INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

MEMORANDUM

February 1, 2000

To : Chairman, Research Review Committee

Through : Division Director, LSD F. Qadri

From : Dr. Kaisar Ali Talukder

P.I. Protocol No. 99-039 : Molecular epidemiology of *Shigella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complications.

I would like to include Dr. A.S.G. Faruque, Scientist, CSD, as an investigator in the above ongoing USAID Funded Project for its smooth running.

I would appreciate it very much if you would kindly approve the inclusion of Dr. Faruque as one of the Co-investigator of the above Protocol.

Thank you.

Cc: Dr. A.S.G. Faruque, CSD

Approved at the PRC meeting on 19/2/2000

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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

17 November 1999

To : Dr. Kaiser Ali Talukder
Laboratory Sciences Division

From : Professor V. L. Mathan
Chairman, Research Review Committee

Sub Approval of protocol # 99-039

I am pleased to inform you that the Research Review Committee in its meeting held on 15th November 1999 approved your protocol # 99-039 entitled "Molecular epidemiology of *shigella dysenteriae* type 1 strains associated with haemolytic uraemic syndrome (HUS) and other complications."

Thanking you and wishing you success in running the above mentioned study.

cc: Division Director
Laboratory Sciences Division

Principal Investigator: Last, first, middle _____Talukder KA_____

International Centre for Diarrhoeal Disease Research, Bangladesh

FOR OFFICE USE ONLY
Protocol No: Date:

RRCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:

RESEARCH PROTOCOL
99-039

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)
Molecular epidemiology of *Shigella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complications

2a. Name of the Principal Investigator(s) (Last, Middle, First).	**2b. Position / Title**	**2c. Qualifications**
Talukder KA	Asstt. Scientist	Ph.D.

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
Laboratory Sciences Division

4. Contact Address of the Principal Investigator
4a. Office Location:
ICDDR,B, GPO Box 128, Dhaka -1000
Bangladesh

4b. Fax No: 880 2 872529/883116
4c. E-mail: katalukdar@hahoo.com
4d. Phone / Ext: 871751-60/2405, 2406

5. Use of Human Subjects	**5a. Use of Live Animal**	**5b. If Yes, Specify Animal Species**
Yes [ ]	Yes [ ]	
No [x]	No [x]	

6. Dates of Proposed Period of Support
(Day, Month, Year - DD/MM/YY)

ASAP---Two years from starting date

7. Cost Required for the Budget Period
7a. Ist Year ($): 20,735 2nd Year ($): 15,806 Overhead: 9,135

7b. Direct Cost ($) 36,541 Total Cost ($) 45,676

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well as by the external reviewers.The protocol has been revised accordingly to the reviewers comments and is approved.

Prof. V. I. Mathan
Name of the Division Director

Signature

4/11/99
Date of Approval

9.Certification by the Principal Investigator
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

10. Signature of PI
Kaisar Ali Talukder

Date: 4-11-99

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☐ Check here if appendix is included

Principal Investigator: Last, first, middle _____Talukder KA_____

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (TYPE TEXT WITHIN THE SPACE PROVIDED).

Principal Investigator DR. KAISAR ALI TALUKDER.

Project Name: Molecular epidemiology of *Shigella dysenteriae* type 1strains associated with haemolytic-uraemic syndrome (HUS) and other complications.

Total Budget US $ 45,676 Beginning Date ASAP Ending Date two years from Starting date.

Shigellosis represents one of the most severe forms of acute bacterial gastroenteritis, which can also lead to some extraintestinal complications like haemolytic-uraemic syndrome (HUS), encephalopathy, and septicemia due to invasion of the organism into the bloodstream. Although *Shigella dysenteriae* type 1 has been associated with HUS more commonly than the other serotypes, only a small fraction of all the cases suffering from this infection will develop HUS or the other severe complications like encephalopathy and septicemia. Although host factors may play important roles in determining whether or not a patient would develop a serious complication, it is equally important to examine whether some particular genetic element is common among the strains of *Shigella dysenteriae* type 1 that cause HUS and other complications. Pulsed-field gel electrophoresis (PFGE) has recently emerged as a useful tool for studying molecular epidemiology of various microorganisms causing disease in humans. In our previous studies, PFGE analysis of *Shigella dysenteriae* type 1 strains has revealed numerous fragment profiles (29, 30). This study seeks to examine and compare the PFGE profile of *S dysenteriae* type 1 strains isolated from cases of shigellosis, which develops HUS, or encephalopathy, or septicemia with those, which do not develop such complications. In addition, the results will also be helpful to analyse whether those strains, which show resistance against multiple antibiotics also, show any common pattern in respect of their PFGE profiles. The results of this study may therefore lead to a better understanding of the pathogenesis of *S. dysenteriae* type 1 causing HUS and other severe complications, with an opportunity to study the genetic profiles of the multidrug resistant strains.

