Attachment 1. 69

Date 14.04.87

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Sadequr Rahman

Trainee Investigator (if any) _____

Application No. PCC/2/87

Supporting Agency (if Non-ICDDR,B) _____

Title of Study Exam. of a possible correlation between the phage pattern and plasmid profile of ETEC strains.

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

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

1. Source of Population:
   (a) Ill subjects — Yes (No)
   (b) Non-ill subjects — Yes (No)
   (c) Minors or persons under guardianship — Yes (No)
2. 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)
3. 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)
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — Yes (No)
   (b) Procedures to be followed including alternatives used — Yes (No)
   (c) Physical risks — Yes (No)
   (d) Sensitive questions — Yes (No)
   (e) Benefits to be derived — Yes (No)
   (f) Right to refuse to participate or to withdraw from study — Yes (No)
   (g) Confidential handling of data — Yes (No)
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)
5. Will signed consent form be required:
   (a) From subjects — Yes (No)
   (b) From parent or guardian (if subjects are minors) — Yes (No)
6. Will precautions be taken to protect anonymity of subjects — Yes (No)
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ___ Protocol (Required)
   ___ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.
4. The study will be carried on specimens already collected in the laboratory for the cholera vaccine trail and other bacteria. No patients will be involved. (PTO)

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

Sadequr Rahman
Principal Investigator

Trainee

SECTION - I: RESEARCH PROTOCOL

1.	TITLE	: EXAMINATION OF A POSSIBLE CORELATION BETWEEN THE PHAGE PATTERN AND PLASMID PROFILIE OF ETEC STRAINS.
2.	PRINCIPAL INVESTIGATOR	: Dr. Sadequr Rahman* Assistant Professor, Department of Biochemistry, University of Dhaka (Thesis Supervisor), Dhaka.
	CO-INVESTIGATORS	: Mrs. Yasmin Ara Begum Prof. K.A. Monsur (Thesis Co-Supervisor) Dr. Zia Uddin Ahmed (Consultant)
3.	STARTING DATE	: January 01, 1987 or date of approval.
4.	COMPLETION DATE	: December 31, 1987 after one year from date of approval.
5.	TOTAL DIRECT COST	: $ 4200/-
6.	AVAILABILITY OF FUNDS	: Available
7.	SCIENTIFIC PROGRAM HEAD	: This protocol has been approved by the LSE Division.

Signature of the Scientific Division Head _____

Date _____

* As per recommendation of SRC of PCC Dr. Sadequr Rahman will be the P.I. and Mrs. Yasmin Ara Begum will be the Co-P.I.

SECTION - I: RESEARCH PROTOCOL

1. TITLE : EXAMINATION OF A POSSIBLE CORRELATION BETWEEN THE PHAGE PATTERN AND PLASMID PROFILE OF ETEC STRAINS.

2. PRINCIPAL INVESTIGATOR : Ms. Yasmin Ara Begum

CO-INVESTIGATORS : (i) Dr. Sadequr Rahman
Assistant Professor
Department of Biochemistry
University of Dhaka
(Thesis Supervisor)

The protocol is interesting & worth pursuing.
Sadequr Rahman

: (ii) Prof. K.A. Monsur
(Thesis Co-Supervisor)

(iii) Dr. Zia Uddin Ahmed
(Consultant)

3. STARTING DATE : January 01, 1987

4. COMPLETION DATE : December 31, 1987

5. TOTAL DIRECT COST : $ 4200/-

6. AVAILABILITY OF FUNDS : AVAILABLE

7. SCIENTIFIC PROGRAM HEAD : This protocol has been approved by the BLS Division

Approved and recommended by Academic Committee (15.1.87) Dept. of Biochemistry Dhaka University 15/1/87

Chairman Dept. of Biochemistry Dhaka University

Signature of the Scientific Division Head: _____

Date: _____

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1. TITLE : EXAMINATION OF A POSSIBLE CORRELATION BETWEEN THE PHAGE PATTERN AND PLASMID PROFILE OF ETEC STRAINS.

