological agents from clinical as well as bacteriological evaluation.

C. METHODS AND PROCEDURES:

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) operates a rural-based hospital at Matlab, 45 km South-East of Dhaka.

Both male and female patients, 6 months to 60 yrs of age, consulting at the treatment Centre with history of watery diarrhoea not exceeding 48 hrs and not receiving any antibiotics during this period, are eligible for the study. Watery diarrhoea will be defined as 3 or more bowel movements of loose or liquid stools in 24 hrs. Patients having watery diarrhoea will be divided into four age groups: 6 m - 2 yrs, 3-5 yrs, 6-12 yrs and 13-60 yrs. Each group having 50 cases and a total of 200 cases will be studied throughout a year.

Seperate registration will be maintained for dysentery patients. Dysentery will be defined as 3 or more bowel movements of bloody-mucoid stools in 24 hrs accompained by fever, lower abdominal pain or tenesmus. Only 50 cases of adult patients, age ranging 13-60 years, of either sex, having dysenteric symptoms, will be incorporated into this study.

Patients will be selected by a systematic sampling of all patients hospitalized. Randomization will be done using the hospital registration number. First case will be selected from the random table using the last two digits of hospital registration number. Consequative three cases (first two cases of watery diarrhoea and following single case of dysentery) will be taken into study in each day between 8:00 AM and 4:00 PM. If any of these three cases has any associated complications or anyhow does not fit into criteria for inclusion, the next case will be taken into study.

Prior consent will be taken from each patient. The parents of the children patients will be informed of the study. If they agree to participate will be included in the study.

Criteria for inclusion:

a) Pre-hospitalization illness not exceeding 48 hrs.

b) No history of antibiotic use during present illness.

c) Age of patients not beyond 6 months and 60 years in case of watery diarrhoea and not below 13 years in case of dysentery.

d) No associated complications.

Criteria for exclusion:

a) Age beyond 6 months to 60 yrs for watery diarrhoea and children patients below 13 yrs in case of dysentery.

b) Pregnant women

c) Malnourished patients (wt.for age $>3^{0}$).

d) History of antibiotic use during present illness.

e) Another significant disease process requiring specific therapy present from the begining:- eg. Bronchopneumonia, nepritis, seizure, meningitis, toxaemia, septicaemia, amoebiasis and giardiasis.

Clinical Procedures:

Immediately after admission, medical history will be taken by a physician collaborater (Form I). Thorough clinical examination will be done by the physician immediately after admission of the patient and clinical findings will be recorded (Form II).

Before therapy begins, a stool sample will be taken. Amoebiasis and giardiasis cases will be immediately dropped from the present study. A rectal swab for culture and sensitivity, urine for analysis and blood for white cells, differential and platelet counts will be taken for further diagnosis and to find out underlying infection, if any, present at the begining. If any patient develops sudden high temperature or any other allerming symptoms during the course of illness, routine tests will be repeated accordingly.

Weight in kg. will be taken on admission and every morning during hospitalization. Strict intake and output of fluid will be measured on 8 hourly schedule. Exact quantity of stool, urine and vomitus will be measured on volumatric method. Rectal temperature and vital signs will be taken on admission and every 4 hours. Hydration status will be assessed initially by the trained paramedics as routine procedure. Intravenous fluid and electrolyte therapy, oral rehydration solution (sucrose-based WHO solution) and diet will be prescribed as indicated and in conformity with our usual practice.

No extra medications will be given. Drugs that might affect the patients' clinical course, such as opiates and kaolin-pectin mixtures will not be used.

A daily record will be kept for each patient noting the stool characteristics (volume, consistency, presence of blood and mucus), fever, presence of abdominal pain, and hydration status. Physicians during their routine patient visits will find out any clinical abnormalities or complications present at the bigining or developed during the course of illness. Whenever a septic condition is suspected the patient will be immediately dropped from the study and a blood sample for culture will be taken for conformity. A patient can be removed the study if he/she develops any serious complication (toxaemia, septicaemia, seizure, uraemia, intestinal obstruction etc.) or is pursuing a progressively worsening course. When such situation arise, the study drug will be stopped and appropriate therapy will be started according to clinician's best judgement.