KEY PERSONNEL (List names of all investigators including PI and their respective specialites)

Name	Professional Discipline/ Specialty	Role in the Project
1. Kaisar Ali Talukder, PhD	Molecular Biology	Overall responsibility for the project
2. K.M.A. Jamil, MBBS, PhD.	Medicine	Examine patient history and entry data of CRF
3. Wasif A. Khan, MBBS	Medicine	Examine patient history and entry data of CRF
4. M.John Albert, PhD, MRCPath	Microbiology	Provide strategic and academic feedback
5. Prof. V.I. Mathan, MD, PhD,FRCP, FMA, FNA	Gastroenterology	Provide strategic and academic feedback

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

We propose to study the molecular epidemiology of *Shigella. dysenteriae* type 1 strains isolated from patients with or without HUS, encephalopathy or septicemia. We hypothesize that the severity of illness is associated with some specific strains of *S. dysenteriae* type 1, which are clonally related. This will be determined by PFGE profile analysis.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1. To compare *S. dysenteriae* type 1 strains isolated from shigellosis patients developing HUS and encephalopathy, and septicemia with those without such complications, by pulsed-field gel electrophoresis (PFGE) of genomic DNA.

2. To examine if a specific DNA profile is found to be significantly correlated with a set of clinical manifestations.

3. To study the antimicrobial susceptibility pattern of different PFGE types of *S. dysenteriae* type 1 and examine if any particular genetic element is found common among the multidrug resistant strains.

4. To examine from the above analysis possible clonal relationship between the prevailing HUS strains in the community.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is

no sufficient information on the subject, indicate the need to develop new knowledge. Also include the significance and rationale of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

Bacillary dysentery caused by the invasive enteric bacteria *Shigella* is a major public health problem throughout the developing world and is a significant cause of morbidity and mortality particularly in children below 5 years of age. *S. dysenteriae* type 1 is responsible for over two million cases of severe dysentery in the world every year, and an estimated 250,000 deaths, many of which occurred during epidemic outbreaks. An epidemic among Rwandan refugee camps in Goma, Zaire, in 1994, killed an estimated 30,000 persons in a period of two weeks. Among the different species of *Shigella*, *S. dysenteriae* type 1 produces Shiga toxin and is associated with the most severe disease in young children which includes leukemoid reaction and haemolytic uraemic syndrome (6, 10, 20 23). *Shigella* encephalopathy and septicemia caused by this organism are two other dreaded complications of shigellosis which significantly increase morbidity and mortality due to this infection. Factors that determine the development of HUS in shigellosis are not clearly understood. In our recently conducted study on shigellosis, out of 124 *S. dysenteriae* type 1 isolates, haemolytic uraemic syndrome and leukaemoid reaction developed in 26 (21%) and 33 (27%) patients respectively (21). In another study seizure was witnessed in the hospital in 13 (8%) of 157 patients infected with *S. dysenteriae* type 1 (14). Varied clinical presentations, and development of complications occurring in a fraction of infected persons indicates that there is a role of both host factors as well as microbial factors. It is possible that the severity of the disease is different when different subtypes of *S. dysenteriae* type 1 strains, which are yet to be identified, cause infections.

The role of shiga toxin (*stx*) genes for the pathogenesis of enterohemorrhagic *Escherichia coli.* Several investigators (8, 10) have studied enterohemorrhagic *E. coli* (EHEC) with severity of disease. It has been reported by Bonnet *et al.* (5) that non-o157:H7 stx_2-producing *E. coli* strains are associated with sporadic cases of HUS in adults. To the best of our knowledge there are no detailed studies with *Shigella dysenteriae* 1 causing HUS and other complications.

