tachment 1.

Date 6/7/98

ETHICAL REVIEW COMMITTEE, ICDDR,B.

incipal Investigator Motiur Rahman

Trainee Investigator (if any) x

plication No. 98-015

Supporting Agency (if Non-ICDDR,B) SIDA/SAREC

tle of Study Molecular characteri-zation and antimicrobial resistance of Helicobactor pylori strains.

Project status:
(x) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

rcle 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)
Yes Abstract Summary (Required).
Yes 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)
Yes 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.

(PTO)

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

Motiur Rahman

Principal Investigator 6/7/98

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: Date:

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)

Molecular characterization and antimicrobial resistance of *Helicobactor pylori* strains.

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

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

LSD and CSD

4. **Contact Address of the Principal Investigator**	
4a. Office Location: **Laboratory Sciences Division (LSD)**	**4b. Fax No: 87 25 29** **4c. E-mail:** motiur@icddrb.org **4d. Phone / Ext: 871751-60 Ext 2405**

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

6. Dates of Proposed Period of Support *(Day, Month, Year - DD/MM/YY)*	**7. Cost Required for the Budget Period**		
When fund is available – Three years from the starting date.	*7a. 1st Year ($):* **18,740**	*2nd Year ($):* **22,514**	*3rd Year:* **18,417**
	7b. Direct Cost ($) 59,671	**Total Cost ($) 59,671**	

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 by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

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

Signature

6/7/98
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 requeried progress reports if a grant is awarded as a result of this application.

10. Signature of PI

Motiur Rahman

Date: 6/7/98

Principal Investigator: Last, first, middle **Rahman Motiur**

Table of Contents

Page Numbers

[x] Check here if appendix is included

Principal Investigator: Last, first, middle **Rahman Motiur**

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

Motiur Rahman

Project Name: **Molecular characterization and antimicrobial resistance of *Helicobactor pylori* strains.**

Total Budget	Beginning Date	Ending Date
59,671 $	When fund is available.	Three years from the starting date.

Hypotheses:

1. *Cag* pathogenicity island is associated with the gastroduodenal ulcer caused by *H. pylori* infection.
2. Antimicrobial resistance among *H. pylori* is responsible for treatment failure.
3. *H. pylori* is transmitted through contaminated water.

Objectives:

1. To compare the *H. pylori* strains isolated from patients with duodenal ulcer, gastric cancer and non-ulcer dyspepsia with respect to pathogenicity.
2. To study the transmission of *H. pylori* through water in Bangladesh.
3. To study the antimicrobial susceptibility of *H. pylori* strains.

Background:

H. pylori colonizes about 50% of the world population. In developing countries 80% of the population is infected by the age of 20 years and is inversely related to the socioeconomic condition. Transmission of *H. pylori* is extensively studied but is not yet fully understood. Oral-oral and fecal-oral transmission has been proposed. *H. pylori* does not causes an acute disease after infection but its persistent colonization results in chronic active gastritis, gastric and duodenal ulcer and gastric cancer. The bacterial factors necessary for colonization of the hostile gastric environment and for the virulence of the pathogen are poorly understood. The putative virulence factors include urease, vacuolar cytotoxin, flagella and gene products encoded by a 40 kb pathogenicity island e.g. *cag*A, *pic*A and *pic*B. An increase in prevalence of metronidazole and clarithromycin resistance among *H. pylori* has been reported. Excessive and improper use of antibiotics for treatment and prevention, over-reliance on medication, and abuse and overuse of antibiotics have favored the selection of resistant microorganisms and their dissemination.

Design and methods:

H. pylori will be isolated from patients with gastroduodenal ulcer, non-ulcer dyspepsia and gastric cancer. The isolates will be analysed for putative virulence factors e.g. vacuolar cytotoxin and Cag A protein. The genotypes of *Cag*A and *Vac* will be analysed. The distribution and genotype of the virulence factors of the isolates in all three groups will be compared. The susceptibility of *H. pylori* isolates to commonly used drugs will be examined by disk diffusion and E-test. Water samples from community will be analysed as a possible mode of transmission in the community.

Principal Investigator: Last, first, middle **Rahman Motiur**

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

Name	Professional Discipline/ Specialty	Role in the Project
1. Dr. Motiur Rahman	Microbiologist and Molecular biologist	Principal Investigator
2. Dr. John Albert	Microbiologist	Coinvestigator
3. Dr. Shafiqul Sarker	Physician	Coinvestigator
4. Dr. Noor Haque Alam	Endoscopist	Coinvestigator
5. Dr. Firdausi Qadri	Immunologist	Coinvestigator
5. Dr. Sawkat Hassan	Immunologist (BIRDEM)	Coinvestigator
6. Dr. Lars Engstrand	Molecular Biologist (SMI, Sweden)	Coinvestigator

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.

1. *Cag* pathogenicity island is associated with gastroduodenal ulcer caused by *H. pylori* infection.
2. Antimicrobial resistance among *H. pylori* is responsible for treatment failure.
3. *H. pylori* is transmitted through contaminated water.

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 *H. pylori* strains isolated from patients with duodenal ulcer, gastric cancer and non-ulcer dyspepsia with respect to pathogenicity.
2. To study the transmission of *H. pylori* through water in Bangladesh.
3. To study the antimicrobial susceptibility of *H. pylori* strains.

