

REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

Principal Investigator Dr. M. U. Khan Trainee Investigator (if any) \_\_\_\_\_

Application No. 80-009 Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_

Title of Study Epidemiological Study of Multiply Antibiotic Resistant Cholera Project status:  
Dacca (  ) 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 abortion  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

- Will signed consent form be required:
  - (a) From subjects  Yes No
  - (b) From parent or guardian (if subjects are minors)  Yes No
- Will precautions be taken to protect anonymity of subjects  Yes No
- Check documents being submitted herewith to Board:
  - Umbrella proposal - Initially submitted overview (all other requirements will be submitted with individual studies)
  - Protocol (Required)  Pilot
  - Abstract Summary (Required)
  - Statement given or read to subjects of 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:
- 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.
  - Examples of the type of specific questions to be asked in the sensitive areas.
  - An indication as to when the questionnaire will be presented to the Board for review.

I hereby request to obtain approval of the Review Board on the Use of Human Subjects for any change in the rights and welfare of subjects before making such change.

M. U. Khan

Principal Investigator

Trainee

SECTION 1 - RESEARCH PROTOCOL

20-009  
Rec'd 1/4/80

Title: **Epidemiological studies of Multiple Antibiotic Resistant  
Vibrio cholerae cases in Dacca.**

Principal Investigator: **Dr. M.U. Khan, Co-Investigator Dr. L.N. Mutanda**

Starting Date: **Pilot ongoing**

Completion Date: **Depends on the availability of the cases.**

Total Direct Cost: **\$ 36,104**

Scientific Program Head:

This protocol has been approved by the Disease Transmission  
Working Group.

\*Signature of Scientific Program Head:

L. C. She

Date:

24 Mar 80

\*This signature implies that the Scientific Program Head takes responsibility for the planning, execution and budget for this particular protocol.

Abstract Summary: (250 words or less)

With the emergence of multiple antibiotic resistant Vibrio cholerae (MARVC) we plan to study the epidemiologic characteristics of the MARVC with respect to geographical distribution, age, sex, seasonality, secondary infection rates, secondary case rates, symptomatology of non-hospitalised cases, duration of illness, its recovery from and resistance in environmental

samples, its potentiality in spreading the disease to the family contacts, neighbourhood contacts and to the sources of water used for domestic purposes as compared to the antibiotic sensitive ones.

We will try to trace the resistant ones, from the stock lot in order to trace their 1st appearance. Initially, we will culture all cases of watery diarrhoea reported to Treatment Centre to isolate the resistant vibrios and select study and control cases.

On isolation of a MARVC we will follow the case family members and culture their stool, water and if available food. The cases will be daily cultured at least for 10 days and history of illness recorded until the positives are found negative for three consecutive days. Samples of water will also be cultured simultaneously. Vibrio isolates will be tested for persistence and transfer of R-plasmid. Presence of R-plasmid in environmental vibrios and also after laboratory passages in culture media will be investigated. Families with antibiotic sensitive V. C. cases will be studied as controls.

Analysis of the data of the multiple-antibiotic resistant vibrios will be made and the results will be compared with those obtained from sensitive control El Tor cholera cases. The potentiality of MARVC as a risk factor to the community will be examined.

8. Reviews :
- a. Ethical Review Committee : \_\_\_\_\_
  - b. Research Review Committee : \_\_\_\_\_
  - c. Director : \_\_\_\_\_
  - d. BMRC : \_\_\_\_\_

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION

1. Objective : The objective of this study is to understand the characteristics of the new MARVC as regards its epidemiologic similarity or dissimilarity with the sensitive strains in establishing infection and producing disease and its potentiality as a risk to the community and environment. Persistence or Transfer of R-plasmid will be tested.

Background : Since the inception of ICDDR, B (former CRL)

Vibrio cholerae of both serotypes and biotypes were found sensitive to conventional broad spectrum antibiotics. Tetracycline was found to be effective, economic and available for treating cholera cases(1) and preventing secondary cases in contacts. Upto the recent times there is only one report of an outbreak with tetracycline resistant Vibrio cholerae from Tanzania(2).

In December last(1979) one cholera case was found not responding to treatment with tetracycline in Matlab hospital. Subsequent drug-sensitivity testing, revealed that V. cholerae was resistant to tetracycline, ampicillin, kanamycin, streptomycin and trimethoprim sulphamethoxazole. Although multiple antibiotic resistant Vibrio cholerae were reported from Tanzania, Africa, the pattern of antibiotic resistance exhibited by our strain was different. This

observation has warranted a search for resistant vibrios from our stock cultures. 42 MARVCs have been found; some dating back from August, 1979. These MARVCs belong to both serotypes, Inaba and Ogawa, and biotype El Tor.