Laboratory Procedures:

Daily stool will be collected from each sample patient for culture. If stool samples are not available, rectal swab will be taken. All specimens will be plated immediately and incubated. Culture specimens will be processed for pathogenic vibrios, Shigella and Salmonella using standard methods. Vibrio like colonies identified on trypticase-tellurite-gelatin plates will be further characterized in terms of biochemical, serotypic and salt tolerance properties and will be classified as Vibrio cholerae group O:1, organisms.[32]

From each culture, 10 lactose-positive colonies with typical E. coli morphology will be saved from Mackonkey's agar plates and will be pooled on nutrient agar slants. These pools will be tested with the chinese hamster overy cell assay for heat-labile toxin (LT) and with the infant mouse a say for heat-stable toxin (ST).[33] A portion of each specimen will be incubated into a Campy-BAP media (brucella agar base, with 5% sheep blood and the following antimicrobial concentrations per litre: vancomycin 10 mg, trimethoprim 5 mg, polymyxin B 2500 I.U., amphotericin B 2 mg and cephalothin 15 mg) for isolation of Campylobacter. All plates will be incubated in candle jars at 42°C for 48 hrs. Identification of organisms Campylobacter jejuni will be done according to standard criteria.[34]
A second rectal swab will be taken from each patient and will be refrigerated in phosphate-buffered saline for subsequent testing by enzyme-linked immunosorbent assay (ELISA) for Rotavirus antigen.[35]

Stool cultures will be continued during hospitalization until these are negative for three consequtive days. When it continues to be positive after 5 days of treatment the case will be dropped and no further culture will be done.

Daily stool microscopy will be performed on three consequtive days to see vegetative ameba and giardia and to determine when the stool becomes free from leukocytes (10 leukocytes /hpf) and RBC in case of dysentery.

Routine complete blood count and urine analysis will be dome on admission and repeated as necessary.

Therapy:

Patients of both the groups of watery diarrhoea and bloody-mucoid diarrhoea will recieve either the study drug 'Bioflorin' or 'Placebo' which will be dispensed in double blind form and allocated as scheduled after randomizstion.

Drug and placebo will be prepared as capsule and syrup. Patients between 6 months to 9 years will recieve syrup and the adults above 9 years will recieve capsule. Therapy will be only in oral route in three-dose schedule for three days.

One capsule of the study drug 'Bioflorin' will contain 75 millions of a pure lyophilized culture of SF 68 strain of Streptococcus faecium. The test syrup preparation for children will contain 3.75×10^7 bacterium in each ml.

The placebo capsule and syrup will contain some bland preparation not affecting the illness and will be identical in appearence with the study drug.

Clinical evaluation:

A line list of physical findings will be recorded each day for each patient. Characters of diarrhoea and associated symptoms (appetite, vomiting, abdominal pain or tenesmus) will also be looked for.

General appearence of patient, hydration status, vital signs, lungs findings (creps or rhonchi and breath sound), presence or absence of abdominal distention and bowel sound, and neurological features (conciousness, seizure etc.) are main clinical variables which will be checked daily by the P.I. Other findings will be recorded according

to Form II everyday.

Stool characters describing no. of motions in 8 hours, amount of stool during that period, its consistency, colour, and presence or absence of mucus or blood will be recorded carefully. The time required for 50% reduction in frequency and quantity of loose stool will be assessed. Total clinical cure will be considered by consequative three normal stools.

Presence of diarrhoea after five days of initiation of treatment will definitely be considered as clinical failure. Again, gradual detorioration of general condition and thereby removal of the patient from the study will be treated as clinical failure.