Multiresistance of *Shigellae* is common in Bangladesh. Pivmecillinam (selexid) is one of the most effective oral drugs, which is widely used for treating shigellosis with about 90% sensitivity against all strains of *Shigella.* Currently, resistance against pivmecillinam in *S. dysenteriae* type 1 has been found to be about 5% in the Dhaka hospital of ICDDR,B and 10% in Matlab. Studies conducted elsewhere suggested that most of the strains harboured easily mobilisable resistance plasmid (9, 15) but some strains exhibited non-transferrable drug resistance located on the chromosome (9). Very recently Rajakumar K. *et.al.* (26, 27) have reported that a spontaneous 99 Kb chromosomal fragment showed chromosomally encoded antibiotic multi-resistance in *Shigella flexneri.* They also showed that this 99-Kb fragment was involved in reduction of contact haemolytic activity of chromosomally encoded antibiotic multi-resistance in *Shigella* spp. Thus it is important to know if there is any difference in the susceptibility patterns among the different PFGE types of *S. dysenteriae* type 1 strains for epidemiological studies.

In order to be able to study the epidemiology of *S. dysenteriae type* 1 strains causing HUS and other complications, isolates have to typed below the pheno- or genospecies level. Several

different typing method can be used for strains discrimination, e.g. molecular methods, such as PFGE, ribotyping, RAPD, plasmid profiling and PCR. Analyses of plasmid content and antimicrobial susceptibility pattern have been used in the past for strain discrimination of certain *Shigella* sp. (28, 33). However, these methods have limitations as plasmids are unstable, and in many instances, antimicrobial resistance is encoded by plasmid (19). These methods have now been superseded by ribotyping, which relies on polymorphism in the regions surrounding rRNA (1i) and pulsed-field gel electrophoresis (PFGE) of whole chromosomal DNA (31). In the latter, chromosomal DNA is digested with a restriction enzyme. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness. PFGE has been employed successfully for strain discrimination of a variety of bacteria including *E.coli* 0157:H (3), *Shigella sonnei* (7, 16) and *Shigella dysenteriae 1* (29,30). Recently Blaser *et al* (5) and Strockbine *et al.* (25) showed that the restriction fragment length polymorphism (RFLP) of the Stx gene indicated at least three separate clones of the *Shigella dysenteriae* 1 strains. Very recent we found that the epidemic isolates yieided two types each with two subtypes, whereas the endemic isolates and culture collection yielded eight types with numerous subtypes (30). But to our knowledge, there has been no study where this technique was used for strain discrimination of *S. dysenteriae* 1 causing HUS and others complications. Therefore, we will use the utility of PFGE in typying *S. dysenteriae* 1 isolates. We will also include some *Escherichia coli* 0157:H7 reference strains to find commonalities between *Shigella dysenteriae* type 1 strains associated with complications and *Escherichia coli* 0157:H7.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Sourch of strains

We propose to study 200 strains collected both from Dhaka and Matlab. Strains of *Shigella dysenteriae* type 1 which have been collected from previous studies, on going studies and from the 2% surveillance system of ICDDR,B will be tested. Information regarding the clinical aspects of the patients from whom the strains have been isolated will be obtained from archived data and from Principal Investigators of the respective studies.

Strains confirmation

S. dysenteriae type 1 isolates will be confirmed by biochemical reactions and agglutination with specific antiserum (32).

PFGE

For molecular typing PFGE will be performed by standard method (22). In brief, bacterial cells on an agar medium will be directly embeded in low melting agarose (Bio-rad Lab, Calif, USA). After appropriate preparation, genomic DNA in 1% clean-cut agarose (Bio-Rad) will be digested with restriction enzymes (Gibco BRL, Gaithersburg, MD) and incubated overnight at 37°C. DNA will be electrophoresed on 1% pulsed-field-certified agarose (Bio-Rad) as described previously (2) but with different pulse times. The DNA size standards will be used are the bacteriophage lambda-ladder consisting of concatamers starting at 48.5 kbp and increasing to 1000 kbp (Bio-Rad), and *Saccharomyces cerevisiae* chromosomal DNA ranging from 225-2200 kbp (Bio-Rad). Determination of band size will be measured by measuring the migration distances of the bands and extrapolation to a standard curve by plotting migration distances against the logarithmic molecular sizes of the DNA size standards.

