Attachment 1.

Date _____

ETHICAL REVIEW COMMITTEE, ICDDR,B.

26

Principal Investigator Dr. S.Q. Akhtar

Trainee Investigator (if any) _____

Application No. 81-067

Supporting Agency (if Non-ICDDR,B) _____

Title of Study Isolation and Characterization of anaerobic bacterial flora from Diarrhoeal Patients.

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).

1. Source of Population:
   - (a) Ill subjects — (Yes) No
   - (b) Non-ill subjects — Yes No
   - (c) Minors or persons under guardianship — Yes No
2. 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
3. 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
4. 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: N/A
   - (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 — N/A 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. — NA
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. — NA

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

S. Q. Akhtar
Principal Investigator

Trainee

81-047

SECTION I - RESEARCH PROTOCOL Rec'd 10-11-81

1. Title: "Isolation and characterization of anaerobic bacterial flora from diarrhoeal patients".

2. Principal Investigator: Dr. S.Q. Akhtar

. Co-Investigators: Drs. K.M.S. Aziz, S.C. Sanyal P. Speelman, M.I. Huq, Hasan Ali, Akbar Ali

3. Starting Date: January 1, 1982.

4. Completion Date: December 31, 1982.

5. Total Direct Cost: $ 30780.00 (Staff commitment $ 14734.00)

6. Scientific Programme Head:

This Protocol has been approved by the DTWG

Disease Transmission Working Group.

Signature of Scientific Programme Head: [signature]

Date: 10/11/1981

7. Abstract Summary:

This study intends to isolate and characterize anaerobic bacterial flora from diarrhoeal patients. Uptil now we know approximately 80% of the causes of diarrhoeal illness. The other 20% or more is still unknown. Most of the bacterial pathogen responsible for diarrhoea are either aerobic or facultative. Due to difficulties of isolation and cultivation of anaerobic bacteria no significant studies were carried out on anaerobic bacterial flora in diarrhoeal disease patients.

As the isolation, identification and toxin assays of anaerobic bacterial flora from diarrhoea patients would be a very extensive

one, the work would be carried out in different phases. During the 1st year search would be made only for C. perfringens and C. difficile which are established intestinal pathogens from hospitalized severe diarrhoea patients, patients with colitis but having no established pathogen and also from children of 1 year, clinically diagnosed as toxic, necrotising or pseudonembramous enterocolitis. During subsequent years the other anaerobic bacteria might be taken up, if thought necessary. At the beginning only biochemical characterization would be done, for extensive and specific studies gas liquid chromatographic studies would be applied later. Stool samples and rectal swabs would be cultured under anaerobic conditions. After identification these two anaerobes would be subjected for toxin testing.

8. Reviews :

a. Ethical Review Committee : _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

SECTION II - RESEARCH PROTOCOL

A. INTRODUCTION

1. Objectives:

The main objective of this study is to explore whether anaerobic bacteria are responsible for diarrhoeal illness in Bangladeshi population. Another aim is to set up anaerobic techniques in our laboratory to facilitate the isolation of anaerobic organisms and hence to train laboratory personnels on this aspect.

2. Background:

Due to the difficulty in culturing anaerobic organisms, literature survey shows that work on attempts at anaerobic isolation of pathogenic bacteria from diarrhoeal patients is rare (Falsen et al, 1980; Chang et al, 1980; Batts et al, 1980). Currently a few laboratories are performing some work on the isolation and characterization of toxigenic anaerobic bacterial pathogens (Larson et al, 1978; Bartlett et al, 1978, George et al, 1978). Using a special selective medium Falsen et al (1980), has shown 3% isolation of Clostridium difficile from diarrhoeal patients. They also reported that any change of the normal bacterial fecal flora due to antimicrobial treatment or enteric infections like Salmonella increases the possibilities of isolating C. difficile. The majority of cases with diarrhoea and C. difficile were self-healing and not servere. Larson et al (1978) has recently reported that in many cases toxin-producing clositridia caused

pseudomembranous entero-colitis. Toxin producing clostridia has also been reported to produce pseudomembranous entero-colitis in patients treated with antibiotic (Bartlett, 1978). Bartlett et al (1979) has been able to detect C. difficile toxin from stool samples of 98% patients with pseudomembranous entero-colitis and 15% of pateints with antibiotic-induced diarrhoea wilthout sings of pseudomembranous enterocolitis. Until recently C. difficile was considered non-pathogenic for humans (Bartlett et al, 1079; Larson et al, 1978).