Bacteriological evaluation:

Daily bacteriological data will be checked to see what pathogen is most actively inhibited. Isolation of Shigella, Salmonella, or Cholera from stools more than three days after initiation of therapy and isolation of ETEC, Rota or Campylobacter after five days will be arbitrarily considered to be bacteriological failure.

D. SIGNIFICANCE:

This study will generate newer approach in diarrhoea management if the drug is found effective. It is expected that it might reduce drug-induced complications as well because the product is a lactic acid producing bacterium, the normal inhabitant of human gut. The findings of this study will be of great interest to health personnel in Bangladesh as well as in other countries.

E. FACILITIES REQUIRED:

1. No new office space is required.
2. Personnel - Supervisor, Study Physician - The Investigators themselves supervise and perform the job of study physician - part-time.
3. No new laboratory space is needed.
4. Hospital support - No new facility required. The study will utilize the current facilities that are presently offered to patients by ICDDR,B.
5. Animal resource - for ST and LT determinations, approximately 50-100 mice will be needed per month.
6. Logistid support - none
7. Major items or equipments - No major item or equipment will be needed except the reagents for ELISA assay and mice for ST and LT determinations.
8. Other special requirements - culture materials, medicine, computer tapes and stationery will be needed.

F. COLLABORATIVE ARRANGEMENTS:

The study will be carried out in collaboration with WHO, Geneva.

Data Analysis:

A line list of physical findings and bacteriological data will be recorted each day after admission. Watery diarrhoea and dysentery cases will be analysed seperately.

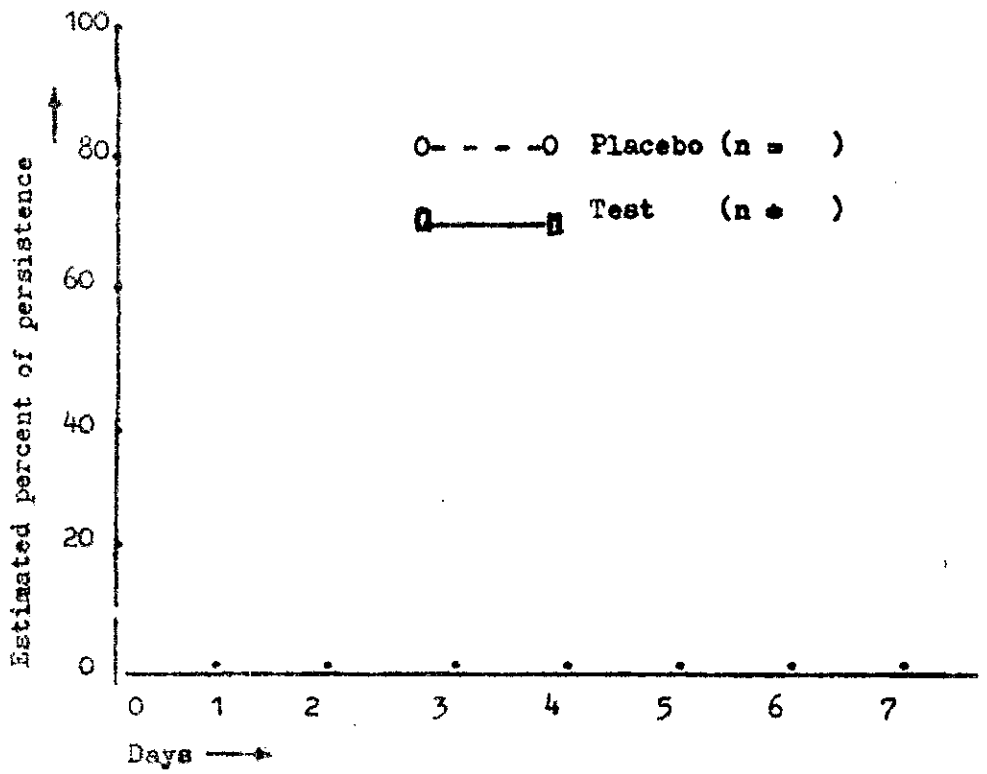
1) Test group and Placebo group will be compared for number of patients, sex, age and clinical details for homogenicity:-