Preparation of chromosomal DNA

Chromosomal DNA from *Shigella dysenteriae* 1 strains will be extracted by using the proceedure described by Marmur (18).

Detection of Shiga toxin (Stx) genes and copy number by Southern blot hybridization

The Stx gene will be detected by southern blot hybridization (17). The chromosomal DNA from each strain will be extracted and digested with *EcoR*1 restriction enzyme (24). After electrophoresis the DNA will be transferred to positively charged nylon membrane (Boehringer Mannheim). The oligonuecleotides will be synthesized using a Beckman Oligo-1000 DNA synthesizer available in our laboratory. Two primers homologous to the A-subunit gene of *stx/slt-1* (24) will be used to amplyify a 680-bp fragment. The sequence of the 5' primer is GACAGGATTTGTTAACAGG, and the 3' primer is TTCCAGTTACACAATCAGGC. The polymerase chain reaction (PCR) will be done according to procedure as described earlier (13). The amplified PCR product will be labeled with digoxigenin-dUTP (DIG) (Boehiringer Mannheim) using a random primer DNA labeling kit (Boehringer Mannheim). DIG-labeled probes will be recovered by ethanol precipitation, resuspend in TE buffer (10mM Tris-HCL, 1mM EDTA, pH 8.0) and store at -20°C until used. Immeiately prior to use, the probes will be denatured to a single-stranded DNA by boiling for 10 min and then chilling on ice to prevent renaturation. Hybridization of

the blots and development of the blots with anti-DIG-alkaline phosphatase will be done according to the instructions provided in the DIG DNA Labeling and Detection kit (Boehringer Mannheim). Hybridized probe and number of copies of *stx* gene will be determined by following the manufacturer instruction (Bohringer Mannehim).

Southern blot analysis with probes linked to resistance determinants

After PFGE, DNA both from sensitive and multi-drug resistance of *Shigella dysenteriae* 1 strains prepared according to the protocol described previously, will be transferred on to a positively charged nylon membrane (Boehringer Mannheim) and blot will be prepared according to the protocol described by Rajakumar K. *et.al.* (26, 27). Probe for specific antibiotic marker as described (26, 27), will be labelled with digoxigenin-dUTP (DIG) according to the protocol described by the manufacturer (Boehringer Mannheim). Hybridization of the blots and development of the blots with anti-DIG-alkaline phosphatase will be done according to the instructions provided in the DIG DNA Labeling and Detection kit (Boehringer Mannheim).

ELISA for Shiga toxin (Stx)

The quantities of Stx produced by a strain representative of each goups of PFGE pattern will be determined by ELISA method as described by Gouveia *et al (10).*

Antimicrobial Susceptibility Test:

The antimicrobial susceptibility test will be done by the method of Bauer *et al.* (4) using commercially available disks (BBL Microbiology system) at specific antibiotic concentrations.

Interpretation of clinical data

The clinical features of the patients enrolled will be recorded in a data sheet and analysed later to categorize them as severe and non-severe cases. Those developing the following complications will be termed as severe: (i) haemolytic-uremic syndrome, (ii) renal failure without complete HUS, (iii) convulsion, coma or other neurologic manifestations, (iv) septicemia and/or shock

Interpretation of PFGE band patterns

We will use the utility of PFGE in typing *S. dysenteriae* 1 isolates. As one approach, Tenover *et al.* (31) proposed that strains be grouped as indistinguishable, closely related, possibly related, or

different based on the number of restriction fragment differences when compared with outbreak strain. Isolates will be considered genetically indistinguishable if they possess PFGE patterns with the same number and same size of bands. Closely related strains differ by changes consistent with a single genetic event (2-3 band differences), and possibly related strains differ by changes consistent with two independent events (4-6 band differences). Unrelated strains differ by three or more independent genetic events (≥7 band differences). Genetic events are defined by deletion, addition or substitution of bases. The use of PGFE of *Not*1-digested DNA fragments will be applied for typing of *S. dysenteriae* type 1 isolates (30, 31).

