Title of Study: "Isolation and characterization of Vibrio mimicus from aquatic environments of Bangladesh"

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

5. Will signed consent form be required:
   (a) From subjects Yes No
   (b) From parent or guardian Yes No

6. Will precautions be taken to protect subject 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.

Principal Investigator

Supporting Agency (if Non-ICDDR,B) X

New Study
Continuation with change
No change (do not fill out rest of form)

Date: 02-9-84

Ethical Review Committee, ICDDR,B.
SECTION I - RESEARCH PROTOCOL

1. TITLE: Isolation and characterization of Vibrio mimicus from aquatic environments of Bangladesh. (The work of this protocol will be submitted by the Principal Investigator as a dissertation for the partial fulfilment of M.Sc. (thesis) degree of the Dept. of Microbiology under the University of Dhaka).

2. PRINCIPAL INVESTIGATOR: Md. Afzalur Rahim Chowdhury

3. CO-INVESTIGATOR: Mr. Zeaur Rahim

4. CONSULTANT Dr K.M.S. Aziz Dr Bradford A. Kay

5. STARTING DATE: 1st October, 1984

6. COMPLETION DATE: 1st October, 1985

7. TOTAL DIRECT COST US$ 2583.60

8. SCIENTIFIC PROGRAM HEAD:

This protocol has been approved by the Working Group.

Signature of the Scientific Program Head Date

9. ABSTRACT SUMMARY

This study will be carried out from the selective study points to assess the prevalence of V. mimicus in the aquatic environments of Bangladesh. The isolated environmental strains will be compared with the patient isolates in terms of their biochemical behaviors, enzymatic activities, enterotoxigenicity and antibiogram.

02 JUL 2002
10. **REVIEWS:**

   a) Ethical Review Committee: __________________________

   b) Research Review Committee: __________________________

   c) Director __________________________
SECTION II - RESEARCH PLAN

INTRODUCTION:

1. Objective:

The main objectives of this protocol is to findout:

a) Prevalence of *V. mimicus* in aquatic environments of Bangladesh &

b) The similarities of environmental isolates with the patient isolates in terms of biochemical behaviors, enzymatic activities, enterotoxigenicity and antibiogram.

2. Background:

*Vibrio cholerae* 01 has long been known as the aetiological agent of Asiatic Cholera. During the early period, the classical biotype of *V. cholerae* 01 was established as the causative agent of cholera but subsequently the El Tor biotype was also shown to be a causative agent. Serotypes which fail to agglutinate with *Vibrio cholerae* polyvalent 0-1 antiserum i.e. those other than serotype 0-1 were established as NAG or non-cholera vibrios (1). However, their role in producing disease was not recognized but their biochemical and the serological similarities grouped them with *Vibrio cholerae* group (1,3,4,5).

Recent studies on more than 50 sucrose negative *Vibrio* species (Davis et al, 1980, 1981) defined the biochemical parameter by determining DNA relatedness of biochemically atypical strains to a typical *V. cholerae* (6). Thus, the sucrose-negative strains were not *V. cholerae*, but they were designated as a new species, the *V. mimicus* (7,8).

*V. mimicus* has been regarded as a serious pathogen for human being because it causes various diseases (9,10,11,12,13,14,15). It causes
cholera-like syndrome in Asiatic countries (6,15). Shanderma (1983) isolated *V. mimicus* from the ear of patients with otitis (10) and from patients with diarrhoea, nausea, vomiting and abdominal cramps, with fever, headache and bloody diarrhoea (10,14,15,16).

Like *V. cholerae*, *V. mimicus* has also been isolated from various environments like river, brackish and sea water, sea food (e.g. raw oyster, shellfishes) from various parts of the world (6,7,10,17,18). Davis, *et al* (1981) studied 51 strains of *V. mimicus* from various sources including two strains from aquatic environment of Bangladesh (6). Currently in ICDDR,B Investigators of the protocol entitled, "Ecology and survival of *V. cholerae* and related pathogenic vibrios in the aquatic environments of Bangladesh during cholera epidemic and interepidemic periods", isolated many strains of *V. mimicus* (unpublished data). Sanyal *et al* (1983) isolated *V. mimicus* from 13 patients with watery diarrhoea and one with bloody mucoid stool (15).

It is now suspected that *V. mimicus* is transmitted through water in Bangladesh to cause disease like other members of the *V. cholerae* non O1 (19,20,21). Although some investigation has been done with patient isolates of *V. mimicus*, we know little about the environmental isolates of *V. mimicus* in Bangladesh.