By January 9, two MARVCs were also isolated at Dacca hospital. One of them was traced back to Matlab endemic area and the other belonged to Bajitpur of Mymensingh which is about 70 miles up stream and north of Matlab. Later another case in Dacca and one in Munshiganj were isolated.

Clinically, the patients harbouring the multiple antibiotic resistant V. cholerae fail to respond to 12 doses of tetracycline, purge longer and require larger quantity of I. V. fluid.

New cases among the purging family and neighbouring contacts have been isolated. We have also found one dugwell positive for MARVC in Tongi, one sistern and one latrine positive for MARVC around Munshiganj family. Incidentally, this area is up stream and on the western side of Padma and Matlab. Though one man has relations in Matlab no history of definite relationship could be established. However, the drug sensitivity tests of 16 samples received from

distant areas of Bangladesh by Dr. Farida Huq of public health microbiology laboratory did not reveal any resistant strain.

The sudden emergence of MARVC is suggestive of involvement of R-plasmid in the environment. It has been found <sup>that</sup> ~~an~~ cell resistant to all four drugs could in a single conjugal event, transfer the genetic determinants of all these resistances to a sensitive recipient''(3). The MARVC isolated are thus a potential risk for treatment with antibiotics and may cause longer sufferings. In addition, 'the transmission of drug resistant factors could occur not only between the organisms of the same species but intraspecifically as well''(3). The E. coli may thus act as recipient bacteria causing more problem. 'Very important from the clinical stand point is the frequency with which R-factors are passed from non-pathogenic to pathogenic bacterial species''(4). The 'R-factors usually mediate resistance to several antibiotics at once. Thus, the presence of R-factors puts limitations on antibiotic usage''(5). All these tell about the importance of R-factors; and in a country like Bangladesh where diarrhoea and cholera are highly endemic the existence of R-factor in natural samples or in clinical cases may increase considerably the

morbidity and mortality from diarrhoea caused by these MARVC. 'In 1935, (when there was no antibiotics) enterobacteria caused only 12% of all bacteremic cases and deaths. In 1965 (when there were many antibiotics) they caused 55% of the cases and 67% of the deaths''(6). If the R-factors persist or are transferred from one bacterium to another both in nature or in human it will undoubtedly be a concern for all. Therefore, considering the importance of the situation this MARVC should be studied from all possible directions.

3. Rationale :

Epidemiological characterisation of the MARVC is one of the most important studies. This will help understand the pattern and may help adopt control measures. The clinical features of cases of MARVC is also equally important, so are experimental studies to determine the duration of plasmid carriage. In a previous study(9) plasmid carriage was higher in Bangladesh organisms (43%) than in the Chesapeake Aeromonas (9%). Antibiotic resistance was highest in areas of greatest human impact and could well prove to be a serious human health problem in these areas in the future.

B. SPECIFIC AIMS :

1. To determine the incidence by age, sex and seasonality of cholera caused by MARVCs.
2. To determine the rates of secondary infection and secondary cases in family contacts of MARV cholera cases and in controls.
3. To determine the extent of spread to the neighbourhood families and to the environment; and also to see the possible vehicles of transmission.
4. To see the persistence of MARVCs in comparison with the sensitive ones in human and in environmental samples as a risk factor.
5. To describe the duration and severity of the symptoms of the non-hospitalised cases.
6. To try to transfer R-plasmids to recipient organisms.

C. METHODS OF PROCEDURE :

1. Study population : As we expect only a few cases of MARVCs all such cases will be taken up for study initially from the Treatment centre or hospitals. When sufficient cases are



available then the cases will be selected on systematic or random basis not to exceed one index case per day. The hospitalised cases, their family members, their neighbours with diarrhoea and the control families will serve as study population. The controls will be selected from the antibiotic sensitive hospitalised age and sex matched cases. Diarrhoea cases in the neighbourhood of MARVC cases will be cultured. The families of MARVC cases from the neighbourhood will be studied as usual. We will study 80 MARVC case families and 80 control families of sensitive V. cholerae cases.