	Placebo group	Test group	Statistical analysis
No. of cases			
Sex M			
F			
Age 6 m - 2 yrs.			
3 - 5 yrs.			
6 - 12 yrs.			
13 - 60 yrs.			
Clinical remarks at the start of treatment			
Duration of diarrhoea (days)			
Bowel movement (average no. a day)			
Mucus in faeces (no. of cases)			
Blood in faeces (no. of cases)			
Presence of fever (no. of cases)			
Stool cultures (no. of cases)			
Duration of treatment (days)			
Mean			
Range			

2) The number of cured cases at the end of treatment in both groups will be analysed.

3) The number of failure cases in both groups will be compared in relation to microorganisms isolated.

4) Estimated percentage of persistence of diarrhoea according to time and type of treatment will be compared.



5) Persistence frequencies of the diarrhoea in the compared groups according to the age of patients will be analysed.

	Placebo group					Test group				
Persistence days	1	2	3	4	>4	1	2	3	4	>4
Age										
6 m - 2 yrs.										
3 - 5 yrs.										
6 - 12 yrs.										
13 - 60 yrs.										

6) Bacterial strains isolated in the stools of the patients recovered within 48 hrs. will be compared in two groups.

Bacterial strain	Placebo group		Test group	
	n	%	n	%

7) The overall results of stool cultures expressed as a cumulative percentage of negative findings at each time of examination will be analised.

Stool cultures	Placebo group				Test group			
	n	days (%)			n	days (%)		
		2	5	7		2	5	7
Total								

REFERENCES

1. Rohde, J.E., and R.S. Northrup. Taking science where the diarrhea is. In: Acute Diarrhoea in Childhood. Ciba Fndn. Symp.42:339,1976.

2. Chen L.C., Rahman M, Sarder AM. Epidemiology and causes of death among children in a rural area of Bangladesh. Int J Epidemiol 1980; 9:25-33.

3. Black RE, Merson MH, Rahman ASMM, et al. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. J Infect Dis 1980; 142:660-4.

4. Walsh JA, Warren KS. An interim strategy for disease control in developing countries. N Engl J Med 1979; 301:967-74.

5. W.B.Greenough III, Oral Replacement Therapy in Diarrhea: Consensus and Controversy. ICDDR,B Scientific Report No. 14.

6. Stool BJ., Glass RI., Huq MI., Khan MU et al., Surveillance of patients attending a diarrhoeal disease hospital in Bangladesh. Br. Med. J. 23 Oct 1982, 285, 1185-1188.

7. Ryder, R.W., Sack, D.A., Kapikian. A.Z., McLanghlin, J.C., Chakraborty, J., et al., Enterotoxigenic Escherichia coli and reovirus-like agent in rural Bangladesh. Lancet 1:659-663, 1976.

8. Anoymous. Rotavirus gastroenteritis (editorial). Br. Med. J. 2:784-785, 1977.

9. Kapikian, A.Z., Kim, H.W., Wyatt, R.G., et al. Human reovirus like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N. Engl. J. Med. 294:966-972, 1976.

10. Hieber, J.P., Shelton, S., Nelson, J.D., Leon J., Mohs, E. Comparison of human rotavirus disease in tropical and temperate settings. Am. J. Dis. Child. 132:853-858, 1978.

11. Rahman ASMM. Experience in treating cholera at a rural hospital. J Pak Med Asso 1969; 19:366-372.

12. Rahman M.M., Aziz K.M.S., Patwari Y., Munshi M.H. Diarrhoeal mortality in two Bangladeshi villages with and without community-based oral rehydration therapy. Lancet October 20,1979 pp. 809-812.