2. PRINCIPAL INVESTIGATOR : Ms. Yasmin Ara Begum

CO-INVESTIGATORS : (i) Dr. Sadequr Rahman
Assistant Professor
Department of Biochemistry
University of Dhaka
(Thesis Supervisor)

: (ii) Prof. K.A. Monsur
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7. SCIENTIFIC PROGRAM HEAD : This protocol has been approved by the ELS Division

Signature of the Scientific Division Head: David A. Sack

Date: 6 Jan 1987

ABSTRACT

Enterotoxigenic Escherichia coli is a major cause of diarrhoeal disease throughout the world. The ability to secrete exterotoxins either a low molecular weight heat stable (ST) or a high molecular weight heat labile (LT) toxin is usually plasmid mediated. Phage sensitivity pattern is a potentially simple method of detecting the identity or otherwise of most strains of E. coli from a mixture of colonies. Plasmid profile is another such useful tool. Recently we obtained from a single patient, isolates of ETEC that gave three different phage patterns. These isolates also gave three different plasmid profiles.

The study wants establish if the above observation is generally true. If enterotoxigenic E. coli, (ETEC) isolated from the same individual, which are found to be different in their phage pattern are also found to have different plasmid profiles, this will confirm that the individual has simultaneous infection with more than one toxigenic strains of E. coli. This will indicate the necessity for further studies to explain how this may have happened.

SECTION - II: RESEARCH PLAN

A. INTRODUCTION:

1. Objective:

The main objectives of the proposed study are:

a) to examine a sample of ETEC isolates from the same patient for their phage-sensitivity pattern and plasmid profile.

b) to examine the extent of correlation between these two properties.

2. Background:

Escherichia coli population is normally present in the gut of men and animals as a changing mixture of many different strains (1, 2, 3). It has been observed that, using the phage lysotype as a marker, it may be possible to establish the identity or otherwise of particular strains present in such a mixture (4). Plasmid profile analysis has proven to be another useful tool for identifying the putative strain in epidemiological studies of infectious diseases in various microorganisms (5, 6). Electrophoresis of plasmid DNA in agarose gel provides a simple and useful method for such identification.

In human, there are 4 recognized classes of Escherichia coli that cause diarrhoeal diseases; enteropathogenic,

enterotoxigenic, enteroinvasive and recently recognized enterohemorrhagic or verotoxin producers. Plasmids have been found to be associated with each of these recognized pathogens.

Studies have shown that moderately large plasmid in E. coli code for virulence factors viz ST/LT. Other studies have shown that most naturally occurring strains of E. coli also contain small (0.8-10) Mdalton non-conjugative plasmids. Genetic transfer study showed that ST production may be transferred to 30% of the transconjugants selected on the basis of drug resistance (7). The LT DNA region is known to be a part of a large, transmissible plasmid which could be transferred to wild type E. coli and also to E. coli K-12 (8). However, the plasmids that encode LT are large, about 50×10^6 daltons in mass (9). It has also been suggested that the toxin genes may also be carried by transposable genetic elements. Because of these, toxin production can be both an easily transferable and an unstable trait.

Both LT and ST toxin genes are capable of being transferred from one (toxigenic) strain to another. It is thus possible that this may be the explanation for the presence of toxigenic strains with more than one phage pattern in the same subject. This possibility will be further strengthened if, in the same individual, toxigenic strains with different phage patterns are also found to have different plasmid profiles. Such a finding will have considerable academic and clinical significance. In that case it will justify further study to identify this

mechanism. It is possible that some of the toxigenic strains are only short term carriers of toxigenicity and the commonly recognized ETECs are really those in which the toxigenicity, once acquired, tends to remain as a stable characteristic.

If the above findings are found to be of common occurrence, it will provide a strong justification for further studying:

i) the detailed mechanism of transfer of toxigenicity among the E. coli strains in the intestine;

ii) the stability of toxigenic characters acquired by strains not commonly labelled as ETECs.