Principal Investigator: Last, first, middle **Rahman Motiur**

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).**

Helicobacter pylori, a Gram negative spiral shaped microorganism colonizes about 50% of the world population (Taylor *et al.,* 1991). The bacteria are extremely well adapted to their ecological niche, the gastric mucosa, having to compete with only a few microbial species. *H. pylori* usually does not cause an acute disease after infection, but its persistent colonization of the G.I tract inevitably results in gastritis and duodenal ulcer and plays an important role in the development of gastric cancer. It is now recognized as the major factor for the development of chronic active gastritis and also accepted to play a major role in the pathogenesis of gastric and/or duodenal ulcer and in the genesis of gastric adeno-carcinoma and malt lymphoma.

Results of a recent study on the prevalence of *H. pylori* infection in Bangladesh using C^{13} urea breath test have shown that 85% of the study population were infected with *H. pylori* (Sarker *et al.*, 1995). In a similar study in children between the ages of 0 to 3 months and 4 to 12 months, 60% and 80% of the study population respectively were found to be positive for *H. pylori* (Sarker *et al.,* 1997, personal communication). Recent reports from developing and developed countries have showed a striking variability in *H. pylori* associated gastroduodenal pathology. In Bangladesh, peptic ulcer diseases are very common but chronic atrophic gastritis and gastric cancer appear to be low. Whereas the reverse picture is common in Peru and Japan (Bardhan *et al.*, 1994; Ching *et al.*, 1995).

Epidemiological data show that the rate at which a population acquires *H. pylori* infection is higher in the developing countries than in developed countries; infection is inversely related to the socioeconomic condition, and clusters of infection are found in families with children (Taylor *et al.,* 1991). In developing countries, 80% of the population is infected with *H. pylori* by the age of 20, while in developed countries infection increases with age from 20% at the age of 30 and 50% at the age of 60. Clustering of cases in low socioeconomic group and within families indicates intrafamilial spread. But such clustering could be due to a common source of infection, person to person transmission or a genetic predisposition to infection. A positive correlation between *H. pylori* infection and the density of living and age has been observed (Malaty *et al.*, 1991).

Transmission of *H. pylori* has been extensively studied but is not yet fully understood. Oral-oral and fecal-oral transmission of the bacterium has been proposed. *H. pylori* has been detected in feces, saliva, dental plaque and in the drinking water suggesting the presence of a common environmental source (Enroth *et al.*, 1995; Hulten *et al.* 1996).

Optimum therapy for eradication of *H. pylori* from patients to-date consists of bismuth subcritrate and two antimicrobial agents including metronidazole and clarithromycin. However, several reports in recent years show an increase in the prevalence of metronidazole and clarithromycin resistance strains. The selection of a resistant microorganisms and their dissemination have been favored by excessive and improper use of antibiotics for treatment and prevention, over-reliance on medication, and abuse and overuse of antibiotics. Beside these, use of inappropriate antimicrobials and improper combination of antimicrobials increase the risk for development of antimicrobial resistance among the bacteria.

The bacterial factors necessary for colonization of the hostile gastric environment and for the virulence of the pathogen are poorly understood. The putative virulence factors include urease, vacuolar cytotoxin, flagella and gene products encoded by a 40 kb pathogenicity island e.g *cag*A, *pic*A and *pic*B (Tummuru *et al.*, 1993; 1995; Antherton *et al.*, 1995). Despite the clear association between *H. pylori* infection and gstroduodenal disease, the majority of the infected population shows no symptom suggesting that variation in the host susceptibility as well as microbial pathogenicity might be crucial to the outcome of infection. Thus, genetic predisposition, immune response, the age of acquisition of the infection and environmental factors are the determinants that affect the outcome of infection, as are the strain differences in the microbe with respect to the presence and expression of putative virulence factors.

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).**

Study Site: This study will be conducted in ICDDR,B and BIRDEM hospital.

Recruitment of patients: *H. pylori* from three groups of patients will be studied. Consecutive patients attending the Dept. of gastroenterology, BIRDEM with gastric/duodenal ulcer, gastric cancer, and non-ulcer dyspepsia will be selected. Patient will be informed about the study and those who are willing to participate will be asked for written consent. Patient will undergo endoscopic examination and will be grouped into gastroduodenal ulcer and non-ulcer dyspepsia group and biopsy sample will be collected. For patients with gastric carcinoma tissue sample will be collected during surgery.

Inclusion criteria:
Age: 18 to 55
Sex: Both male and female
History of epigastric pain/discomfort and duodenal ulcer.
Gastric cancer patients who will undergo surgery.

Exclution criteria:
Known contraindication for endoscopy.
Use of proton pump inhibitor in recent weeks.
Previous abdominal surgery.

Isolation of strain: Selected patients will undergo endoscopic investigation and four biopsy specimens will be taken from gastric antrum by a sterilized endoscope. Serum will also be collected from the patients. The biopsy specimen will be tested for *H. pylori* by CLO test.

Culture of *H. pylori*: Immediately after collection biopsy sample will be homozinised and cultured on Brucella agar containing 5% yeast extract, 7% horse blood, vancomycin (mg/ml) and amphotericin (15mg/L) in microaerophilic condition (0_2 5%, Co_2 15%, N_2 80%) at 37C for 5 days. *H. pylori* will be confirmed by Gram staining, urease test, catalase test, oxidase activity and by PCR using primers specific for 16S rRNA(Hulten *et al.*, 1996).

