

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date \_\_\_\_\_

Principal Investigator Mrs. Khaleida Haider Trainee Investigator (if any) \_\_\_\_\_

Application No. 85-018 Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_

Title of Study: "Plasmid Profile Analysis of Epidemic Shigella Dysenteriae type 1 strains" 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 Cttee. for review.

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

Khaleida Haider  
Principal Investigator

Trainee

85-018  
25/7/85

## SECTION I - RESEARCH PROTOCOL

1. Title : Plasmid profile analysis of Shigella dysenteriae type I strains.
2. Principal Investigator : Mrs. Khaleda Haider  
Co-Investigators : Dr. M.I. Huq  
Dr. Bradford A. Kay
3. Starting Date : When budget approved
4. Completion Date : One year from the date of starting
5. Total direct cost : US \$ 15,635.00
6. Scientific Program Head :

This protocol has been approved by Disease Transmission Working Group.

Signature of the Program Head : David A. Sack

Date : July 10, 1985

7. Abstract Summary :

This study seeks to examine and compare the plasmid profile of S. dysenteriae type I strains isolated during epidemic and non epidemic periods from different geographical locations of the world. In addition, antibiotic resistances will be examined to determine if a particular resistance pattern may serve as a plasmid marker.

Previously, we have shown that in S. dysenteriae type I and S. flexneri there are multiple plasmids present with varying molecular weights(1). Plasmid profile analysis showed that 96% of the S. dysenteriae type I strains isolated during epidemic periods in Dhaka, Bangladesh harboured four plasmids in common, with approximate molecular weights of 140, 6, 4 and 2 Mdal.

However, *S. dysenteriae* type 1 strains isolated from individual cases during non-epidemic periods showed different banding patterns. In addition, other species of *Shigella* which gave diverse plasmid profiles were also different from the plasmid pattern of epidemic-associated strains of *S. dysenteriae* type 1.

We propose to further investigate the apparent genetic similarities among epidemic strains of *S. dysenteriae* type 1 and to determine if these strains possess unique plasmid profiles from non-epidemic strains. In order to accomplish this, we will examine the plasmid profiles of three hundred strains of *S. dysenteriae* type 1 isolated during epidemic and non epidemic periods from various locations of Bangladesh. In addition, approximately 200 strains of *S. dysenteriae* type 1 from individual cases and from different geographical areas which have experienced epidemics such as India, Burma, Sri Lanka, Mexico and Peru will be solicited for inclusion in this study from investigators worldwide. Each strain received will be examined for its plasmids as well as its antibiotic resistances.

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION

#### 1. Objectives :

- a. To obtain the plasmid profiles of Shigella dysenteriae type I strains from sporadic cases and epidemics from geographically diverse areas.
- b. To compare the plasmid profiles of S. dysenteriae type I from sporadic cases with those from epidemics.
- c. To obtain the antibiotic resistances of S. dysenteriae type I isolates from sporadic cases and epidemics.
- d. To compare the antibiotic and plasmid profiles to determine if antibiotic markers associate with a unique plasmid profile(s) from epidemic and/or non-epidemic associated strains.

#### 2. Background

Plasmid profile analysis has proven to be a useful tool in epidemiological studies of infectious diseases by various microorganisms (2,3,4). Electrophoresis of plasmid DNA in agarose gel provides a simple and useful tool to aid in the identity of epidemic strains of bacteria. Since plasmids can be transferred from one bacterial species to another, it is sometimes the plasmid rather than the bacterial strains which is

epidemic. Unlike other methods of identifying bacteria, such as phage type, serotype, biotype and antibiogram, this approach can be used to identify epidemic strains of bacteria. Epidemic plasmids have been known to spread through several different species(3). Brunner and colleagues (2) compared the effectiveness of plasmid profile analysis with phage typing and antibiotic resistance patterns. They found plasmid analysis to be rapid, relatively simple to perform, as specific as phage typing and superior to biotyping and antibiotic resistance patterns.