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

Existing Hospital, laboratory and office facilities of ICDDR,B are adequate to carry out the project. This is a laboratory-based study, which will be carried out with stored clinical isolates, isolates from on going projects and from 2% surveillance system. The project will require some bacterial culture facilities and molecular biology facilities to perform the techniques, such as, PFGE, Southern blot hybridization, cloning, ELISA etc. The mentioned facilities are available in the Bacteriology and Molecular biology laboratory of Laboratory Sciences Division. The data for patient's history are available in the hospitals record book. A computer will be needed for analysis the experimental data.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

We will use different molecular biology techniques to analyze data for comparison the disease severity of the patients infected with *S. dysenteriae* type 1. The proportion, trends and patter obtained using different techniques will be compared using the Chi-squared test, Fisher exact test or other appropriate statistical test. These test will be used to compare different patters obtained from the patients within the groups and between the different groups in different degrees of disease severity. Statistical package used will be SPSS for Windows.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

N/A

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. **Adeleye, I.A.** 1992. Conjugl transferability of multiple antibiotic resistance in the three genra of Enterobacteriaceae in Nigeria. J. Diarrhoeal Diis Res. 10:93-96.

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3. **Arbeit, R.D., Arthurs, M., Dunn, R., Kim, C., Selander, R.K., and Goldstein R.** 1990. Revolution of recent cvoidtionary divergence among *Escherichia coli* from related lineges: the application of pulsed field electrophoresis to molecular epidemiology. J. Infect. Dis. 161:230-235.

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29. **Talukder K.A. and M.J. Albert**. 1998. Molecular analysis of *Shigella dysenteriae* type 1 strains by using pulsed-field gel electrophoresis. JDDR, **16(2)**: 141.

30. **Talukder K.A., D.K. Dutta and M.J. Albert.** 1999. Evaluation of pulsed- field gel electrophoresis for typing of *Shigella dysenteriae* type 1. J. Med. Microbiol. **48: 781-784.**

31. **Tenover, F.C., R.D. Arbeit, R.C. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing, and B. Swaminathan**. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233-2239.

32. **World Health Organization**. 1987. Programme for control of diarrhoeal disease (CDD/93.3, Rev. 1), p.9-20. *In*: Manual for laboratory investigation of acute enteric infections. World Health Organization, Geneva.

33. **Yagupsky, P., M. Loeffleholz, K. Bell, and M.A. Menegus**. 1991. Use of multiple markers for investigation of an epidemic of *Shigella sonnei* infections in Monroe County, New York. J. Clin. Microbiol. 29:2850-2855.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The study will provide further understanding the disease pathogenesis and may help the clinicians at an early stage in a more aggressive management of patients infected with *S. dysenteriae* type 1 with a specific type of strains causing HUS and other complications. It is planned to present the results of this study both in national and international conferences and to publish the data in an international journal.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (DO NOT EXCEED ONE PAGE)

Nil

Biography of the Principal Investigator

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Dr. Kaisar Ali Talukder	Assistant Scientist LSD, ICDDR,B, Dhaka, Bangladesh	10th Novemver, 1954

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Tokyo	Ph.D.	1993	Molecular Biology
University of Dhaka	M.Sc	1977	Biochemistry
University of Dhaka	B.Sc (Hons)	1976	Biochemistry

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1. Senior Technician (Research), Microbiology Branch, ICDDR,B, March 1981- Nov. 1985.
2. Research Officer, Microbiology Branch, ICDDR,B, December 1985 – October 1987.
3. Research Student: Department of Bacteriology, Institute of Medical Science, University of Tokyo, October 1987 – March 1989.
4. Doctoral student: Department of Bacteriology, Institute of Medical Science, University of Tokyo, April 1989 – March 1993.
5. Research Officer, Laboratory Sciences Division, ICDDR,B, April 1993 – April 1994.B
6. Assistant Scientist, Laboratory Sciences Division, ICDDR,B, May 1994 to till.

Publications of Dr. Kaisar Ali Talukder

1. Okada N, Sasakawa C, Tobe T, Yamada M, Nagai S, **Talukder KA**, Komatsu K, Kanegaseki S, Yoshikawa M.1991. Virulence-associated chromosomal loci of *Shigella flexneri* identified by random Tn*5* insertion mutagenesis. Mol Microbiol ; 5:187-195.