13. Nalin D.R., Cash R.A., Islam R., Molla M., Phillips R.A., Oral maintenance therapy for cholera in adults. Lancet August 17,1968, pp. 370-373.

14. Snyder J.D., Yunus M., Wahed M.A., and Chakraborty J. Home-administered oral therapy for diarrhoea: a laboratory study of safety and efficacy. Royal Society of Tr Med Hyg Vol. 76, No.3, 1982 pp. 329-333.

15. Islam M.R., Greenough W.B., Rahman M.M., Choudhury A.K.A. and Sack D.A. Lanon-gur (common salt and brown sugar) oral rehydration solution in the treatment of diarrhoea in adults. J Tro Med Hyg 1980, 83, 41-45.

16. Azurin JC and Alvero M. Field evaluation of environmental sanitation measures against cholera. Bull WHO 1974; 51:19-26.

17. Khan M.U., Interruption of shigellosis by hand washing. Royal Society Tro Med Hyg Vol. 76, No. 2, 1982.

18. Mosley WH. The role of Immunity in cholera. A review of epidemiological and serological studies. Tex Rep Biol Med 1969; 27:Suppl 1:227-241.

19. Hossain M.M., Glass R.I., Khan M.R. Antibiotic use in a rural community in Bangladesh. Interna J Epidemic Vol 11. no.4 pp. 402-405.

20. Glass R.I., Huq M.I., Lee J.V., et al. Plasmidborne multiple drug resistance in Vibrio cholerae serogroup O1, Biotype El Tor: Evidence for a point-source outbreak in Bangladesh. J Inf Dis Vol. 147, no. 2. Feb 1983. pp 204-209.

21. Mutanda L.N., Kibriya A.K.M.G. and Mansur M.N. Pattern of Shigella flexneri serotypes and drug-resistance in Dhaka. Indian J Med Res 73, Jan 1981, pp 8-12.

22. Rahman M.M., Huq I., Dey C.R., Kibriya A.K.M.G. and Curlin G. Ampicillin-resistant shiga bacillus in Bangladesh. Lancet, March 9, 1974.

23. Zaman K., Yunus M, Baqui A.H., Hossain K.M.B. and Khan M.U. Co-Trimoxazole-resistant shigella dysenteriae type 1. Outbreak in a family in rural Bangladesh. Lancet 1983 Vol 11. 796-7.

24. Garrod, L.P., et al.: Antibiotic and Chemotherapy. 4a Ed. Churchill, Livingstone, 1973.

25. Pictrusko, R.E.: Pharmacology of diarrhoea. Amer. J. Hosp. Pharm. 36:757, 1979.

26. Lewenstein, A., Frigeric, G., and Moroni, M.: Biological properties of SF68, a new approach for the treatment of diarrhoeal disease. Curr. Therap. Res. 26:967-981, 1979.

27. Underdahl, N.R., Torres-Medina, A., Doster, A.R. The effect of S. Faecium C-68 in control of E.Coli-induced diarrhoea in Gnotobiotic pigs. In Press T. Amer. Vet. Med. Ass. 1982.

28. Gaetano Bellomo, Antonio Mangiagi, Luigi Nicastro and Giuliano Frigerio. A controlled double-blind study of SF68 strain as a new biological preparation for the treatment of diarrhoea in Pediatrics. Current Ther. Res. vol. 28, No. 6, Dec., 1980.

29. E.Camarri, A., Belvisi, G., Guidoni, G. Marini, G.Frigerio. A double-blind comparison of two different treatments for acute enteritis in adults. Chemotherapy 27: 466-470 (1981).

30. Bongetta R., Quirino T., Ortisi G. et al. The colonization of Streptococcus faecium in human intestinal tract after oral administration. Estralto dal. Boll. 1st Sieroter. Milanese.60, 5, 1981.