The nature of the toxin elaborated by *V. mimicus* in causing gastroenteritis is still not clearly defined. Spira (1977) isolated *V. mimicus* which produced cholera toxin (CT) (13), or one of the closely related toxins (12,13,23) which is usually designated as "CT-like toxin". There is also
evidence that *V. mimicus* may produce a toxin unlike CT but similar to that of *E. coli* ST toxins (11,24,17). Nishibuchi et al (1983) reported the elaboration of enterotoxin from environmental and patient isolates of *V. mimicus* using rabbit ileal loop and suckling mice model (17). Sanyal et al showed that culture filtrates of *V. mimicus* strains caused increased capillary permeability in rabbit skin, fluid accumulation in rabbit ileal loops, and changed morphology in Chinese hamster ovarian cells (10,15). The culture filtrates of environmental strains of *V. mimicus* are known to produce a toxin similar to the ST of *E. coli* (6,17,25). On the basis of their pathogenic mechanism, strains of *V. mimicus* are now divided into two groups: one producing a toxin immunobiologically similar to cholera toxin and the other producing a heat stable toxin unlike CT-toxin (15). However, the biochemical properties and nature of the toxins of *V. mimicus* from aquatic environments of Bangladesh are still not worked out thoroughly.

There are many reports on the drug sensitivity pattern of so-called non-cholera vibrio (26,27), but only two reports are available on *V. mimicus* (6,15) and most of these included only patient isolates. They observed that *V. mimicus* isolated from stool of diarrhoeal patients were sensitive to Tetracycline, Gentamicin, Streptomycin, Chloramphenicol, Ampicillin, Carbenicillin, Cephalothin (6), Streptomycin, Tetracycline, Ampicillin, Chloramphenicol, Cotrimazole, Kanamycin, Gentamicin (15). There is no information on the resistance pattern of *V. mimicus* isolated from Bangladesh environments.
3. **Rationale:**

The rationale underlying this research protocol is to study the prevalence of _V. mimicus_ in aquatic environments of Bangladesh and to characterize them in respect to their biochemical behaviors, enzymatic activities, enterotoxigenicity and antibiogram to know the similarities of environmental strains and strains isolated from patients.

**B. SPECIFIC AIM:**

The specific aim of this protocol is to:

i) isolate the _V. mimicus_ strains from aquatic environments of Bangladesh,

ii) determine if the environmental strains are enterotoxogenic,

iii) compare biochemical properties, enzymatic activities and drug sensitivity pattern of the environmental and the patient isolates of _V. mimicus_.

**C. MATERIALS AND METHODS:**

1) **Sampling points:** Water and soil sediments will be collected from Buriganga River at Babu Bazar ghat and Dhanmondi lake near Kalabagan Lake Circus (Dhaka City).

2) Surface water will be collected from the sampling sites in presterilized 4 oz. glass bottles for bacteriological analysis and for better isolation moore swab will also be used. Soil sediments will be collected with the help of ICDDR,B constructed core sampler and taken into presterilized 4 oz. glass bottles. Then the samples will be transported to the laboratory in an insulating foam box provided with ice bags.
3) Bacteriological analyses:

Bacteriological analyses of both water and soil sediments will be carried out for the isolation of *V. mimicus*. Patient isolates of *V. mimicus* will be collected from the Microbiology Branch of ICDDR,B for comparative studies.

i) Isolation of *V. mimicus*:

Water samples will be filtered through 0.45/μm membrane filter (Millipore Corp, Bedford, Mass (29)) and the filter paper with retained bacteria on the surface will be enriched in bile peptone broth for 18–24 hours at 37°C. Then one loopful of growth from the enriched broth will be streaked on TTGA (28), TCBS agar medium and incubated at 37°C for 18–24 hours for isolation of *Vibrio*. Typical *Vibrio* like colonies from TTGA and TCBS agar will be identified through conventional biochemical reactions and only sucrose and V-P negative *V. cholerae* non-01 will be identified as *V. mimicus* (6,17).

Soil sediments will be enriched in bile peptone broth and *V. mimicus* will be isolated following procedure mentioned above.

4) Test of enterotoxicity:

Test of enterotoxicity of *V. mimicus* will be conducted in various models as follows:

i) Heat-labile enterotoxin: Following standard procedure, heat-labile enterotoxin will be detected by:-

a) adult rabbit ileal loop test (30),

b) skin permeability test (31),

c) Chinese hamster ovarian cell line (CHO) (32)
ii) Heat-Stable enterotoxin: Will done in "suckling mice assay"(2).

5) Enzymatic activities:
Using API ZYM micromethod system the comparative study of environmental and patient isolates will be done for the following 19 enzymes:
Phosphatase alkaline, Esterase (C4), Leucine arylamidase, Valine arylamidase, Cystine arylamidase, Trypsin, Chymotrypsin, Phosphatase acid, Phosphoamidase, \( \alpha \) galactosidase, \( \beta \) galactosidase, \( \beta \) glucuronidase, \( \alpha \) glucosidase, \( \beta \) glucosidase, N-acetyl - \( \beta \) glucosaminidase, \( \alpha \) mannosidase, \( \alpha \) fucosidase.