2. Okada N, Sasakawa C, Tobe T, **Talukder KA**, Komatsu K, Yoshikawa M. 1991. Construction of a physical map of the chromosome of *Shigella flexneri* 2a and the direct assignment of nine virulence-associated loci identified by Tn*5* insertions. Mol Microbiol ; 5:2171-2180.

3. Haider K, Kay BA, **Talukder KA**, Huq MI. 988. Plasmid analysis of isolates of *Shigella dysenteriae* type 1 obtained from wide geographical locations. J Clin Microbiol ; 26:2083-2086.

4. Haider K, Huq MI **Talukder KA,** Ahmad QS.1989. Electropherotyping of plasmid deoxyribonucleic acid (DNA) of different serotypes of *Shigella flexneri* strains isolated in Bangladesh. Epidem Infect ; 102:421-428.

5. Haider K, Chatkaeomorakot A, Kay BA, **Talukder KA**, Taylor DN, Escheverria P, Sack DA.1990 Trimethoprim resistance gene in *Shigella dysenteriae*1 isolates obtained from widely scattered locations of Asia. Epidemiol Infect; 104:219-228.

6. Albert, M.J., N.A. Bhuiyan, **Talukder KA,** A.S.G. Faruque, S. Nahar, S.M. Faruque, M. Ansaruzzaman, and M. Rahman. 1997. Phenotypic and genotypic changes in *Vibrio cholerae* O139 Bengal. J. Clin. Microbiol. **35**:2588-2592.

7. **Talukder K.A**. and M.J. Albert. 1998. Molecular analysis of *Shigella dysenteriae* type 1 strains by using pulsed-field gel electrophoresis. JDDR. 16 (2): 141.

8. **Talukder K.A**., D.K. Dutta and M.J. Albert.1999. Evaluation of pulsed- field gel electrophoresis for typing of *Shigella dysenteriae* type 1. J. Med. Microbiol. 48; 781-784.

Principal Investigator: Last, first, middle _____Talukder KA_____

Detailed Budget for New Proposal

Project Title:Molecular epidemiology of *Shiella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complications.

Name of PI: KAISAR ALI TALUKDER

Protocol Number: Name of Division: Laboratory Sciences Division

Funding Source: USAID Amount Funded (direct): US$ 36,541 Total: US$45,676
Overhead 25%, US$ 9135

Starting Date: ASAP Closing Date: Two year from starting date

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	Position	Effort%	Salary	1st Yr	2nd Yr	Total
	Kaisar Ali Talukder	NO-B	50		5388	5688	11076
	Wasif A. Khan	NO-A	5		539	566	1105
	K.M.A. Jamil	NO-B	5		500	525	1025
	Sr. Research Technician	GS-IV	50		2214	2325	4539
	Laboratory Attendant	GS-1	25		723	728	1451
	Sub Total				9,364	9,832	19,196
	Supplies and Materials (Description of Items)						
	Chemicals and media				4000	2500	6500
	Office supplies				150	150	300
	Stock items				750	750	1500
	Non-stock supplies				3500	2000	5500
	Equipment (Computer)				2500	-	2500
	Sub Totals				10,900	5,400	16,300

	Library Service charges	23	24	47
	Travel (local)	250	200	450
	Training Workshop, Seminars			
	Printing and Publication	148	300	448
	Xerox, Fax, Postage	50	50	100
	SUB TOTAL	471	574	1,045

	Direct cost	20,735	15,806	36,541
	Overhead 25%			9,135
	Total cost			45,676

3-11-99
M. Rehman Chowdhury
Senior Budget & Cost Officer
ICDDR, B, Mohakhali
Dhaka-1212, Bangladesh.

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

1. **Equipment:** For data analysis we need computer.

2. **Chemical and media:** Chemicals and media need for isolation and identification of bacteria, DNA analysis, hybridization etc.

3. **Travel**: For collection of samples from Matlab station, ICDDR,B, we need to travel Matlab & Dhaka.