Chracterization of strains:

Virulence factor expression: Expression of virulence factor will be analysed by SDS-PAGE and immunoblotting. Whole bacterial lyset will be separated on 10% SDS and will be transferred to nitrocellulose membrane. Immunoblotting will be done by using rabbit anti *H. pylori* antibodies and the expression of Cag, Vac and heat shock protein will be analysed. Expression of known virulence factor will be compared in isolates from all three different groups of patients.

Assay for cytotoxicity: All strains collected will be analyzed for the production of vacuolar cytotoxin by cytotoxicity assay. *H. pylori* isolates will be grown in Brucella broth containing 5% FCS in a microaerophilic atmosphere for 72h. Culture supernatants will be concentrated by filtration through 0.2um pore-size filters. Confluent monolayers of Hela cells will be incubated with serial dilution of culture supernatant in tissue culture medium (1:1 to 1:8) containing NH_4Cl. Vacuolation will be measured by neutral red uptake and will be assessed by light microscopy.

VacA: The presence of *vacA* will be investigated in all isolates by colony blot hybridization using a *VacA* specific probe generated by cloning the conserved C-terminal region of the *vacA*.

Genotyping of *vac*A: The *vacA* genotype of the clinical isolates will be determined by PCR using s1a, s1b and s2specific primers for signal sequences and m1 and m2 specific primers for the middle region of *vacA* gene (Atherton *et al.*, 1995).

Pathogenicity island: Most strains collected from patients with duodenal ulcer contain a 40 kb pathogenicity island carrying the *CagA* gene, encoding the immunodominant CagA protein of unknown function as well as other genes including *picA and picB*. Using the known sequences of *cagA, picA* and *picB* genes primers will be designed to amplify the *cagA, picA* and picB genes (Doorn *et al.*, 1998; Celik *et al.*, 1998).

Histopathological evaluation of the biopsy specimens: Biopsy sample collected from patients will be examined for the degree of inflammation. One μm thick paraffin embedded tissue sections will be deparafinized and stained with Giemsa stain. Tissue sections will be examined for the degree of inflammation using the standard methods. The correlation between the degree of inflammation and expression of virulence will be analysed.

Comparison of strains collected from patients with gastroduodenal ulcer, gastric cancer and non-ulcer dyspepsia: Strains collected from all three groups will be analyzed by 2D-gel electrophoresis and will be analysed. Any novel protein or proteins expressed in one group but not in other group will be N-terminally sequenced. From the amino acid sequence corresponding DNA sequence will be generated and will be used to screen the complete gene sequence of *H. pylori*. If significant homology is obtained then the corresponding open reading frame will cloned and further analyzed.

Detection of *H. pylori* in water supply by IMS PCR: Water samples (50 ml) will be collected from house holds supplied by municipal corporation in Nandipara and from other sources like ponds and wells. Water samples will be centrifuged at 20,000xg for 30 min and will be resuspended in one ml PBS. *H. pylori* will be fished out with immunomagnetic beads coated with polyclonal rabbit IgG raised against a mixture of *H. pylori* rod and coccoid forms (Nilsson *et al.*, 1996). Presence of *H. pylori* will be detected by PCR assay based on the primers designed from *H. pylori* adhesin subunit encoding gene and 16S rRNA gene (Enroth *et al.*, 1995).

Antimicrobial susceptibility test: The susceptibility of *H. pylori* strains will be tested by disk diffusion, agar dilution method and by E test. Two fold serial dilutions of antimicrobials will be incorporated into the medium as follows, ampicillin and erythromycin (0.32 - 0.0025mg/L), rifampicin and tetracycline (4.0 - 0.15 mg/L) clathramycin (0.2 – 2 mg/L) and metronidazole (8.0 - 0.03 mg/L). *H. pylori* strains will be suspended in PBS at a dilution 10^6 cfu/ml and applied as 0.001 ml aliquots to the surface of the plates yielding a final inoculum of 10^3 cfu/spot. For disk diffusion method duplicate plates will flooded with 1ml of broth containing 10^4 to 10^6 cfu/ml *H. pylori*. After removal of excess of fluid, plates will be dried at 37C and discs (AB Biodisk, Sweden) containing ampicillin 10 μg, erythromycin 15 μg, gentamycin 30μg, rifampicin 5μg, tetracycline 30μg clarithromycin and metronidazole 10, 5, and 1μg will be applied. Inhibition zone diameter will be measured after 3 and 5 days incubation at 37C.

Sample size: Assuming an 80% prevalence of *H. pylori* infection (background information) the minimum number of patients included in this study will be 90 (precision 2%, confidence level of 95%).

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).**

The study will be carried out in LSD of ICDDR,B and SMI Stockholm, Sweden. LSD has all necessary equipments for caring out the research, incubation facilities instruments for molecular biology is available here in the laboratory. We are planning to buy one Thermal cycler (PCR) equipment. Microbiology laboratory in SMI has the most modern *H. Pylori* incubation and all others essential lab facilities. Patient's material (biopsy sample, blood etc) will be collected from BIRDEM Hospital and the available facilities are optimum for the present study.

Principal Investigator: Last, first, middle **Rahman Motiur**

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares 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).**

Data will be analyzed by the investigators themselves, using the statistical packages Epi-Info, Version 6 Centers for Disease Control, Atlanta, Ga, USA or any other suitable statistical programme. If necessary, the advice of a specialist in biomedical statistics will be requested.

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.