2. Specific collection : The families of the index cases will be visited at the earliest moment and within 2 days. Families will be censused, other relevant data recorded and samples of stool or rectal swabs will be obtained from all members of the family. If any purging neighbour is found his rectal swabs will be cultured. The swabs will be streaked on Monsur's plate and then kept in Bile pepton media for enrichment. History of travel, common meals, past diarrhoea and recent diarrhoeal death in the family and in the neighbourhood will be obtained. If any neighbour is found to be MARVC positive his family also

will be taken up for further family study. While taking RS everyone would be asked about the presence of diarrhoea. Clinical symptoms will be recorded from all MARVC positive cases who are not hospitalised. Severe cases found in the family or neighbourhood will be referred to hospital for I. V. therapy and the mild and moderate ones will be treated with oral saline. The families would be followed normally for 10 days. But members having MARVCs should be continued till 3 negative swabs are obtained. The laboratory will adopt usual technique for culture and antibiotic sensitivity tests (disc and MIC). The MARVCs will be used by other investigators for R-plasmid characterisation. The domestic water samples from container and sources like tank, pond, canal or tubewells will be taken for culture daily. In addition, latrine samples from the family and purging neighbours will be obtained for similar culture. If the left over food, which has been earlier consumed by the index, is available will be sampled for culturing. This should be collected in sterile vials with bile pepton media(10 ml). Water will be collected in 100 ml vials containing triple strength bile pepton media.

spread, route of transmission, clinical pictures, and significant differences with the existing epidemiological knowledge etc.

D. SIGNIFICANCE :

The emergence of MARVC is a significant change in that, the duration of diarrhoea, which could be cut down in the past to one third by using antibiotics, will not likely be affected by current therapy with tetracycline and as such there will be greater loss of working hours, longer morbidity and potentially higher family and environmental spread. This strain may cause epidemics of cholera in the future involving large number of people; hence it is important to learn epidemiologic and clinical characteristics of MARVC. The epidemiological characteristics of this new strain has not been studied anywhere. Therefore, this study will be an important and timely undertaking for this laboratory.

E. FACILITIES REQUIRED :

1. Office space - already exists. No new space is needed.
2. Laboratory space - already exists.
3. Hospital resources - none needed for this study. Severe cases

from families will be treated as usual procedure.

Mild cases will be treated in homes with oral saline.

4. Animal resources - none is needed for this study.
5. Logistic support - One transport with a driver will be needed daily if there are cases to be followed. Services of ricksha, baby taxi, country boat or motor launches may be needed if the residences of the patients are beyond the approach of a car.
6. Major items of equipment - none required.
7. Other - none required.

F. COLLABORATIVE ARRANGEMENTS :

No collaboration with other institution is expected now. The authorship of any publication from this study will include the following persons :

Dr. M. U. Khan and Dr. L. N. Mutanda.

## REFERENCES

1. Greenough, W. B. Gordon, R. S. et al. Tetracycline in the treatment of cholera. *The Lancet*, Feb. 15, 1964, pp. 355-357.
2. Mhalu, F. S. Mmari, P. W. Ijumba, J. Rapid emergence of El Tor *Vibrio cholerae* resistant to antimicrobial agents during the first six months of fourth cholera epidemic in Tanzania. *Lancet*, 1979; pp. 345-347.
3. Johnson, et al(1973). Text book of microbiology, Twentieth Edition, William Burrows Ed., p. 180.
4. Weinstein, L. (1975). *Pharm. Basis Therap. Fifth Edition*, Goodman, Gilman, Editors p. 1090.
5. Benveniste, R. Davies, J. (1973). *Annu. Rev. Biochem.*, 42: 471.
6. Finland, M. (Oct. 1973). *Post graduate Med.* 54(4): 175.
7. Mosley, W. H. Ahmed, S. et al(1968) *Bull. WHO.* 38: 777-785.
8. Khan, M. U. Shahidullah, M. Pattern of Intrafamilial spread of cholera, symposium on cholera, Karatsu, Japan, US-Japan Cooperative Medical Science Conference, 1978. P: 30-34.
9. Dr. McNicol, et al. Isolation of Drug-resistant *Aeromonas Hydrophila* from the Aquatic Environment. McNicol, L. N. et al. (submitted *Antimicrobial Agents and Chemotherapy*).