B. SPECIFIC AIMS:

a) To further evaluate the use of phage pattern as an identification marker for E. coli strains by examining also their plasmid profile. The assumption here is that if two strains give two phage patterns, they are different. If these strains also give two different plasmid profiles, the validity of this assumption will be reinforced.

C. MATERIALS AND METHODS:

In the vaccine trial field study three individual toxin positive Escherichia coli colonies from the same patient with watery diarrhoea will be tested for their phage sensitivity patterns (4). If these different colonies give more than one phage patterns, then they will also be tested for their

plasmid profile. Groups of E. coli strains with similar phage pattern will also be tested for their plasmid profile to find out if the plasmid profile is also the same.

Test for phage sensitivity pattern:

The test strains, originally from the cholera vaccine trial, are preserved in stab culture in tryplicase soya agar. Strain for this study will be transferred from this stab culture to glycerine broth and preserved at - 70 °C. The phage sensitivity pattern for each strain will be tested by the method described by Monsur et al. (4) using 31 phages. Test will also be carried out to observe if there is inherent variability in the pattern among the different colonies of same origine.

Isolation of plasmid DNA and agarose gel electophoresis:

Plasmids will be extracted by a modified version of the technique of Birnboim and Doly (10), and electrophoresed by using standard procedures.

The size of the plasmid DNA will be estimated by including strains with plasmid of known molecular wt. in the same electrophoretic run.

Two strains of E. coli will be used as a source of plasmids of known molecular weight, (V-517; containing eight plasmid species ranging in size from 1.2 to 35.8 Megadalton and pdk-9 containing 105 and 140 Megadalton (Mdal).

D. **SIGNIFICANCE:**

1. If ETEC strains isolated from a particular individual are found to have different phage sensitivity pattern and also different plasmid profile this will reinforce the assumption that the individual has simultaneous infection with more than one ETEC strain. The study may also enable us to identify in our strains the plasmid involved and also enable us to plan further study relating to the mechanism of transfer for this plasmid.

2. Good correlation between phage pattern and plasmid profile will also strengthen the value of phage pattern as an identification marker.

COLLABORATION

This is a collaborative study with the Unviersity of Dhaka. The work will be carried out at ICDDR,B by Mrs. Yasmin Ara Begum in partial fulfillment of the degree of M. Phil. of the University of Dhaka. Dr. Sadequr Rahman of the Department of Biochemistry, University of Dhaka, will act as her supervisor. It is hoped that the study will eventually be funded by the PCC. But the ELS Division is requested to process the initial clearance and funding till such time that the mechanism of formal funding and approval by PCC gets completed.

SUMMARY FOR ETHICAL REVIEW COMMITTEE

Enteroitoxigenic Escherichia coli is a major cause of diarrhoeal disease throughout the world. The ability to secrete exterotoxins either a low molecular weight heat stable (ST) or a high molecular weight heat labile (LT) toxin is usually plasmid mediated. Phage sensitivity pattern is a potentially simple method of detecting the identity or otherwise of most strains of E. coli from a mixture of colonies. Plasmid profile is another such useful tool. Recently we obtained from a single patient, isolates of ETEC that gave three different phage patterns. These isolates also gave three different plasmid profiles.

The study wants establish if the above observation is generally true. If enterotoxigenic E. coli, (ETEC) isolated from the same individual, which are found to be different in their phage pattern are also found to have different plasmid profiles, this will confirm that the individual has simultaneous infection with more than one toxigenic strains of E. coli. This will indicate the necessity for further studies to explain how this may have happened.

The study will be carried out only on stool/RS received from the vaccine trial or on laboratory specimens received for other studies. No patients will be involved. Hence no consent from is required.

Reference

1. Cruickshank, R., Duguid, J.P. Marmion, B.P., and Swain, R.H.A., 1973. Medical Microbiology 12th ed. rol. 1 pp. 328.

2. Sears, H.J. and Brownlee, I. 1952. Further observations on persistence of individual strains of Escherichia coli in the intestinal tract of man. J. Bact. 63: 47-57.