Shigellosis is a major cause of mortality in developing countries(6,7). In the past 15 years there have been reports of dysentery outbreaks caused by multiply resistant S. dysenteriae type I strains in several developing countries throughout the world. The first of these epidemics started in Guatemala in 1969 and spread to El Salvador, Nicaragua, and Mexico during the next two years(8). Likewise, there was an epidemic in Dhaka, Bangladesh due to S. dysenteriae type I between July 1972 and December 1973(9). In June 1973, there was another outbreak of bacillary dysentery in Bangladesh, this time on St. Martin's Island in the Bay of Bengal(10).

Multiply drug resistant S. dysenteriae type I strains were have been isolated from patients with bacillary dysentery in Karnataka and Kerala during 1976 and 1977 (11), in Vellore between 1973 and 1977 (12), in Bangalore in 1978 and 1979 (13), and in West Bengal in 1984 (14), all of these places being in India.

In Sri Lanka there was an outbreak due to S. dysenteriae type I in 1976-78 (14), at about the same time there was a similar outbreak in the Colombo region (15).

High mortality was reported from Somalia in 1963-64 due to an epidemic caused by S. dysenteriae type I (17). Currently, a major epidemic due to multiply resistant S. dysenteriae type I has been ongoing since 1979 in north-east Zaire (18).

In Bangladesh, an epidemic due to S. dysenteriae type I was recognized in April Dhaka in April 1983(19). A similar outbreak in the Rangpur district was reported in 1985(20).

Among the Shigella dysenteriae type I is apparently unique in its virulence, antibiotic resistance and epidemic potential. For these reasons we have selected this organism as the subject of this study.

### 3. Rationale

Past studies at ICDDR,B and other institutions worldwide have established the usefulness of plasmid profiles as epidemiologic and research tools. Comparison of plasmid and antibiotic profiles of S. dysenteriae type I from worldwide sporadic and epidemic-associated cases has the potential for supplying the following information :

- a. The identification of a unique epidemic-associated plasmid profile(s)

- b. The identification of potential virulence-associated plasmid(s).
- c. The identification of virulence-associated antibiotic profile(s).
- d. The identification of antibiotic resistance(s) associated with potential virulence plasmid(s).

The identification of these characteristics may indicate areas of future research on the elaboration of plasmid-mediated virulence of S. dysenteriae type I.

#### B. SPECIFIC AIMS

- a. To obtain a collection of Shigella dysenteriae type I strains from epidemic and non-epidemic dysentery cases from geographically diverse sources.
- b. To determine the plasmid profile and antibiotic resistance pattern of each strains.
- c. To compare the plasmid and antibiotic profile between epidemic and non-epidemic strains as well as between different geographic regions.
- d. To seek to identify for further study a plasmid or plasmids which are potentially linked to epidemic-associated strains, or which may serve as a marker of epidemic-associated strains for epidemiological purposes.

### C. MATERIALS AND METHODS

Five hundred strains of Shigella dysenteriae type I isolated during epidemic and non-epidemic periods from various parts of the world will be included in this study. The date and place of isolation will be noted as well as its epidemiologic associations. Antibiotic resistance patterns of all strains will be assessed by the agar disc diffusion method (21). Commercially available standard antibiotic discs (BBL, Cockeysville, MD 21030, USA) containing Ampicillin (10 ug), Chloramphenicol (30 ug), Tetracycline (30 ug), Streptomycin (10 ug), Kanamycin (30ug), Gentamycin (10ug) and Trimethoprim-sulfamethoxazole (1.25 + 23.75 ug) will be used. Nalidixic acid (30 ug) will also be included. The control strain of Eschericia coli, (ATCC-25922), will be used in each experiment.

Plasmids will be extracted according to the method of either Kado and Liu(22) or Birnboim(23) which allows the detection of small and large plasmids. Plasmids will be separated by electrophoresis on 0.7% agarose (at 30 mA for 150 min) and will be visualized by staining with ethidium bromide (0.1 ug/ml) solution for 20 min and destained in distilled water for one hour. Gels will be photographed with a Polaroid MP4 camera. Approximate molecular weights will be calculated by comparing plasmids of known molecular weights (E. coli V-517, and Pdk-9, and R1) with those isolated.