2. SUPPLIES AND MATERIALS :

<u>I T E M</u>	<u>UNIT COST</u>	<u>AMOUNT</u>	<u>TAKA</u>	<u>DOLLAR</u>
Rectal swab culture for cholera	Tk. 11.00	5000	55,000	-
Ballons	Tk. 25.00	5	125	-
Vibriocidal titre	Tk. 2.00	1000	2,000	-
Stationaries	Tk. -	-	5,000	-
Multivitamins	Tk. 100	6	600	-
Oral salt	Tk. 1.00	300	300	-
Candies	Tk. 12.00	20	240	-
Bags	Tk. 150.00	3	350	-
Umbrellas	Tk. 75.00	6	450	-
IBM Cards	Tk. 25/100	2	-	50.0
Teps	Tk. 100.0	1	-	100.0
Sub total			Tk. 64,065	150.00

3. EQUIPMENT

Filing Cabinet	Tk. 1,500.00	1	1,500	-
Sub total			Tk. 1,500	-

4. PATIENT HOSPITALISATION

Tk. 140.00	75 days	10,500	-
Sub total		Tk. 10,500	-

5. OUTPATIENT CARE

Family contacts care -

with oralyte and Vitamin	Tk. 20.00	50	<u>1,000</u>	-
			Sub total Tk. 1,000	-

6. ICDDR TRANSPORT

Mileage - Dacca

Transport M 40 X 350 X 3	Mile 3.00		<u>42,000</u>	-
			Sub Total Tk. 42,000	-

7. TRAVEL AND TRANSPORTATION OF PERSONS

Local Travel		Tk. 6,000	-
International travel		Tk.	\$ 5,000
	Sub total	<u>Tk. 6,000</u>	<u>\$ 5,000</u>

8. TRANSPORTATION OF THINGS

	\$ 100
Sub total	<u>\$ 100</u>

9. RENT, COMMUNICATION & UTILITIES

	Tk. 100
Sub total	<u>Tk. 100</u>



10. PRINTING AND PUBLICATION

Tk. 6,000    \$ 300

Sub total

Tk. 5,000    \$ 300

11. OTHER CONTRACTUAL SERVICE

Nil

12. CONSTRUCTION, RENOVATION, ALTERATION

Nil

## ABSTRACT

### EPIDEMIOLOGY OF MARV CHOLERA CASES, DACCA

The purpose of this study is to understand the new MARVs from epidemiological points of view. There is no existing data on the epidemiological characteristics of the MARVs. The study will provide with knowledge required for patient care, care of the family contacts, care of the environment and above all it will help in planning control of this disease. The age, sex, geographical, seasonal distribution of the cases and infected contacts and also the persistence of diarrhoea and presence of MARVs in nature will be revealed.

1. The patients may be of either sex or of any age. As the number of cases are expected to be small all cases will have to be taken into study. The family contacts and the neighbourhood contacts will be exposed to the index cases. Therefore, they will have to be included in the study. To compare the MARVs antibiotic sensitive cases will be needed as control. The consent will be obtained from them and in cases of infants, it will be obtained from their parents.
2. There is no risk in taking rectal swab or stool for culture. While taking blood (one or two drops) from the finger tips absolute sterility will be maintained to protect the subjects from any infection. There is no risk at all in obtaining finger stick blood for testing.
3. Strict sterility of swab stick and the pricking needling (used once only) will be maintained. Before pricking the finger will be sterilised with spirit swab.
4. The study does not include any confidential information. However, the data collected on illness and other family history will not be published by name and address. The data will be kept in strict

control and no information of any kind will be supplied to sources which might be objectionable to the subjects.

An informed consent will be obtained when the team will visit the families in their homes. In case of a minor the consent will be obtained from the legal guardian.

a. Signed consent will be obtained.

b. No information will be withheld from the subject about the findings.

6. The study will involve an interview. The initial interview will be with the patient in the hospital. The final interview will take place in the house of the subjects. This will include the history of diarrhoea, travel, eating out, attending common meals, symptomatology, SES, age and sex of the members of the family and the purging neighbours.

7. The study will provide benefit to the hospitalised cases and their family members. They will be provided with appropriate treatment either at home or at Treatment centre. The cost of treatment will not be charged. In addition when they will remain in hospital they will be provided with diet free of charge. In certain severe cases may be lifted to the hospital free of charge. The children whose RS etc. will be collected would be provided with candy or balloons. Thus the benefits will definitely outweigh the little risk.

8. The activity requires use of some records from hospital about their illness and care. It also requires 50 lamda (nearly 3 drops) of finger tip blood for testing and rectal swab for culture.

The entire process will not require more than half an hour time.

STATEMENT TO BE READ TO THE SUBJECT  
WHEN VERBAL CONSENT IS OBTAINED

Doctors at the Cholera hospital are studying a new type of vibrio which is responsible for cholera. This organism has been found in your family member/in your neighbour's family/in the water you use for domestic purposes. They are trying to find out the age and sex of the people the organisms commonly attack, changes in the blood after attack, duration of persistence in human and in the water, manifestation of the illness they produce, the mode of transmission and the way they can be interrupted. We will help you in treatment as much as practicable.