Very recently Bartlett (1981) demostrated toxin in stools of patients who had antibiotic-associated diarrhaea or collitis. C. difficile has been found responsible for toxin production and the toxin could be neutralized by C. sordellii antitoxin. Many investigators have observed that in animal models all animals appeared to have a similar etiologic mechanism in which there was a toxin in the stool that could be neutralized with C. sordellii antitoxin (Silva 1979; Bartlett et al, 1978, Fekety et al, 1979). Intracecal injection of either the organism or the pertialy purified toxin produces an analogous disease in experimental animal models (Bartlett, 1977). Same toxin was found in the stool specimen from patients with antibiotic associated PMC. Reports in the literature provides evidence for C. difficile being responsible for producing this toxin. Willey and Bartlett (1979) observed that stool cultures from these patients almost invariably yield this organism which produces a cytotoxin neutralizable by C. sordellii antitoxin. In vitro production of similar or identical

toxin has also reported by Bartlett (1978b). From Bartletts recent review it is apparent that 100% of the PMC patient showed the presence of toxin producing C. difficile and about 20% ih patients with antibiotic-associated diarrhaea in which there are relatively mild symptoms and normal endoscopic results. C. difficile has been established as a common enteric pathogen only in associated with the diarrhaeal complications of antibiotic usage.

Epidemiological studies by Nord and Heimdahl (1979) on healthy individual indicate that C. difficile can be isolated from stools in 2% of adults. Mullingan et al (1980) studied the epidemiological aspects of C. diddicile induced diarrhoea and colitis and reported C. difficile as a cause of antimicrobial agent-associated diarrhoea and colitis. Cultures of the hospital environments of six of eight patients whose fecal cultures were positive for C. difficile yielded this organism, whereas cultures of control hospital sites were almost invariably negative. Relatively high counts of toxigenic C. difficile are present in the feces of patients with C. difficile induced diarrhaeal disease. Chang et al (1980) also reported about diarrhoea caused by C. difficile toxin. Batts et al (1980) reported about treating antibiotic-associated C. difficile diahhroea with oral vancomycin.

In man, C. perfringans type. A is a common cause of food poisoning, which follows the ingestion fo heat resistant spores contaminating inadequately cooked food. Many cases of life-threatening clostridial enterotoxemias

have been reported in human being. As early as 1933 Glenny et al reported that C. perfringens types B and C produces beta toxin which was lethal but nonhemolytic. Intracutaneons injection in guinea-pigs showed purplish, localized dermonecrosis. Cell free products of C. perfringens have shown fluid accumulation in rabbit loop and production of diarrhoea in rabbits (Duncau and Strong, 1969). Hauschild et al (1971) has shown rapid detection of C. perfringens enterotoxin in a modified ligated intestinal loop technique in rabbits. He has also been able to induce diarrhaea by enterotoxin of C. perfrigens type A in monkeys (Hanschild et at, 1071). Yamamoto et al (1979) observed that C. perfringens enterotoxin when inoculated into ligated intestinal loop of mice, caused marked distension due to fluid accumulation. The fluid accumulation was proportional to the dose of enterotoxin.

Recently studies on the pathophysiology of antibiotic-associated pseudomembraneous colitis have emphasized the importance of clostridia as human intestinal pathogens. Volsted-Pederson et al (1976) Howard et al (1977), Kliegman (1979a) reported the isolation of clostridia from the clinical specimens of infants with necrotizis interocloits (NEC). Kliegman et al (1979) has described clostridia as pathogens in neonatal necrotizing interocolitis. They identified 51 neonates with necrotizing enterocolitis which gave an over all incidence rate of 5.1% of all patients admitted to the neonatal intensive care unit. C. perfringens was isolated from 14% of those patients. Fatal necrotizing enterocolitis due to C. perfringens

type C has been first reported in Germany. Pig-bel is a well-studied necrotozing enteritis with similar pathologic finding and severity associated with C. perfringes type C toxin occuring in children and adult of New Guinea (Lawrence et al 1979).