4. **Personels:** No new recruitment is needed. Salary support is budgeted for five staffs who are essential to carry out the work.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

N/A

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☐
2. Approval of the Division Director on Face Sheet ☐
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☐
4. Table on Contents ☐
5. Project Summary ☐
6. Literature Cited ☐
7. Biography of Investigators ☐
8. Ethical Assurance ☐
9. Consent Forms ☐
10. Detailed Budget ☐

Reviewer 1

Title

Summary of Referee's Opinion: Please specify your opinion on available the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank score		
	High	Medium	Low
Quality of Project	–		
Adequacy of Project Design	–		
Suitability of Methodology	–		
Feasibility within time period	–		
Appropriateness of budget	–		
Potential value of field of knowledge	–		

CONCLUSIONS

I support the application:

a) Without qualification ☐ –

b) With qualification ☐

- on technical groups ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee:

Signature _____ Date September 23, 1999

Position:

Professor of International Health and Medicine

Institution:

Johns Hopkins University School of Hygiene and Public Health

Detailed comments

Please briefly provide your comments on the [illegible] with special attention to the originality and [illegible] the proposed [illegible] the justification of financial support [illegible] modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title: Molecular Epidemiology of Shigella dysenteriae type 1

PI: KA Talukder

Reviewer: R. Bradley Sack, M.D., Sc.D.

The author has a unique collection of *Shigella dysenteriae* type 1 strains and wishes to look at their genetic profile to determine whether one can identify unique genetic markers on these strains that correlate with clinical illness, particularly the HUS syndrome.

In the introduction it is stated that *S dysenteriae* killed 30.000 persons in two weeks in Zaire. Most of these deaths were due to cholera, not Shigella.

It will be important to have the expert clinical interpretation of the archived clinical data. This expertise will be available.

Dr. Talukdar has had excellent training in the genetic studies he proposes to do in the laboratory.

Reviewer 2

PI: Dr. K.A. Talukder, Assistant Scientist, Laboratory Sciences Division, ICDDR,B

Reviewer: Dr. G. Balakrish Nair, Deputy Director, National Institute of Cholera and Enteric Diseases, Calcutta, India

Reviewer 2

Title: Molecular epidemiology of *Shigella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complications

Summary of Referee's Opinion: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design		✓	
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget		✓	
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) **Without qualification** ☐

b) **With qualification** ☐ ✓

- **on technical groups** ☐ ✓
- **on level of financial support** ☐

I do not support the application ☐

Name of Referee: Dr. G. Balakrish Nair

Signature _____ Date October 17, 1999
Position: Deputy Director

Institution: National Institute of Cholera and Enteric Diseases, P-33, CIT Scheme XM, Beliaghata, Calcutta - 700010, India

Detailed comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

This project proposal attempts to test an interesting hypothesis that strains of *Shigella dysenteriae* type 1 isolated from shigellosis patients developing HUS and encephalopathy and septicemia might be clonally related and the proposal is designed to examine this by the use of pulsed field gel electrophoresis. The comparison part between *S. dysenteriae* type 1 strains isolated from patients with or without complications is an interesting strategy and would shed useful information on the differences in the molecular configuration of strains of *S. dysenteriae* type 1 associated with complications.

There are a few minor suggestions that the investigator may consider including in the proposal:

1. The investigators should consider including some *Escherichia coli* O157:H7 reference strains in this study given the fact that the O157:H7 strains are most often associated with HUS and other complications in developed countries and try to find commonalities between the *S. dysenteriae* type 1 associated with complications and the O157:H7 strains.

2. A technical problem is that *S. dysenteriae* type 1 is loaded with plasmids which have not been suitably addressed in this proposal and which are likely to interfere with the PFGE analysis if suitable methods are not adopted to exclude them from the analysis.

3. Since molecular typing of a fairly large number of strains of *S. dysenteriae* type 1 is planned, it might be a good idea to include ribotyping and RAPD analysis of some strains of *S. dysenteriae* type 1 with this study to see how the various typing patterns relate to each other.