#### D. SIGNIFICANCE

This study should allow us to determine if a unique plasmid-associated genotype is common to strains of *S. dysenteriae* type 1. If a candidate plasmid profile is identified, the plasmid(s) could be analysed and characterized in depth to assess their phenotypic and virulence-associated properties. This information could significantly add to the understanding of the mechanisms of the pathogenesis of this organism. It is also possible that a unique plasmid pattern could be used as an epidemiologic tool for identifying epidemic-associated strains.

#### E. FACILITIES REQUIRED

No new laboratory space will be needed. The existing laboratory space and equipment used for doing current plasmid studies will be sufficient for carrying out this protocol.

#### F. COLLABORATIVE ARRANGEMENT

It may become necessary, during the course of the protocol, to consult with other investigators working in this field. The interpretation of the plasmid patterns we will generate will require careful examination and analysis. The attached list of prospective donors also represents a group of well established scientists some of which may work in collaboration with us.

## SECTION II

### A. DETAILED BUDGET

#### 1. PERSONNEL SERVICES

| Name                | Position               | % Time used | Annual salary | Project Requirement | TAKA | DOLLAR |
|---------------------|------------------------|-------------|---------------|---------------------|------|--------|
| Mrs. Khaleda Haider | Principal Investigator | 75          | Tk. 79812     | 59,859/-            | -    | -      |
| Dr. M.I. Huq        | Co-Investigator        | 10          |               | -                   | -    | -      |
| Dr. Bradford Kay    | Co-Investigator        | 10          |               | -                   | -    | -      |

Sub total Tk. 59,859/-

#### 2. SUPPLIES AND MATERIALS

|                                   |        |
|-----------------------------------|--------|
| Chemicals and Laboratory supplies | 6500/- |
| Media                             | 1000/- |
| Office supplies                   | 5000/- |
| Polaroid film                     | 600/-  |

#### 3. EQUIPMENT - None

#### 4. PATIENT HOSPITALIZATION - None

#### 5. OUTPATIENT CARE - None

#### 6. ICDDR,B TRANSPORT 100

#### 7. TRAVEL AND TRANSPORTATION OF PERSONS - None

#### 8. TRANSPORTATION OF THINGS - 1000

#### 9. RENT, COMMUNICATION AND UTILITIES - None

Project Requirement  
TAKA DOLLAR

10. PRINTING AND REPRODUCTION

Xerox

Tk. 4000/-

Publication

Tk. 5000/-

11. OTHER CONTRACTUAL SERVICES - None

12. CONSTRUCTION, RENOVATION AND ALTERATION - None

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Total Tk. 73,859/- \$9200/-

# B. BUDGET SUMMARY

|                                             | <u>TAKA</u> | <u>DOLLAR</u> |
|---------------------------------------------|-------------|---------------|
| 1. Personnel Services                       | 59,859      | -             |
| 2. Supplies Materials                       | 5,000       | 8100          |
| 3. Equipment                                | -           | -             |
| 4. Patient Hospitalization                  | -           | -             |
| 5. Outpatient Care                          | -           | -             |
| 6. ICDDR,B Transport                        | -           | 100           |
| 7. Travel of persons                        | -           | -             |
| 8. Transportation of things                 | -           | 1000          |
| 9. Rent, Communication and utilities        | -           | -             |
| 10. Printing and Reproduction               | 9,000       | -             |
| 11. Other contractual services              | -           | -             |
| 12. Construction, Renovation and Alteration | -           | -             |
|                                             | -----       | -----         |
|                                             | Tk. 73,859  | \$ 9200       |
|                                             | =====       | =====         |

Taka Equivalent to US \$ 2735.00

Total Direct costs US \$ 11935.00

Management and Scientific Support  
Costs 31% \$ 3700.00

Grand total US \$ 15,635.00

Conversion Rate US \$ 1:00 = 27.00

Dear Colleague

We are preparing for a WHO-funded project which will require the examination of a large number of Shigella dysenteriae type I (Shigella shiga) strains for their plasmids. these strains must be from a wide range of geographically diverse areas and should represent both isolated as well as epidemic associated cases. We anticipate that this project could identify future areas of research on the virulence and epidemiological interactions of S. dysenteriae type I strains worldwide.