3. Rationale:

Recent investigations though, very few have shown that the anaerobic bacteria specially C. difficile, C. perfringens are to some extent responsible for diarrhoeal illness including pseudomembranous and necrotizing enterocolitis. In ICDDR,B no attempt has been made to isolate anaerobic bacterial pathogens from patients of these syndroms. To explore and contribute to the exixting causes of diarrhoea it has become essential to isolate bacteria from the rectum with an aim to culture anaerobic bacteria. This study is juftifiable for two reasons (1) to explore potential anaerobic toxigenic or invasive bacteria and (2) to establish anaerobic bacteriology in our microbiology laboratory.

B. SPECIFIC AIMS

1. The main aim of this study is to explore whether anaerobic bacteria like C. difficile and C. perfringens are responsible for causing diarrhoeal illness in Bangladeshi population.

2. To set up a laboratory for anaerobic diagnostic work.

C. METHODS AND MATERIALS

Patient selection:

1. First group would include patients (not less than 5 years) hospitalized for persistent diarrhoea with or without other complications. Diarrhaea continuing for more than 7 days dispite of treatment would be considered as persistent diarrhaea. Stool or rectal swab from those patients would be cultured to isolate C. difficile or C. perfringens only when they would have no other bacterial pathogen. Exclusion of rotavirus infection would not be considered.

   First group of patients would be divided into two subgroups. Subgroup A would consit of patients without a clinical history of prolonged antibiotic therapy. Subgroup B would include patients with prolonged history of antibiotic theraphy, diagnosed as pseudomembranous enterocolitis. Total number of patients for both subgroups would not exceed 200 (100+100).

Second group of patients would be selected from Dr. P. Speelmans protocol "Colitis in patients with Campylobactor , V. parahemolyticus and shigella infection". Anaerobic stool culture would be done from these 200 patients included in this protocol. To search for mixed bacterial infection, patients having other bacterial cause would no be excluded.

colonic fluid which Dr. Speelman would draw for his study by endoscopic examination would be cultured to isolate anaerobes, Endoscopy to draw colonic fluid from all negative pateints (group 3 of Dr. Speelman's protocol) would also be performed. Number of patients in this all negative group is expected to be very small and would never include infant or small children (under 5 years). Approximate number of patient in this group is not known.

Third group of paients would include infants under one year of age who clinically present as toxic/necrotiging/pseudomembranous enterocolitis. Number of patients in this group would not exceed 30 (2-3/ months).

Collection of stool samples for culture:

For the time being, until we receive anaerobic cabinet or glove box, R/S or stool sample would be streaked immediately after collection of samples at bed side. After the installation of anerobic cabinet stool specimens would be drawn by catheter and collected under liquid parafin. Culture would be made under anaerobic cabinet as soon as possible. Peach and Haycf (1974) experienced a three fold increase in the isolation rate of anaerobes from clinical specimens when anaerobic cabinet was used instead of an anaeronic jar. Initially an effort would be made to make an anaerobic cabinet locally. Two anerobic caninets will be ordered for long term use.

Setting up the Gaspak Jar:

1. The inoculated plates would be placed in the jar.

2. A disposable anaerobic indicator socket would be opened and placed in the jar so that indicator wick is visible. The indicator rapidly turns blue on exposure to air, but becomes colourless again under anaerobic conditions. Decolorization may take several hours.

3. Disposable hydrogen-carbon-dioxide gernator would be opened, (Gaspak or Gaskit) three generators should be used for the large 36-40 plate jars, the generators were activated and placed upright in the jar.

4. The lid of the jar should be immediately secured and placed in the incubator.

Plates would be inoculated in the usual way, preferably on freshly prepared plates. For culturing strict or otherwise demanding anaerobes it is desirable to use freshly prepared plates since during storage the medium takes up oxygen from the atmosphere in sufficient amounts to prevent the growth of these even though complete anaerobiosis has apparently been obtained in the jar.