The following groups will be studied:

Subject criteria	No of subjects	Age of the patients	Samples	Source
Gastroduodenal Ulcer due to *H. pylori* infection.	30	18-55	Peripheral blood biopsies	BIRDEM
Gastric carcinoma	30	18-55	Peripheral blood Gastric tissue	BIRDEM
Dispepsia due to *H. pylori* infection.	30	18-55	Peripheral blood biopsies	BIRDEM

Adult male and females attending the Dept. of gastroenterology, BIRDEM with gastroduodenal ulcer, gastric carcinoma or non-ulcer dyspepsia will be eligible for the study. After explaining the aim of the study and written consent patients will be included in the study. Intestinal biopsies will be collected from patients who are presenting with gastroduodenal ulcer and dyspepsia and are positive for *H. pylori* as indicated by CLO test. Gastric tissue from patients with gastric carcinoma will be collected during surgery. The study will not interfere with the management and treatment of the patients and none of the procedures will be harmful.

Principal Investigator: Last, first, middle **Rahman Motiur**

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.

No animal will be used in this study.

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.

References:

1. Atherton, J.C., Cao, P., Peek, M.R., Tummuru, KR.M., Blaser, J.M., and cover, L.T. (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori* . J. Biol. Chem. 271:30 17771-17777.

2. Bardhan, P.K,. Islam, M., Mahalanabis, D,. Gry, K. (1994) A study of Helicobacter pylori in Bangladeshi subjects with non-ulcer dyspepsia. Am. J. Gastroenterol 89:1310.

3. Cederbrant, G., Kahlmeter, G., Ljung, A., (1993). The E test for antimicrobial susceptibility of *Helicobacter pylori*. J. Antimicrob. Chemo. 31:65-71.

4. Ching, C.K., Lam, S.K., Chen, B.W., et al (1995) North – South gastric cancer and duodenal ulcer disease gradient in China. Gastroenterology. 108(4):A8.

5. Cover, T.l. and Blaser, M.J. (11992) *Helicobacter pylori* and gastroduodenal disease. Annu. rev. Med. 43:135.

6. Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burroni, D.,Macchia, G., Massone, A., Papini, E., Xiang, Z., figura, N., and Rappuuoli, R. (1993) Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. acad. Sci. USA. 90:5791-5795.

7. Cover, T.L. and Blaser, M.J. (1992) Purification and characterization of the vacuolating cytotoxin from *Helicobacter pylori*. J. Biol. Chem. 267.

8. Crabtree, J.E., Wyatt, J.I., sobala, G.M., Miller, G., Tompkins, D.S., Primrose, J.N. and Morgan, A.G. (1993) Systemic and mucosal humoral responses to *Helicobacter pylori* in gastric cancer. Gut. 34:1339.

9. Doorn L.J.V., Figueiredo C., Rossau R., Jannes G., Asbroeck M.V., Sousa J.C., Carneiro F., and Quint W.G.V. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and Reverse hybridization. J. Clin. Microbiol. 1998. 36:1271-1276.

10. Enroth, H.R. and Engstrand, l. (1995) Immunomagnetic separation and PCR for detection of *Helicobacter pylori* in water and stool specimens. J. Clin. Microbiol. 33:2162-2165.

11. Evans, D.G., Karjalainen, T.K., Evans, D.J.Jr., Graham, D.Y., and Lee, C.H. (1993) cloning, nucleotide sequence, and expression of a gene encoding an adhesion subunit protein of *Helicobacter pylori*. J. Bacteriol. 175:674-683.

12. Ferguson, D.A.Jr., Li, C., Patel, N.R., Mayberry, W.R., Chi, D.S. and Thomas, E. (1993). Isolation of *Helicobacter pylori*. from saliva. J. Clin. Microbiol. 31:2802-2804.

13. Graham, D.Y., Malaty, H.M., Evans D.J.Jr., Klein, P.D., and Adam, E. (1991) Epidemiology of Helicobacter pylori in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status. Gastroenterology 100:1495-1501.

14. Hulten, K., Han, W.S., Enroth, H., Klein, D.P., Opekun, R.A., Gilman, H.R., Evans, G.D., Engstrand, L., Graham, Y.D., El-Zaatari, A.K.F. (1996) Helicobacter pylori in the drinking water in peru. Gastroenterology 110:1031 – 1035.

15. Janet C., Bin Su, Urban T., Yigael F, Thoresson A.C., Engstrant L, Bengt S., bernander S., Normark S. (1998) Virulence and colonization associated properties of *Helicobacter pylori* isolated from children and adolescents. J. Infect Dis Submitted.

16. Kristina H., Han S.W., Helena E., Peter D.K., Antone R.O, Gilman R.H., Dolores G.E., Lars E., Graham D.Y. , Fouad A.K. Zaatari EL. *Helicobacter pylori* in the drinking water in Peru. Gastroenterology 1996;110:1031-1035.

17. Malaty, H.M., Graham, D.Y., Klein, P.D., Evans, D.G., Adam, E., and Evans, D.J. (1991) Transmission of Helicobacter pylori infection. Studies in families of healthy individuals. Scand. J. Gastroenterol. 26:927-932.

18. Nilsson, O.H., Aleljung, P,. Nilsson, P,. Tyszkiewicz, T and Wadstrom, T. (1997) Immunomagnetic bead enrichment and PCR for detection of *Helicobacter pylori* in human stool. J. Microbiol. Methods 27: 73-79.

19. Taylor DN, Blaser Mj, The epidemiology of *Helicobacter pylori* infection. Epidemiol rev. 1991;13:42-59.

20. Tummuru, M.K.R., Cover, T.L., and Blaser, M.J. (1993) Cloning and expression of a high molecular mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect. Immun. 61:1799-1805.