If you would be willing to supply a group of strains for this study we would be most grateful. When submitting the strains, would you please fill out (as for documentation of the isolates.

We appreciate your support and would be most happy to acknowledge your donation of strains in any forthcoming publication from this study. In addition, we would be happy to correspond with you as to our findings.

Thank you.

Sincerely yours,

BRADFORD, A. KAY, DR. P.H.  
Head, Microbiology Branch

BAK:pm

### References

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2. Brunner, F., A. Margadant, R. Peduzzi, J.C. Piffaretti, 1983. The plasmid pattern as an epidemiological tool for Salmonella typhimurium epidemics; comparison with the lysotype. J. Infect. Dis. 148: 7-11.
3. Farrar, W.E. 1983. Molecular analysis of plasmids in epidemiologic investigation. J. Infect. Dis. 148: 1-6.
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5. Chabbert, Y.A., N. Elsolh, M.J. LePors, A. Rousell, J.L. Witchitz, 1979. Plasmid epidemics, In: Grassi, C., Ostino G. eds. Antibiotics and hospitals. Infect. Immun. 35: 625-632.
6. Mata, L.J., E.J. Gangarosa, A. Caceres, D.R. Perera, and M.L. Mejicanos, 1970. Epidemic Shiga bacillus dysentery in Central America, I. Etiologic Investigation in Guatemala, 1969. 122: 170-80.

7. Rahaman, M.M., M.U. Khan, K.M.S. Aziz, M.S. Islam, and A.K.M.G. Kibriya, 1975. An outbreak of dysentery caused by Shigella dysenteriae type 1 on a coral island in the Bay of Bengal. J. Infect. Dis. 132: 15-19.
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17. Cahill KM, Davies JA, Johnson R. Report on an epidemic due to Shigella dysenteriae type 1 in the Somalia interior. Am J Trop Med Hyg. 1966; 15:52-56.
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23. Birnboim HC and Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res 1979; 1513-23.



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BACTERIAL STRAIN INFORMATION

Date \_\_\_\_\_

| Sl. Strain designation | Genus and species | Biotype sero-type etc. | Date of isolation | Place of isolation | Isolated case or epidemic* | Remarks |
|------------------------|-------------------|------------------------|-------------------|--------------------|----------------------------|---------|
| 1                      |                   |                        |                   |                    |                            |         |
| 2                      |                   |                        |                   |                    |                            |         |
| 3                      |                   |                        |                   |                    |                            |         |
| 4                      |                   |                        |                   |                    |                            |         |
| 5                      |                   |                        |                   |                    |                            |         |
| 6                      |                   |                        |                   |                    |                            |         |
| 7                      |                   |                        |                   |                    |                            |         |
| 8                      |                   |                        |                   |                    |                            |         |
| 9                      |                   |                        |                   |                    |                            |         |
| 10                     |                   |                        |                   |                    |                            |         |
| 11                     |                   |                        |                   |                    |                            |         |
| 12                     |                   |                        |                   |                    |                            |         |
| 13                     |                   |                        |                   |                    |                            |         |
| 14                     |                   |                        |                   |                    |                            |         |
| 15                     |                   |                        |                   |                    |                            |         |

\* Strain epidemiologically linked to an outbreak or epidemic

The accompanying bacterial strain(s) are hereby released to/by (mark out one) the International Centre for Diarrhoeal Disease Research, Bangladesh. I do/do not (mark out one) request acknowledgement of their origin if they are cited in scientific publications.

\_\_\_\_\_  
Name (Please Print)

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date