3. Sears, H.J. Brownlee, I. and Uchiyama, J.K. 1950. Persistence of individual strains of Escherichia coli in the intestinal tract of man. J. Bact. 59: 293-301.

4. Monsur, K.A. et al. 1985. Use of Bacteriophage as a marker for identification of freshly isolated individual Escherichia coli strains. J. Diarrhoeal Dis. Res. 8(3): 131-137.

5. Burnner, F.A. Margadant, R. Peduzzi, J.C. Piffaretti, 1983. The plasmid patern as an epidemiological tool for Salmonella typhimurium epidemics, comparison with the lysotype J. Infect. Dis. 148: 7-11.

6. Farrar, W.E. 1983. Molecular analysis of plasmids epidemiologic investigation. J. Infect. Dis. 148: 1-6.

7. Wachsmuth I.K., S. Falkow and R.W. Ryder, 1976. Plasmid mediated properties of a heat stable enterotoxin producing Escherichia coli associated with infantile diarrhoea. Infect. Immun. 14: 403-407.

8. Smith, H.W., and M.A. Linggood 1971. Observations of the pathogenic properties of the K88, H1y and Ent plasmids of Escherichia coli with particular reference to poreine diarrhoea, J. Med. Microbiol. 4: 467-485.

9. So, M., J.H. Crosa, and S. Falkow. 1975. Polynuclestide sequence relationships among Ent. plasmids and the relationship between Ent plasmids and other plasmids. J. Bacteriol. 121: 234-238.

10. Birnboim, H.C. and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinal plasmid DNA.

SECTION III

A. DETAILED BUDGET

1. PERSONNEL			Project Requirement	
Name	Position	% time used	Taka	US$
Ms. Yasmin Ara Begum	Research Officer	30%	-*	-
Dr. K.A. Monsur	C-Supervisor	5%	-*	-
Dr. Sadequr Rahman	Thesis Supervisor (D.U.)	15%	-	1000/-
Dr. Zia Uddin Ahmed	Co-Supervisor	5%	-*	-

* These represent transfer from other areas and thus do not represent additional cost.

2. SUPPLIES

Bacteriological media 1000/-

Supplies and chemicals 2000/-

3. PRINTING AND PUBLICATION 200/-

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Total = Taka 30,000/- $ 3,200/-

No other expenditure under any heading established by ICDDR,B is required.

Tk. 30/- = US$ 1.00

Total $ = 3,200 + 1,000 = $ 4,200.00

CURRICULA VITAE

PRINCIPAL/CO-INVESTIGATOR

1. Surname/Family Name: RAHMAN.

   First name/other names SADEQUR

2. Date of Birth: 7.9.57.

   Place of Birth: DHAKA.

   Nationality: BANGLADESHI.

3. Degrees

Degree	Year	Institution	Discipline
B.A.(HONS)	1979	UNIVERSITY OF CAMBRIDGE, U.K.	BIOCHEMISTRY.
PH.D.	1982	UNIVERSITY OF LONDON, U.K.	BIOCHEMISTRY.

4. Academic Distinctions:

	Degree	Year
X		

5. Present post (Title, Institution, Dates)

   Title: ASSISTANT PROFESSOR, DEPARTMENT OF BIOCHEMISTRY.

   Institution: UNIVERSITY OF DHAKA.

   Dates: 12.1.86-

CURRICULA VITAE

6. Previous posts (Title, Institution Dates)

Title: a. POST-DOCTORAL FELLOW,DEPARTMENT OF BIOCHEMISTRY,

b.

c.

Institution:

a. UNIVERSITY OF TORONTO,CANADA.

b.

c.

Dates:

a. 1.4.83 -31.7.85.

b.

c.