In my opinion, this project proposal is well written, is in a novel area, attempts to test an interesting hypothesis and, therefore, deserves to be supported

Title: Molecular epidemiology of *Shigella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complications

Response to reviewers' comments

The reviewers have unanimously agreed with the hypothesis, objective, appropriateness and design of the project entitled "Molecular epidemiology of *Shigella dysenteriae* type 1 strains associated with haemolytic-uraemic syndrome (HUS) and other complicaions". The reviewer 1 fully supported the proposal without qualification. The reviewer 2 supported the proposal with a few minor suggestions to include in the proposal. We have carefully considered the comments and suggestions made by the reviewers which are as follows:

Reviewer 1

We have corrected the sentence

Reviewer 2

1. The reviewer suggested in his comment 1 to include some *Escherichia coli* 0157:H7 reference strains to find commonalities between *Shigella dysenteriae* type 1 strains associated with complications and *Escherichia coli* 0157:H7 in this study. We will try to include the *Escherichia coli* 0157:H7 reference strains from abroad, since there is no report available about the isolation of *Escherichia coli* 0157:H7 in Bangladesh. This has been included in this study in the last portion of the second para on page 6.

2. Preparation of agarose embeded bacterial DNA for PFGE will be followed by standard method (22) using CHEF Bacterial Genomic DNA Plug Kit (Bio-Rad, Calif, USA). From our past experiences, it has been noted that there is no possibility to interfere plasmid with PFGE (Talukder *et al.*, J Med Sci, 1999; 48: 781-784).

3. PFGE has been applied successfully as the most discriminatory method for strain discrimination of a variety of bacteria. This is explained clearly in the second para on page 6. Recently several papers were published indicating PFGE was the most discriminatory method for molecular typing of bacterial strains compared with ribotyping and RAPD analysis (Harrington *et al.*, Epidemiol Infect, 1999; 122(3): 367-75, {Annexure 1}, Tynkkynen *et al.*, Appl Environ Microbiol 1999; 65(9): 3908-14, {Annexure 2}). For this reason, ribotyping and RAPD analysis will not be included in this study.

Epidemiol Infect 1999 Jun;122(3):367-75

Molecular epidemiological investigation of an outbreak of Campylobacter jejuni identifies a dominant clonal line within Scottish serotype HS55 populations.

Harrington CS, Thomson-Carter FM, Carter PE

Department of Medical Microbiology, University of Aberdeen, Scotland, UK.

Three molecular typing methods, pulsed-field gel electrophoresis (PFGE), ribotyping, and flagellin (flaA) gene typing, were used to discriminate within a group of 28 Campylobacter jejuni, heat-stable serotype 55 (HS55) isolates derived from cases of campylobacter enteritis occurring throughout Scotland, including 9 isolates associated with an outbreak. PFGE was found to be most discriminatory, identifying 6 distinct profiles, followed by ribotyping (5 profiles), and then flagellin gene typing (4 profiles). The coincidence of all three genotypic markers identified a dominant clonal line within the HS55 group, accounting for each of the outbreak strains, and for 9 of the 19 sporadic strains. A second, closely related, clonal line accounted for a further 5 of the sporadic strains, and also included the HS55 reference strain. Identification and monitoring of such clonal lines should facilitate more effective future epidemiological surveillance of C. jejuni.

PMID: 10459638, UI: 99387394

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Appl Environ Microbiol 1999 Sep;65(9):3908-14

Comparison of ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis in typing of lactobacillus rhamnosus and L. casei strains

Tynkkynen S, Satokari R, Saxelin M, Vilpponen-Salmela T, Vaughan E, de Vos W

Valio Ltd. Research and Development Centre, FIN-00039 Valio, Finland.

[Medline record in process]

A total of 24 strains, biochemically identified as members of the Lactobacillus casei group, were identified by PCR with species-specific primers. The same set of strains was typed by randomly amplified polymorphic DNA (RAPD) analysis, ribotyping, and pulsed-field gel electrophoresis (PFGE) in order to compare the discriminatory power of the methods. Species-specific primers for L. rhamnosus and L. casei identified the type strain L. rhamnosus ATCC 7469 and the neotype strain L. casei ATCC 334, respectively, but did not give any signal with the recently revived species L. zeae, which contains the type strain ATCC 15820 and the strain ATCC 393, which was previously classified as L. casei. Our results are in accordance with the suggested new classification of the L. casei group. Altogether, 21 of the 24 strains studied were identified with the species-specific primers. In strain typing, PFGE was the most discriminatory method, revealing 17 genotypes for the 24 strains studied. Ribotyping and RAPD analysis yielded 15 and 12 genotypes, respectively.

PMID: 10473394, UI: 99402728