6) Antibiotic susceptibility testing:
All strains isolated from environments and equal number of patient isolates will be tested for sensitivity against common antibiotics following single antibiotic disc method (22). The following antibiotics will be used: Ampicillin, Chloramphenicol, Tetracycline, Streptomycin, Gentamicin, Trimethoprim and Sulphamethoxazole, Kanamycin.

7) Biotyping:
Each isolates will be biotyped using an API-20E test. Additional test will include: string test, salt tolerance, susceptibility to 0129, hemagglutination with chicken red cells, and production of hemolysins.

8) Analysis of Data
Data will be analysed considering following points:-
1. Monthwise isolation rate of \textit{V. mimicus} throughout the study period;
2. Parallel study will be done by taking 25 strains from environmental isolates & 25 from patient isolates. Similarities and/or dissimilarities among the strains will be taken to describe that some environmental
isolates are identical and some are variable. Considering enterotoxicity (LT, ST or LT/ST), biochemical behaviors, enzymatic activities and drug sensitivity pattern, similar strains will be helpful to state that some environmental strains are causing disease whereas dissimilar strains might be sufficient for the biotyping of *V. mimicus*.

D. **SIGNIFICANCE**

The prevalence of *V. mimicus* in aquatic environments of Bangladesh will be studied throughout the study period which will be helpful to know about these species in the environment. The characterization of the environmental isolates with respect to patient isolates will also help us to know whether or how many of environmental strains are identical to those of pathogenic *V. mimicus*. The various biochemical tests, enzymatic activities & antibiogram will provide additional data to know in detail about this bacterium which might be helpful to control it.

E. **FACILITIES REQUIRED**

1. Office space :- Not applicable
2. Laboratory space : ICDDR,B Training laboratory will be required for most of the study period.
3. Hospital Resources: Not applicable
4. Animal Resources : 50 adult albino rabbit and 50 suckling mice will be needed for the test of enterotoxicity.
5. ICDDR,B Transport : 320 miles of automobile transport @ 14.50/per mile required.
6. Miscellaneous : Not applicable.


Epidemiology, II. Occurrence and survival of V. cholerae

20. Levine, R.J. et al 1976. Cholera is primarily water borne

Lancet, 2: 957.

susceptibility testing by a standardized single disc method.

produced by non-cholera vibrios. John Hopkins Med. J. 131:
403-411.

non-01 V. cholerae enterotoxin and that is identical to cholera

of 0 group 1 non-agglutinating Vibrio cholerae and other vibrios
isolated from cases of diarrhoea in Dhaka, Bangladesh, p.137-153.
In D. Middleton and I. Librn (ed.), Proceeding of the 14th Joint
cholera Research Conference, U.S.-Japan Cooperative Medical Science
Program, Karatsu, Japan, 1978. Toho University, Japan.


The assessment of the prevalence of *V. mimicus* will be carried out from the collected water and sediment samples of the selected points. A comparative study for the biochemical properties, enterotoxigenicity, enzymatic activities, and the antibiogram of the environmental and the patient isolates will be done to find out the similarities between them.

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<td>8. Use of hospital records</td>
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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

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<tr>
<th>Name</th>
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<th>% or no.</th>
<th>Annual salary</th>
<th>Project Requirement</th>
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<tr>
<td>Md. Afzalur Rahim Chowdhury</td>
<td>Prin. Invest.</td>
<td>100%</td>
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<tr>
<td>Mr. Md. Zeaur Rahim</td>
<td>Co-Invest.</td>
<td>5%</td>
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<td>Dr K.M.S. Aziz</td>
<td>Consultant</td>
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<td>-</td>
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<tr>
<td>Jr Bradford A. Kay</td>
<td>Consultant</td>
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2. SUPPLIES AND MATERIALS

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<td>Suckling mice</td>
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<td>CHO</td>
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3. EQUIPMENT: Nil

4. PATIENT HOSPITALIZATION: Nil

5. OUT PATIENT NARE: Nil

6. ICDDR,B TRANSPORT: 320 miles 14.50/mile 4640
7. TRAVEL AND TRANSPORTATION: Nil
8. TRANSPORTATION OF THINGS: Nil
9. RENT, COMMUNICATION Nil
10. PRINTING & REPRODUCTION: US$ 100.00
11. OTHER CONTRACTUAL SERVICES Nil
12. CONSTRUCTION, RENOVATION AND Nil ALTERATION
### B. BUDGET SUMMARY

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US$ 1623.6 + 960 = 2,583.60

Grand total US $ 2,583.60