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.

It is hoped that the information generated from this study will result in better understanding of the role of virulence factors associated with *H. pylori* infection. This study will generate knowledge to understand the mode of transmission of *H. pylori* among Bangladeshi population and it will generate valuable information regarding antimicrobial susceptibility of *H. pylori* isolates. The results of this study will be disseminated in international journals and in international conferences.

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)**

This study is a collaborative one among ICDDR,B, BIRDEM and the infections disease institute (SMI), Karolinska Institute, Stockholm, Sweden. The investigator's of SMI will interact closely in the study by transferring recently adapted techniques for culture of *H. pylori*, and molecular biology technology for genotyping of strains continuos linkers will be maintained by visits of scientists to each others laboratory. New techniques will be introduced at ICDDR, B for rapid detection of carcinogenic strains. One set of strain collected here will be send to SMI Sweden for global comparison of the *H. pylori* isolates. Investigations e.g. culture of *H. pylori* characterisation of strains, genotyping of *vac*A gene, antimicrobial susceptibility, histopathological evaluation of biopsy specimen and detection of *H. pylori* in water will be carried out in ICDDR,B Bangladesh. 2-D gel electrophoresis, detection of novel protein, N-terminal sequencing and comparison of strains will be carried out in SMI, Stockholm, Sweden.

This collaboration will help us to set up a advance molecular biology set up for molecular characterization of *H. pylori* strain isolated from Bangladeshi patients with gastroduodenal ulcer.

Principal Investigator: Last, first, middle **Rahman Motiur**

Biography of the Investigators

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
Motiur Rahman	Assistant Scientist	21st Dec 1961

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

Institution and Location	Degree	Year	Field of Study
Rangpur Medical College, Bangladesh.	MBBS	1985	Medicine
Karolinska Institute, Stockholm, Sweden.	Ph D	1997	Microbiology & Molecular biology

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).**

1985 – 1986	Assistant Surgeon	Ranpur Medical College, Rangpur , Bangladesh.
1986 – 1987	Medical Officer	Islamic foundation Hospital, Dinajpur, Bangladesh.
1987 - 1990	Medical Officer	Thana Health Complex, Birgang, Dinajpur, Bangladesh.
1991- 1994	Research Student	Dept. of Bacteriology, Karolinska Institute, Stockholm, Sweden.
1994 – 1995	Research Assistant	Microbiology & Tumorbiology Centre, Karolinska Institute, Sweden.
1996 – 1997	Research Associate	Microbiology & Tumorbiology Centre, Karolinska Institute, Sweden.
!997 –	Assistant Scientist	Laboratory Sciences Division, ICDDR,B. Bangladesh.

Principal Investigator: Last, first, middle **Rahman Motiur**

Publications

1. Bhuiya, B., **Rahman, M.**, Miah, R.A., Rahman, M . and Albert, M.J. (1998) High prevalence of Ciprofloxacin resistant *Neisseria gonorrheae* among the Commercial Sex Workers in Bangladesh. *J Anti. Chemo* in press.

2. **Rahman, M.**, Jonsson, A.-B., and Holme, T. (1998) Monoclonocal antibodies to the α-Gal-(1-4)-β-Gal-(1- epitope of *Moraxella catarrhalis* lipopolysaccharide react with the type IV pili of *N. meningitidis*. *Microbial Pathogenesis*. 23: XXX – XXX.

3. **Motiur Rahman** , Ann-Beth Jonsson and Staffan Normark. (1997) PilC of pathogenic *Neisseria* is associated with the bacterial cell surface. ***Mol Microbiol 25(1):11-25.***

4. Källström, H,. **Rahman, M.**, and Jonsson, A.-B. (1996) Characterization of a eukaryotic pilus receptor for *Neisseria gonorrhoeae* and *Neisseria meningitidis*. ***Proceedings of the 10th International Pathogenic Neisseria Conference. 289-290.***

5. **Rahman, M,**. Källström, H,. Normark, S,. and Jonsson, A.-B. (1996) Characterisation and surface translocation of pilus associated protein PilC of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. ***Proceedings of the 10th International Pathogenic Neisseria Conference. 304-305.***

6. **Rahman**, M,. and T. Holme, (1996) Antibody response in rabbits to serotype-specific determinant in lipopolysaccharides from *Moraxella catarrhalis*. ***J. Med. Microbiol. 44: 1-7.***

7. **Rahman, M,**. Holme, T,. Jönsson, I,. and Krook, A. (1996) Human immunoglobulin isotype and IgG subclass response to different antigens during infection with *Moraxella catarrhalis*. ***APMIS 105:213-220***
.

8. Edebrink, P, Jansson, P-E, Rahman, M M, Widmalm, G, Holme, T, **Rahman, M**, A.Weintraub. (1996). Structural studies of the O-antigen from the lipoplysaccharide of *Moraxella catarrhalis* serotype B, strain CCUG 3292. ***Carbohydr. Res.295:127-146*** .