31. Carbone, M., and Fera, M.T.: Microbiological properties of streptococcus faecium SF68 strain and its relationship with other microrganisms. Boll. 1st. Sieroter Milon, In press 1980.

32. Colwell RR. Polyphasic toxonomy of the genus vibrio: numerical toxonomy of vibrio cholerae, Vibrio parahaemolyticus and related Vibrio species. J Bact 104:410-433, 1970.

33. Merson MH, Sack RB, Kibriya AKMG, Mahmood A. et al. The use of colony pools in diagnosis of enterotoxigenic, Escherichia coli diarrhoea. J Clin Microbiol. 9:493-497, 1979.

34. Blaser MJ, Campylobacter fetus SSP. jejuni: A laboratory manual. ICDDR,B special publication no. 7, June 1980.

35. Huq MI, Sack DA, Black RE. Working manual for assay of E.Coli enterotoxin and ELISA Assay for Rotavirus antigen. ICDDR,B special publication no. 3, April 1979.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% Time used	Salary Taka	Dollar
Dr. A.K. Mitra	Prin.Investigator	20%	7,640	
Dr. M. Yunus	Co-Investigator	10%	19,600	
Dr. K. Zaman	Co-Investigaror	10%	4,580	
Dr. M.Giashuddin	Co-Investigator	10%	3,820	
Mr.B.Hossain	Co-Investigator	10%	3,000	
To be named	1 Clinical Path.Techn.	10%	1,860	
To be named	1 Programmer	5%	3,000	
		Sub-Total Tk:	43,500	

2. SUPPLIES AND MATERIALS

Item	Unit Cost	Taka	Dollar
Stool culture	250x5x30	37,500	
Rotavirus assay	250x3x15	11,250	
E.Coli toxin (ST,LT)	250x3x14	10,500	
Campylobacter	250x3x35	26,250	
Stool microscopy	250x3x10	7,500	
Routine blood count	250x15	3,750	
Urine analysis	250x10	2,500	
Blood culture	10x40	400	
Stationary, form etc.		10,500	
Medicine		6,500	
Miscellaneous		1,000	
	Sub-Total Tk.	1,17,650	

3. EQUIPMENTS - None

4. PATIENT HOSPITALIZATION For this study, no special patient hospitalization is needed.

5. OUTPATIENT CARE - None

6. ICDDR,B TRANSPORT - None

		Taka	Dollar
7.	TRAVEL AND TRANSPORTATION OF PERSONS	-	
	Local Travel I	5,000	
	International Travel	-	
		5,000	
8.	TRANSPORTATION OF THINGS	2,000	
9.	RENT, COMMUNICATION AND UTILITIES -	None	
10.	PRINTING AND PUBLICATION		
	Forms, Xerox	5,000	
	Special reproduction	5,000	
	Publication	-	300
	Sub-Total:	10,000	300
11.	COMPUTER COST		
	Data entry @Tk.20.00/hr. 15x20x12	3,600	
	Terminal time @Tk.20.00/hr. 10x20x12	2,400	
	Computer time @Tk.20.00/hr. 10x20x12	2,400	
	Sub-Total:-	8,400	
12.	OTHER CONTRACTUAL SERVICES	None	
13.	CONSTRUCTION, RENOVATION, ALTERATION	None	

BUDGET SUMMARY

	Category	Taka	Dollar
1.	Personnel Services	43,500	-
2.	Supplies and Materials	1,17,650	-
3.	Equipments	-	-
4.	Patients Hospitalization	-	-
5.	Outpatient Care	-	-
6.	ICDDR,B Transport	-	-
7.	Travel and Transportation of Persons	5,000	-
8.	Transportation of Things	2,000	-
9.	Rent, Communication and Utilities	-	-
10.	Printing and Publication	10,000	300
11.	Computer Cost	8,400	-
12.	Other Contractual Services	-	-
13.	Construction, Renovation, Alteration	-	-
	Total:-	1,86,550	300