After plates have been inoculated they would be placed under anaerobic conditions as quickly as possible. After 48 hours incubation at 37°C primary characterization of different types of colonies would be done by colony characteristics and biochemical tests. Different types of coloies would then be subcultered in duplicating the thioglycollate broth. Final characterization of individual organisms would be done from this subcultrue. For the 1st phase of the study mainly pure isolates of C.difficile and C. perfringens would be subjected to toxin assays to establish their pathogenicity.

For the detection of toxin whole cell culture filtrate would be used. If these two organisms isolated from patients at ICDDR,B are found to be toxigenic the study would be extended. The 2nd phage of this study would explore the pathogenicity of other anaerobic bacterial flora isolated from diarrhoeal patients.

C. perfringes:

C. perfringens is a non motile gram positive rod, straight with parallel sides with rounded ends, about 4 x 1.5 μm, human type C strains are rather larger and may show filaments and swollen forms. Rapidly growing cells may show coccal forms. Non-spore formers in artificial condition but sporulation takes place by alkaline environment and in the absence of fermentable carbohydrate. Types A and C of the enterotoxin are responsible for diarrhoeal episodes or other colonic diseases in man.

Identification:

Culture characeristics: C. perfrigens is not a strict anaerobe, grows rapidly in the presence of small amount of oxygen. It is one of the most rapidly growing anaerobes, surface growth very often detectable within 4-6 hours of incubation. Growth in deep broth might even be observed after 2 hours incubation.

Colony characteristics:

Colonies are convex, semi-translucent, smooth and with an entire edge. Less commonly, colonies are umbonate with radial striations and a crenated or scalloped edge. Growth usually does not spread over the surface of the medium. Some human type C strains produce rough colonies with characteristic thorn like outgrowths. Complete and partial zones of haemolysis are produced by many strains.

The organism produces diffuse opalescence in egg yolk agar. This can be inhibited by C. perfingens antitoxin. There should be no production of any pearly layer. Cultures on lactose egg yolk milk agar show lactose fermentaion. Grows in cooked meat broth within a few hours producing a fair amount of gas. The meat particles turn pink but with no digestion. In ordinary milk medium rapid fermentation of the lactose occurs, with the subsequent development of a characteristics "stormy clot" reaction.

Biochemical:

All types of C. perfringes ferment glucose maltose, lactose and surcose and are gelatinase producers. They are indole negative and H_2S positive. Major products of methabolism are acetic and butyric acids: butanol is sometimes also produced.

Antigenic types of toxin:

C. perfringens is differentiation into 5 serological types (A-E) according to the types of enterotoxins produced. The toxins are antigenic and anti-toxic sera are used in the routine typing of strains.

Pathogenicity:

Most human C. perfringens infections or intoxications are due to type A strains. Type a strain produce a lethal alpha toxin and all other types produce at least one other major lethal toxin in addition to alpha toxin. Differntiation of types is based on the detection of specific neutralization of toxins from culture fluids. Tests for toxicity and serum neutralization for typing are done in mice.

Human type A and C strains are also thought to be responsible for necrotizing enterocolitis. This organisms cause fluid accumulation in ligated intestinal loops (Duncan and Strong, 1969; Hauscluld et al, 1070; 1971). Toxin would be detected by animal inoculation. Trypsinized centrifuged culure filtrate would be used for animal inoculation. (CDC laboratory manual, P. 17/18). Purified toxin is protein and become inactivated at $60^{o}C$.

Examination of faeces:

The following technique, based on Sutton and Hobbs (1968) suggestion, is easily performable in the facilities of the clinical laboratory and ensures isolation of all appropriate strains of the organism. A thick emulsion of faeces (1: 10) would be made in quarter strength Ringer's solution. Using 10-fold dilutions of this emulsion semi-quantitative counts are performed on neomycin blood agar.

The same 10-fold dilution of the emulsion would be heated at 80°C for 10 min and semi quantitative counts would be performed on neomycin blood agar.