9. **Rahman, M,**. Holme, T,. Jönsson, I,. and Krook, A,. (1995) Lack of serotype-specific antibody response to lipopolysaccharide antigens of *Moraxella* (*Branhamella*)*catarrhalis*. ***Eur. J. Clin. Microbiol. Infect. Dis.14:297-304.***

10. Ann-Beth Jonsson, **Motiur Rahman** and Staffan Normark. (1995) Pilus biogenesis gene, *pilC*, of *Neisseria gonorrhoeae*: *pilC1* and *pilC2* are each part of a larger duplication the gonococcal genome and share upstream and down stream homologous sequences with *opa* and *pil* loci. ***Microbiol. 141:2367-2377.***

11. Edebrink, P,. Jansson, P-E,. Rahman, M M,. Widmalm, G,. Holme, T,. and **Rahman, M.** (1995) Structural studies of the O-polysaccharide from two strains of *Moraxella catarrhalis* serotype C. ***Carbohydr. Res. 266:237-261.***

12. M. **Rahman**, S. Normark, A-B. Jonsson. (1994) Pilus mediated attachment of *Neisseriae gonorrhoeae* and *Neisseriae meningitidis* to host cell receptors. ***Proceedings of the Ninth International Pathogenic Neisseria Conference. 127-128.***

13. **Rahman**, M,. I. jönsson, A. Krook,. and T. Holme (1994) Antibody response to outer membrane antigens of *Moraxella catarrhalis*. ***7th International Congress of Bacteriology and Applied Microbiology. 214.***

14. Edebrink, P,. Jansson, P-E,. **Rahman,** M M,. Widmalm, G,. Holme, T,. Rahman, M,. and A.Weintraub. (1994) Structural studies of the O-antigen from the lipoplysaccharide of *Moraxella* (*Branhamella*) *catarrhalis* serotype A (strain ATCC 25238). ***Carbohydr. Res. 257:269-284.***

15. Jönsson, I,. Holme, T,. Krook, A,. **Rahman, M,.** and Thorén, M. (1992) Variability of surface-exposed antigens of different strains of *Moraxella catarrhalis*. ***Eur. J. Clin. Microbiol. Infect. Dis. 11: 919-922.***

Thesis

Motiur Rahman (1997) Characterization of surface components of *Moraxella catarrhalis* and pathogenic *Neisseria.* ISBN- 91-628-2314-0.

Manuscript Submitted

1. Bhuiya, B., **Rahman,** M., Nahar, S., Miah, R.A, Islam, N., Rahman, K.M., Bogaert, J., Albert, M.J.(1998) Prevalence, Antimicrobial susceptibility, and plasmid content of *Neisseria gonorrhoeae* isolates collected from commercial sex workers in Dhaka city: Emergence of high level ciprofloxacin resistance. To be submitted in ***J Clin Microbiol.***

2. Borelli, S,. **Rahman, M,.** Holme, T,. Alf, A, Lindberg,. and P, -E, Jansson. (1998) *Moraxella* (*Branhamella*) *catarrhalis* and *Haemophilus influenzae* lipopolysaccharides express distinct Gal-α-1-4Gal-β epitopes as determined by the binding of specific *H. influenzae* anti-Gala1-4Galb monoclonal antibodies. Submitted to *Microbial Pathogenesis.*

Principal Investigator: Last, first, middle Rahman Motiur

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: **Molecular characterization and antimicrobial resistance of *Helicobacter pylori* strains.**

Principal Investigator: **Motiur Rahman**

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

International Centre for Diarrhoeal Diseases Research, Bangladesh
Consent form for patients (gastroduodenal ulcer/ non-ulcer dyspepsia) for Endoscopy, blood samples.

Helicobacter pylori is one of the most important etiological agent for gastroduodenal ulcer, non-ulcer dyspepsia and carcinoma stomach. We are currently conducting a study on *H. pylori* in Bangladesh with an aim to identify novel virulence factors essential for pathogenesis. If you are suffering from chronic gastric/duodenal ulcer or non-ulcer dyspepsia, we invite you to participate in the study. Your participation will be highly appreciated.

You will undergo endoscopic examination during the study. The instrument (endoscope) has a flexible rubber tube which will be passed through your mouth to examine the stomach. Necessary medication will be provided for the smooth operation of the instrument and you will not feel any pain but there will be minimum discomfort. During examination four tiny pieces of biopsies will be taken from your stomach. The procedure is not harmful and you will be kept under observation for 4 hours.

We will also collect 4 ml of blood samples on the same occasion. The collection of blood will not be harmful. If you agree to participate in the study please sign your name or your left-thumb imprint at the specified space below.

Thank you for your cooperation

Signature or left-thumb impression of the volunteer.

Date:

Signature of Witness

Signature of investigator

Date:

Date:

আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র
মহাখালী, ঢাকা ।

সম্মতি পত্র

হেলিকোবেক্টর পাইলোরী প্রদাহ গ্যাসটিক / ডিয়োডেনাল আলসার, নন্-আলসার ডিসপেপসিয়া ও গ্যাসটিক ক্যানসার এর অন্যতম প্রধান কারন । আমরা বর্তমানে এই জীবানুর উপর একটি সমীক্ষা পরিচালনা করছি । আপনি যদি উপরে বর্নিত যে কোন রোগে আক্রান্ত হয়ে থাকেন তাহলে আমরা আপনাকে আমাদের এই সমীক্ষায় অংশগ্রহনের আমন্ত্রন জানাচ্ছি ।