Incremental cost other than personnel = 1,43,050

Conversion Rate US$ 1.00 =Tk. 25.00

Grand Total US$ 6022

MEDICAL HISTORY — FORM 1

	Column
1. Census no. _____ (Village) _____ (Family) _____ (Indiv.)	1-9
2. Age : _____ (Year) _____ (Month)	10-13
3. Sex : M - 1, F-2	14
4. Date of admission : _____ (Day) _____ (Month) _____ (Year)	15-20
5. Duration of diarrhoea : _____ (Days) Not known 999	21-23
6. No.of steel in past 24 hrs.	24-25
7. Consistency: Loose-1, Liquid-2, Watery-3	26
8. Does steel contain blood and/or mucus None-0, mucus-1, blood-2, both-3	27
9. Do you have : Abdominal pain : N=0, Y=1, NK=2	28
Vomiting : N=0, Preceeding diarrhoea-1 Following diarrhoea-2	29
Loss of appetitie : N=0, Y=1, NK=2	30
Fever : N=0, Y=1, NK=2	31
10. Before coming to hospital, did you	
a) Take any medication? N=0, Antibiotic-1 Other (specify) =2, Both=3	32
b) received ORS? N=0, Packet=1, Salt+Sugar=2, Lahsi+Gur=3, Barley+Salt=4, Barely=5, Rice water=6, Rice+Salt=7	33
11. Have you had any of the following in the past month ? N=0, Y=1, NK=2	
a) Measles	34
b) Pneumonia	35
c) Otitis media	36
d) Night-blindness	37
e) Convulsion in last 24 hrs	38
f) Other (Specify)	39
12. Anthropometry -	
a) Admission wt.(kg)	40-42
b) Discharge wt.	43-45
c) Height(cm.)	46-48

PHYSICAL EXAMINATION FORM II

CENSUS NO._____

Physical findings	On admission	2nd day	3rd day	4th day	5th day

1. Gen. appearance :
   1= normal, 2=distress

2. Hydration :
   1=none, 2=mild
   3= moderate, 4= severe

3. Eyes :
   0=normal, 1=dry spot
   2=xerepthalmia,
   3=conjunctivitis.

4. Ears :
   0= normal, 1=Otitis media

5. Mouth and Oral Cavity:
   0=normal, 1=angular stomatitis,
   2= glossitis, 3=thrush,
   4= 1+2, 5=1+3, 6=2+3,
   7=1+2+3

6. Throat :
   0= normal, 1=tonsillitis

7= Edema:
   0= absent, 1=present

8. Chest:
   0= clear, 1=crops, 2=rhonchi,
   3=1+2.

9. Abdomen :
   0= normal, 1=distended, BS=present
   2=distended, BS=sluggish,
   3=paralytic ileus

10. Liver & Spleen :
    0= normal, 1=liver palpable,
    2=spleen palpable, 3=1+2

PHISICAL EXAMINATION -Contd.

11. Rectal Prolapse :
0=absent, 1=present.

12. Neurelegical :
0=normal, 1=semiconscious,
2=unconscious, 3=convulsion,
4=1+3, 5=2+3.

(For patients dropped from study)

A. Complications after treatment started :

1= Pneumonia
2= Urinary tract infection
3= Septicemia
4= Meningitis
5= Other (specify)
6= Absconded

B. Antibiotics used:

1= Ampicillin
2= Penicillin
3= Gentamycin
4= Chloramphenicol
5= Tetracycline
6= Other(specify)

(Similar record from will be used to see clinical course of complicated patients).