One ml of the emulsion would be inoculated into a tube of cooked meat broth and heated at 100°C for 30-60 min.

Plate count cultures and the enrichment culture are incubated anaerobically for 24 hours, and counts obtained. The enrichment cultures is subcultured to neomycin horse blood agar; this would detect the presence of absence of heat resistant spores of C. perfringens.

This procedure ensures the isolation of heat sensitive or resistant strains and helps to recognize various haemolytic variants. An assessement is obtained of whether C. perfringens is present in relatively small or large numbers. For smaples of faeces collected and examined soon after

the illness counts below 10^5 organisms/gm are regarded as low. The faeces of patients afflicated with C. perfringens food poisoning usually give counts of the order of 10^5-10^7 cells/gm.

Slide agglutination tesst with primary culture and also from pure isolated would be done.

C. difficile:

It is a long slender Gram positive bacillus, about 6-8 x 0.5 um in size. Produces large, oval, subterminal spores that distend the bacillary body. C. difficile is most commonly encountered in the faeces of infants.

Identification:

It is a strict anaerobe,colonies are 2-3 mm in diameter after 48 hours incubation, slightly raised, white, opaque and circular with an entire margin, non-haemolytic on blood agar, entirely non-proteolytic, and is egg yolk negative.

Biochmeical:

Ferments glucose, but not maltose, lactose or sucrose, Does not produce indole and H_2S. Products of fermentation are multiple and complex and include small amounts of acetic, isotutyric, isovaleric, valeric, butyric and isocaproic acids. C. difficile is unusual in that it is tolerant to

cresol, which it produces during growth (Hafiz and Oakley, 1976). This characteristics is helpful for its isolation from mixed cultures. Para-cresol (0.2%) added to an enrichment broth allows selective growth of C. difficile (Hafis et al, 1975). Pathogenicity test would be done by animal inoculation.

Medium:

For primary isolation neomycin blood agar, thioglycollate agar would be used. Subculturing for isolated colonies would be done on the same media.

D. SIGNIFICANCE:

Recent reports in lliterature show the involvement of anaerobic bacterial pathogens for diarrhoeal illness, The significance of the study:

(1) Is the potential contribution to further understanding of the unknown causes of diarrhoeal illness caused by anaerobic bacteria, particularly by C. difficile and C. perfringens in Bangladesh. (2) Additionally through this work we expect to set up anaerobic technology in our laboratory for continuin routine anaerobic diagnostic work which would also be singnificant for advancement of research in this area at ICDDR,B.

E. FACILITIES REQUIRED:

1. Office Space: Already provided
2. Laboratory Space: Already provided

3. Hospital Resources: 500 patients

4. Animal Resources: Rabbits, mice

5. Logistic Support: Yes

6. Equipment: Anaerobic cabinet or glove box, and anaerobic jars.

7. Other Requirements: Chemical and Gas pack

F. COLLABORATIVE ARRANGEMENTS:

P.I has written to DR. Jesteenson, Department of Microbiology, University of Copenhagen, for his suggestion. Dr. Jesteenson was one of the course instructors in the Clinical Bacteriology Course held in our centre during December, 1979 organized by WHO/DANIDA. If collaboration with Dr. Jesteenson is not possible, P.I would communicate other laboratories where advance anaerobic facilities are available.

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Differential characterstics of C. perfringens and C. difficile (Ref: Biochemical tests for identification of Medical Bacteria by J.F. MacFaddin. P:355)

Testing Media	C. difficile	C. perfringens
Aerotolerant	-	-
Haemolysis, 5% SBA	-	+ (dbuble zone of haemolysis- α & β)
Spore shape and location	Sub-terminal oval	Sub-terminal oval
Giclatin liquefaction, 22°C	+ (slow)	+
Nitrate reduction	-	Variable
Motilitis	+	-
Cooked meat digestion	-	-
Serum	-	-
Milk	-	Acid, clost, gas (stormy fermitation)
Indole	-	-
H_2S	-	-
Christensen's urease	-	Variable
Lecithinase	-	+
Lipase	-	-
Esculin hydrolysis	-	-
Starch hydrolysis	-	+
Voges-ProsKauer	-	Variable