রোগ নির্ণয় ও হেলিকোবেক্টর পাইলোরীর উপস্থিতি প্রমানের জন্য যথাক্রমে এন্ডোস্কপি ও পাকস্থলির ঝিল্লির বায়োপসি পরীক্ষার জন্য গ্রহন করা হবে । এন্ডোস্কপি যন্ত্রের একটি স্থিতিস্থাপক নল আপনার মুখ দিয়ে পাকস্থলিতে প্রবেশ করানো হবে । এই পরীক্ষার জন্য প্রয়োজনীয় ঔষধ পত্র আমরা আপনাকে সরবরাহ করবো । এই পরীক্ষা আপনার স্বাস্থের জন্য ক্ষতিকর নয় তবে আপনি সামান্য ব্যাথা অনুভব করতে পারেন । পরীক্ষার সময় আমরা আপনার পাকস্থলি ঝিল্লির চারটি নমুনা গ্রহন করবো এবং এই পরীক্ষার পর চার ঘন্টা আপনাকে পর্যবেক্ষন এর জন্য অবস্থান করতে হবে ।

আমরা আপনার নিকট থেকে সেরোলজীর জন্য চার সিসি রক্ত সংগ্রহ করবো । ইহাতে আপনার সম্মতি থাকলে আমরা আপনাকে এই সম্মতি পত্রে স্বাক্ষর করার জন্য অনুরোধ করছি ।

ধন্যবাদ

১। ক্লায়েন্টের স্বাক্ষর

তাং ————————

গবেষকের স্বাক্ষর

তাং ————————

২। স্বাক্ষীর স্বাক্ষর

তাং ————————

Principal Investigator: Last, first, middle

Detailed Budget for New Proposal

Project Title: **Molecular characterization and antimicrobial resistance of *Helicobactor pylori* strains.**

Name of PI: **Motiur Rahman**

Protocol Number: Name of Division: **Laboratory Sciences Division**

Funding Source: **SIDA/SAREC** Amount Funded (direct): Total: **59,671**

Overhead (%)

Starting Date: Closing Date:

When fund is available (Sept 98) 3 years from the starting date.

Strategic Plan Priority Code(s) :

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Position	Grade	Effort%	Salary	1st Yr	2nd Yr	3rd Yr
1.	Assistant Scientist 5444-5	NOB	20%	810	-	1,994	2,091
2.	Senior lab. Tech 2357-2	GS – 4	25%	380	1,140	-	-
3.	Senior research officer 3571-7	GS - 6	100%	510	-	6,120	6,426
	Sub Total				**1,140**	**8,114**	**8,517**
	Consultants				-	-	-
	Local Travel				300	700	300
	International Travel				2,500	-	2,800
	Sub Total				**2,800**	**700**	**3,100**
	Supplies and Materials (Description of Items)						
1.	Media and plates				1,500	2,500	1,500
2.	Chemical, primer and enzymes				3,000	5,000	2,000
3.	Tissue culture media and plastic materials				1,000	4,500	2,000
	Sub Totals				**5,500**	**12,000**	**5,500**

Principal Investigator: Last, first, middle

	Other Contractual Services			
		300	300	100
	Repair and Maintenance	300	200	300
	Rent, Communications, Utilities	-	300	-
	Training Workshop, Seminars	-	-	500
	Printing and Publication			
	Staff Development			
	Sub Total	600	800	900

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests	-	-	-
	Microbiological tests	600	600	-
	Biochemistry Tests	-	-	-
	X-Rays	-	-	-
	Patients Study	-	-	-
	Research Animals	-	-	-
	Biochemistry and Nutrition	-	-	-
	Transport	200	200	200
	Xerox, Mimeographs etc.	200	100	200
	Sub Totals	**1,000**	**900**	**400**
	Other Operating Costs			
	Capital Expenditure (Computer) (Incubator)	2,700 5,000	-	-
		7,700		
	TOTAL DIRECT COST	**18,740**	**22,514**	**18,417**

✓ ✓ ✓

6/7/98

Md. Bozlur Rahman
Senior Budget & Cost Officer
ICDDR, B, Mohakhali
Dhaka-1212, Bangladesh

Principal Investigator: Last, first, middle **Rahman Motiur**

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.

The estimated budget represents the minimum minimal estimation of the cost.

1. All reagents and equipment will be purchased on the budget code assigned to the study protocol.
2. Office supplies will be purchased on the same budget code.
3. During the first year one research assistant (25%) and during 2nd and 3rd year one senior research officer (100%) will recruited.

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)

Principal Investigator: Last, first, middle Rahman Motiur

Check List

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

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

Detailed Budget [X]

Title: Molecular characterization of Helicobacter pylori strains isolated from patients with duodenal ulcer, gastric cancer and asymptomatic carries.

Summary of Referee's Opinions: 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	X		
Adequacy of Project Design	X		
Suitability of Methodology	X		
Feasibility within time period	X		
Appropriateness of budget	X		
Potential value of field of knowledge	X		

CONCLUSIONS

I support the application:

a) without qualification [X]

b) with qualification [ ]

- on technical grounds [ ]
- on level of financial support [ ]

I do not support the application [ ]

Review of the proposal for a scientific project entitled: "Molecular characterization of Helicobacter pylori strains isolated from patients with duodenal ulcer, gastric cancer and asymptomatic carriers" which represents a collaboration between SMI, Stockholm Sweden with Dr. Lars Engstrand and Professor Staffan Normark as principal investigators and ICDDR,B, Dhaka, Bangladesh with a research group consisting of Dr. Motiur Rahman as investigator, three coinvestigators and Dr. John Albert as coordinator and coinvestigator.