LABORATORY RESULTS FORM III

CENSUS NO._____

Stool Culture: 1st day 2nd day 3rd day 4th day 5th day

1. V. cholera group O:1
   O=N, 1= ..N, 2= OG

2. NAG
   O=N, 1= non O:1
   2= parahaemolyticus,
   3= fluvials,
   4= plesiomonus,
   5= aeromonus

3. Shigella:
   O=N, 1= dysen. type 1
   2= dysen. type II,
   3= dysen. type 3-10,
   4= flex, 5= boydi,
   o= sonnei

4. Salmonella:
   O=N, 1= S. typhi,
   2= other (specify)

5. ETEC:
   O=N, 1= ST, 2= LT, 3=1+2

6. Ca pylobacter:
   O=N, 1=Y

7. Rotavirus:
   O=N, 1=Y

Stool Microscopy: 1st day 2nd day 3rd day

PH : 1 = alkaline, 2=acid

RBC : O=N,1=1-9,2=10-19
3=20-49, 4=50+

FL/hpf : O=N,1=1-9,
2= 10-19,3=20-49,4=50+

Ent. hystolytica:
O=N, 1=Y

Giardia :
O=N, 1=Y

Hook-worm :
O=N, 1=Y

Ascaris :
O=N, 1=Y

Strong.Stercoralies :
O=N, 1=Y

Other (specify) :
O=N, 1=Y

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র উদরাময় চিকিৎসার কার্যকরী পদ্ধতি উদ্ভাবনের জন্য খাওয়ার স্যালাইন ও শিরায় স্যালাইনের সাথে সাথে বিভিন্ন ঔষধ দ্বারা চিকিৎসা উদ্ভাবনের জন্য গবেষণা চালিয়ে যাচ্ছে। বায়োফ্লোরিন দ্বারা বিভিন্ন দেশে গবেষণা করে দেখা গেছে, এই ঔষধ উদরাময় নিরাময়ের ক্ষেত্রে একটি কার্যকরী সংযোজন। উপরন্তু এই ঔষধের পার্শ্বপ্রতিক্রিয়া নেই বলে প্রমাণিত হয়েছে। শিশু ও প্রাপ্তবয়স্ক উভয়ের ক্ষেত্রে পরীক্ষা চালিয়ে এই প্রমাণ পাওয়া গেছে।

আমরা বায়োফ্লোরিন ও প্লাসেবো (ঔষধ-সদৃশ, কিন্তু কোন কার্যক্ষমতা নেই) দিয়ে শিশু ও প্রাপ্তবয়স্ক উভয়ের মধ্যে উদরাময় চিকিৎসার জন্য গবেষণা চালাবো।

এই গবেষণার জন্য আপনার / আপনার শিশুর কাছ থেকে ভর্তির সময় ও প্রতিদিন মল ও রেক্টাল সোয়াব এবং শুধু ভর্তিকালীন মূত্র ও আঙ্গুলের অগ্রভাগ থেকে সামান্য রক্ত নেয়া হবে। আপনার / আপনার শিশুর রোগ নির্ণয়ের জন্য এই ব্যবস্থা প্রয়োজন।

আপনি যদি গবেষণায় অংশগ্রহণে রাজী নাও থাকেন তবুও আপনাকে হাসপাতালের অন্যান্য রোগীর মত চিকিৎসা দেওয়া হবে। এছাড়াও যেকোন অসুবিধা দেখা দিলে কিংবা আপনি চাইলে গবেষণা চলাকালীন সময়েও আপনি গবেষণা থেকে নিজেকে প্রত্যাহার করতে পারেন। এতে করে আপনার স্বাভাবিক চিকিৎসার কোন ক্ষতি হবে না।

অতএব, উপরের বক্তব্য সম্পূর্ণ বুঝে থাকলে যদি আপনি / আপনার শিশুকে গবেষণায় অংশগ্রহণে করতে রাজী থাকেন তাহলে নীচে আপনার স্বাক্ষর / বৃদ্ধাঙ্গুলের ছাপ দিন।

…কের স্বাক্ষর
…খ ______

রোগীর স্বাক্ষর / বৃদ্ধাঙ্গুলের ছাপ
তারিখ ______