7. Academic & Research Awards, Consultant & other posts

X

8. Other University & Institutional Posts

X

9. Current Research Interests including details of Projects of which Applicant is Principal Investigator.

A) INVESTIGATION OF THE STORAGE PROTEINS OF RICE.

B) INVESTIGATION OF EXTRACELLULAR POLYSACCHARIDES OF RHIZOBIUM.

10. Publications & Communications

SEE ATTACHMENT.

List of Publications:

Ph.D. Thesis: The Synthesis of Seed Storage Proteins in Barley. University of London, 1982.

Articles:

1. Grzelczak, Z., Rahman, S., Kennedy, T.D., Lane, B.G. (1985). Compartmentation of Germin, its translatable mRMA and its biosynthesis among the roots, stems and leaves of wheat seedlings. Canadian Journal of Biochemistry ~~(in press)~~. 63, 317-327

2. Kreis, M., Shewry, P.R., Forde, B.G., Rahman, S., Miflin, B.J. (1985). ~~Homology between barley, wheat and rye storage protein genes.~~ J. Molec. Biol. ~~(in press)~~. Molecular Evolution of Seed Storage Proteins of 183, 499-502 Barley, Rye and Wheat.

3. Shewry, P.R., Forde, B.G., Kreis, M., Rahman, S., Miflin, B.J. (1984). The structure and expression of barley storage protein genes. Kulturpflanze 32, 61-70.

4. Rahman, S., Kreis, M., Forde, B.G., Shewry, P.R., Miflin, B.J. (1984). Hordein gene expression during development of the barley (Hordeum vulgare L.) endosperm. Biochem. J. 223, 315-322.

5. Miflin, B.J., Forde, B.G., Kreis, M., Rahman, S., Forde, J., Shewry, P.R. (1984). Molecular biology of the grain storage proteins of Triticeae. Phil. Trans. Roy. Soc. B, 304, 333-339.

6. Kreis, M., Shewry, P.R., Forde, B.G., Rahman, S., Bahramian, M.B., Miflin, B.J. (1984). Molecular analysis of the effects of the lys 3a gene on the expression of Hor loci in developing endosperms of barley (Hordeum vulgare L). Biochem. Genet. 22, 231-255.

7. Shewry, P.R., Kreis, M., Rahman, S., Forde, B.G., Miflin, B.J., (1983). Biochemical evidence for two sub-families of genes at the Hor-2 locus. Barley Genetics Newsletter 13, 35-37.

8. Rahman, S., Kreis, M., Forde, B.G., Shewry, P.R., Miflin, B.J. (1983). Nutritional control of storage protein synthesis in developing grain of barley (Hordeum vulgare L.). Planta 159, 366-372.

9. Miflin, B.J., Rahman, S., Kreis, M., Forde, B.G., Blanco, L., Shewry, P.R. (1983). The hordeins of barley: developmentally and nutritionally regulated multigene families of storage proteins. In: Structure and Function of Plant Genomes. Ed. O. Ciferri and L. Dure III. New York. Plenum Press. pp. 55-92.

10. Kreis, M., Shewry, P.R., Forde, B.G., Rahman, S., Miflin, B.J. (1983). Molecular analysis of a mutation confering the high-lysine phenotype on the grain of barley. Cell 34, 161-167.

11. Kreis, M., Rahman, S., Forde, B.G., Shewry, P.R., Miflin, B.J. (1983). Sub-families of hordein mRNA encoded at the Hor-2 locus of barley. Mol. Gen. Genet. 191, 194-200.

12. Rahman, S., Shewry, P.R., Miflin, B.J. (1982). Differential protein accumulation during barley grain development. J. Exp. Bot. 33, 717-728.

In Preparation:

1. Rahman, S., Kennedy, T.D., Grzelczak, Z., Lane, B.G. (1987). Molecular cloning of Germin, a growth related protein from wheat embryos.

2. Kos, E., Rahman, S., Grzelczak, Z., Kennedy, T.D., Lane, B.G. (1987). Complete Sequence of Germin deduced from cDNA clone.