You can participate or refuse to cooperate with the study or you can ask any question in this respect. Your refusal will not affect the treatment of your illness in our hospital. Information collected from you and your specimen will not be given to anyone other than yourself and the doctors will combine it with information obtained from other patients. We would like to know information on socioeconomic status, age, sex, illness, travel of members, and also would require your stool specimen or rectal swab and finger stick blood twice for culture and examination and water and food samples when required. If you agree please put your signature or LTI.

I agree to cooperate/I allow my ward to cooperate  
Signature : \_\_\_\_\_  
or, LTI of \_\_\_\_\_  
Date \_\_\_\_\_

ଅଭିଯୋଗ ପତ୍ର

ପିଲାତ ଶିକ୍ଷକ ଶାନ୍ତ ଭଣ୍ଡାରି ଓ ଅଧ୍ୟାପକଙ୍କୁ ନାନାପ୍ରକାର  
ନିରାକାର୍ଯ୍ୟ ଓ ଅସମ୍ଭବ କାର୍ଯ୍ୟକ୍ରମ ନିମ୍ନ ସଂସ୍ଥାରେ ଲେଖି  
ଦିଆ ଯାଉଛି, ଏହା ଅଧିକାରୀଙ୍କୁ ସୂଚାଇ ଦିଆଯାଉ, ଲେଖି  
କରିବାପାଇଁ ଅନୁରୋଧ କରାଯାଉଛି ଏହା ଲେଖିକାର କାର୍ଯ୍ୟ ଓ  
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ଏ ପାଇଁ କୌଣସି ପଦକ୍ଷେପ ନେବାକୁ ଅନୁରୋଧ କରାଯାଉଛି।

ଏହାପାଇଁ ପାଠ୍ୟପୁସ୍ତକ (୧୦ ମିନ) ଆମନାତରୁ କିଛି କାର୍ଯ୍ୟ  
କାର୍ଯ୍ୟକ୍ରମ କିଛି, ଏହାକୁ ଏହାପାଇଁ ମାନି ନିମ୍ନ ସଂସ୍ଥାରେ  
ଆମେ ୧୦୦ ଲୋକ ଏହା ପଢ଼ିବାକୁ ଲେଖି ୧୦୦, ୧୫୦  
ଆମନାତରୁ (୧୫୦), ନାନାପ୍ରକାର କାର୍ଯ୍ୟ, ମାନିତ୍ର ଏହା  
ଏହାପାଇଁ ଆମା ପଢ଼ିବାକୁ, ଏହାକୁ ପଢ଼ିବାକୁ ଆମନାତରୁ ବାଧ୍ୟକରି  
ଏ ଆମିକି ଲେଖି କିଛି ୧୫୦, ଏହାକୁ ଆମନାତରୁ ଲେଖି  
ଏହାକୁ ଏ ଲେଖି ୧୦୦ ଲେଖି ଏହାପାଇଁ ଏ ଆମନାତରୁ  
ଏହାକୁ ଏହା ଲେଖିକାର କାର୍ଯ୍ୟକ୍ରମ ନେବାକୁ ନାହାନ୍ତି।

ଏ ପଢ଼ିବାକୁ ଲେଖିକାର କାର୍ଯ୍ୟକ୍ରମ ଆମନାତରୁ ୧୦୦ ଓ  
୧୫୦ ଲୋକ ଏହାକୁ କାର୍ଯ୍ୟକ୍ରମ ଏହାକୁ ଲେଖି ଆମନାତରୁ  
ଏହାକୁ ଏହାକୁ, ଏହାକୁ ଲେଖି ଏହାପାଇଁ ଲେଖିକାର (୧୦୦  
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ଲେଖି କାର୍ଯ୍ୟକ୍ରମ ଲେଖି ଏହାକୁ ୧୦୦ ଲେଖି କାର୍ଯ୍ୟକ୍ରମ  
ଲେଖିକାର ମିଳାଏ।

ଆମର ଆମନାତରୁ କାର୍ଯ୍ୟକ୍ରମ/କାର୍ଯ୍ୟକ୍ରମ କାର୍ଯ୍ୟ (୧୫୦) ଏହା ମିଳାଏ।

- ନାମ -
- ଠିକଣା -
- ଠିକଣା -
- ଠିକଣା -