Carbohydrate Characterization of C. difficile and C. Perfringens (Ref: Biochemical tests for Identification of Medical Bacteria by J.F. MacFaddin P:356)

Carbohydrates	C. difficile	C. perfringens
Glucose	Acid	Acid
Lactose	-	Acid
Maltose	-	Acid
Mannitol	-	-
Sucrose	-	Acid
Salicin	Variable	Variable
Arabirose	Variable	-
Dulcitol	-	Variable
Galactose	-	Acid
Inositol	Variable	Acid
Mannose	Acid	Acid
Raffinose	-	Variable
Ribose	Variable	Variable
Sorbitol	Variable	-
Trechalose	Variable	Variable
Xylose	Acid	-

SECTION III - BUDGET

A. DEATAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% Efforts	Annual salary	Project Requirements Taka	Dollar
Dr. S.Q. Akhtar	Principal Investigator	35%	67,200	23,520	-
Dr. K.M.S. Aziz	Co-Investigator	5%	-	-	$ 3105
Dr. S.C. Sanyal	"	5%	-	-	$ 2267
Dr. M.I. Huq	"	5%	-	-	$ 2687
Dr. P. Speelman	"	5%	-	-	$ 2867
Mr. Akbar Ali		10%	80,000	8,000	-
Dr. Hasan Ali		5%	60,000	3,000	-
Mr. Kibriya		20%	60,000	12,000	-
Ms. Soheli Akhtar		40%	36,000	14,400	-

2. SUPPLIES AND MATERIALS

Gas pack		$ 2,000
Chemical		$ 3,500

3. EQUIPMENTS

Glove Box		$ 4,000
Anaerobic Jar	$ 160/Jar	$ 640

4. HOSPITALIZATION

Nil

5. OUTPATIENT

Nil

6. TRANSPORT

Nil

7. TRAVEL

One trip to or from a laboratory with advanced anaerobic facilities	$ 3,000

8. TRANSPORTATION OF THINGS

Nil

9. RENT AND COMMUNICATION

Nil

10. PRINTING AND REPRODUCTION

Tk. 1,500

11. CONTRACTUAL SERVICE

Nil

12. CONSTRUCTION

Nil

13. ANIMAL REQUIREMENT

Tk. 45,000

B. BUDGET SUMMARY

		TAKA	DOLLAR
1.	Personnel	60,920.00	10,926.00
2.	Supplies		5.500.00
3.	Equipment		4,640.00
4.	Hospitalization	-	
5.	Outpatients	-	
6.	ICDDR,B Transport	-	
7.	Travel Persons		3,000 00
8.	Transportation things		
9.	Rent/Communication	-	
10.	Printing/Reproduction	1,500.00	
11.	Contractual Service	-	
12.	Construction	-	
13.	Animal requirement	45, 000.00	
		1,07,420.00	24,066.00

Grand Total: US$ 30,780.00

ABSTRACT SUMMARY

This study intends to isolate and characterize anaerobic bacterial flora from diarrhoeal patients. Uptil now we know approximately 80% of the causes of diarrhoeal illness. The other 20% or more is still unknown. Most of the bacterial pathogen responsible for diarrhoea are either aerobic or facultative. Due to difficulties of isolation and cultivation of anaerobic bacteria no significant studies were carried out on anaerobic bacterial flora in diarrhoeal disease patients.

As the isolation, identification and toxin assays of anaerobic bacterial flora from diarrhoea patients would be a very extensive one, the work would be carried out in different phases. During the 1st year search would be made only for C. Perfringens and C. difficile which are established intestinal pathogens from hospitalized severe diarrhoea patients, patients with colitis but having no established pathogen and also from children of 1 year, clinically diagnosed as toxic, necrotising or Psendonembramons enterocolitis. During subsequent years the other anaerobic bacteria might be taken up, if thought necessary. At the beginning only biochemical characterization would be done, for extensive and specific studies gas liquid Chromatographic studies would be applied later. Stool samples and rectal swabs would be cultured under anaerobic conditions. After identification these two anaerobes would be subjected for toxin testing by animal inoculation.