Helicobacter pylori is a major pathogen world-wide and represents the most common infection in human. Since the bacterium was first isolated in 1982 much has been learnt about pathogenic mechanisms but large gaps in our knowledge hamper efficient treatment and prevention. An important piece of missing information is the importance of different strains for different clinical manifestations and in different parts of the world. The project, which involves collection of specimens from individuals in Bangladesh with different manifestations of H. pylori infection and a comparison of these specimens to the corresponding ones available in Sweden, is thus highly relevant.

The project design seems highly adequate although I would like to point out that duodenal ulcer or cancer patients are hardly identified on clinical grounds (section 2.2.). The wording should be modified to state that the two groups of patients with clinical symptoms (groups 1 and 2) will be classified by gastroscopic and microscopic findings. The proposed methodology seems suitable for the project.

I lack, however, a specific statement on where the different parts of the study will be performed, e.g. microscopic examination of biopsies, culture of H. pylori form biopsies, characterization of strains, ELISA and immunoblot and so on. The project plan mention technology transfer and the budget contain purchase of equipment as part of expenses in Dhaka but it remains unclear when and to what extent specimens will be processed in Dhaka. Technology transfer which will allow for further studies in Bangladesh seems to me an important part of the project. For these reason, the proposal should specify the technologies to be transferred and include in the budget the costs of training an investigator from ICCDR,B at the SMI. In other respects, the bugged seems adequate.

The project seems feasible within the time frame. The potential value of the field of knowledge is, of course, enormous considering the impact of the infection in all parts of the world. I strongly recommend this project.

As minor comments to the proposal, some further attention should be given to the list of references where two references are missing and two further do not indicate the journal or the issue where the papers have been published.

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF HELICOBACTOR PYLORI STRAINS

~~MATTUR~~

M. RAHMAN
AND OTHERS

1998-015

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 thay are justified.

(Use additional pages if necessary)

Title: Molecular characterization of Helicobacter pylori strains Isolated from patients with duodenal ulcer, gastric cancer and asymptomatic carriers

PI:

Reviewer: .....

See attached sheet

Title: Molecular characterization of Helicobacter pylori strains isolated from patients with duodenal ulcer, gastric cancer and asymptomatic carriers.

Summary of Referee's Opinions: 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	X		
Suitability of Methodology	X		
Feasibility within time period		X	
Appropriateness of budget			X
Potential value of field of knowledge		X	
	X		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification ☐

- on technical grounds ☒
- on level of financial support ☒

I do not support the application ☐

Name of Referee:

Signature: Per ... Date: 98-06-02

Position:

Institution:

Dept of Gastroenterology and Hepatology
Karolinska Hospital
SE-171 76 Stockholm
Sweden

;view of the project: Molecular characterization of Helicobacter pylori strains ,olated from patients with duodenal ulcer, gastric cancer and asymptomatic ;arriers

The outlined study describes a well-planned project from a very compotent and skilled Swedish group that has a long time experience in microbiologic Helicobacter research.

Reading the project plan some few points are striking:

1. P 5, spec aims. If the researchers are studying the molecular characterization of Helicobacter strains, why do they choose to compare strains collected from Sweden and Bangladesh? I think it would do with samples from Bangladesh only where it seems that the major sampling should take place..
2. P 5, isol of strains Why are gastric ulcers not studied? There is today great controversy about the nature of the two types of ulcerations, duodenal and gastric. I do not understand to which group the asymptomatic carriers belong. In addition, I do not see the point in studying HP+ patients without clinical symptoms of duodenal ulcer. Does that mean that the have or have not an ulceration?
3. P 7, antimicrobial Why is not HP tested against clarithromycin instead of erythromycin, as this is the mainly used antibiotic in treatment of HP infection?
4. P 8, 1 para Why is the diameter zone measured already after 2-3 days? Generally the HP are considered to be fully cultured after 3-5 days. I think it would be appropriate to let the bacteria grow for a longer period of time in order to get an optimal inhibitable growth which would justify the efficacy of the antibiotics.
5. Budget The budget for personnel at the SMI in Stockholm seems amazingly high. Generally the cost of a skilled lab technician in Sweden will stay below 40.000 SEK.
6. Geography It has not been carefully described where the different parts of the study should be carried out. Will sampling be done in both countries? If so, no informed consent form in Swedish is given.
7. Work On the whole, the budget seems otherwise to be very tight. With the goal of persuing these studies within the time limit and with only one lab technician in Sweden and one in Bangladesh, one has to assume that the rest of the benchwork has to be done by other persons and doctors during free time. This may not be really feasible since doctors are mainly occupied by their regular work during office hours and will not be expected to fulfill a complete lab work in addition to their clinical work. I do not see which persons should do what in this scenario. Please describe.
8. As the study contributes to technical advantage and development for the country of Bangladesh, will the Bangladeshi collaborators receive any kind of degree (PhD?)after having fulfilled the study?

Reviewer 1

1. The patients were grouped on gastroscopic and microscopic finding.
2. Described in collaborative arrangement section.
3. References were corrected.

Reviewer 2

1. Only samples from Bangladesh will be analysed.
2. Patients with gastroduodenal ulcer will be studied. Asymptomatic carriers belong to non-ulcer dyspepsia. The main objective of the study is to distinguish between strains causing ulcer, non-ulcer dyspepsia and cancer, that's why two ulcer groups was not included separately. We are planning for another study where the two groups will be studied.
3. Clarithromycin is included.
4. Zone diameter will be measured after 3 – 5 days.
5. This includes 40% LKP.
6. Described in collaborative arrangement section.
7. Described in collaborative arrangement section.
8. Laboratory personnel from ICDDR, B will be trained in Stockholm.