66

Date

ETHICAL REVIEW COMMITTEE, ICDDR,B.

pal Investigator S.Q. Akhtar

Trainee Investigator (if any) X

ation No. 83-030

Supporting Agency (if Non-ICDDR,B)

of Study Studies on the

genicity of different Shigella

es

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

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

urce of Population:

) Ill subjects Yes No
) Non-ill subjects Yes No
) Minors or persons under guardianship Yes No

NA

es the study involve:

) Physical risks to the subjects Yes No
) Social Risks Yes No
) Psychological risks to subjects Yes No
) Discomfort to subjects Yes No
) Invasion of privacy Yes No
) Disclosure of information damaging to subject or others Yes No

NA

es the study involve:

) Use of records, (hospital, medical, death, birth or other) (Yes) No
) Use of fetal tissue or abortus Yes (No)
Use of organs or body fluids Yes (No)

subjects clearly informed about:

Nature and purposes of study Yes No
Procedures to be followed including alternatives used Yes No
Physical risks Yes No
Sensitive questions Yes No
Benefits to be derived Yes No
Right to refuse to participate or to withdraw from study Yes No
Confidential handling of data Yes No
Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No

NA

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 NA
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)
NA 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)
NA Informed consent form for subjects
NA Informed consent form for parent or guardian
NA 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.

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

S. Q. Akhtar
incipal Investigator

Trainee

SECTION II - RESEARCH PLAN

INTRODUCTION

1. Objective :

The objective of this study is to utilize plasmid analysis to identify invasive factors of different clinical isolates of Shigella spp and serotypes in Bangladesh.

2. Background :

Bacteria of the genus Shigella produce, via the processes of penetration, multiplication and intercellular dissemination, an infection that is normally limited to the epithelial cells of the colon and results in bloody, mucous diarrhoea. Many studies have been performed since Conradi in 1903 reported on Shigella toxin. More recently, interest has been focused on the role of plasmids in the biological properties of Shigella. It has become increasingly evident that plasmids often play an important role in the pathogenic potential of a variety of organisms. The various technical advances that have accrued from the study of the molecular biology of R plasmids can now be directed towards the investigation of plasmid mediated virulence factors (Elwell et al, 1980).

Sansonetti et al, (1981) demonstrated that a large plasmid of approximately 120 Mdal is necessary for invasion in S. sonnei and reported that the virulent form I of S. sonnei strains contain a 120 Mdal plasmid absent from form II derivatives which are always avirulent and devoid of the O side chain. Indirect evidence is available showing that plasmids are involved in the invasion process of S. flexneri and

also has been provided for S. sonnei (Kopecko et al., 1980; Sansonetti et al. 1980). Kopecko et al. (1980) analyzed the plasmid DNA of form I and II cells of four different S. sonnei isolates, obtained from different areas of the world and found a large plasmid (120 Mdal in 3 of the strains) present in form I cells to be absent from form II derivatives. They found that the spontaneous transition from the virulent form I serotype to the avirulent form II in S. sonnei is associated with the loss of a large, unstable plasmid. Reintroduction of this non-self-transferable plasmid by conjugal mobilization into form II recipients reestablished complete form I lipopolysaccharide synthesis. Sansonetti et al (1982) also reported the involvement of a plasmid in the invasive ability of S. flexneri. All invasive S. flexneri strains irrespective of serotype, were found to harbour a large plasmid of 140 Mdal in size. Spontaneous variants that had lost this 140 Mdal plasmid could neither invade HeLa cell monolayers nor produce Keratoconjunctivitis in guinea pigs. These avirulent strains regained virulence after plasmid transfer. Sansonetti's data directly demonstrated that the large plasmid encodes or regulates some functions (s) required for epithelial cell penetration.

A 140-megadalton plasmid (pWR 110), which has previously been associated with virulence in Shigella flexneri, was transferred to E. coli K-12. Segments of S. flexneri chromosomal materials were then transferred to the plasmid-bearing K-12 strains. The virulence of these transconjugant hybrids was assessed in the HeLa cell model, in ligated rabbit ileal loops, or in the Sereny test. A K-12 strain which harbored only pWR110 invaded HeLa cells, produced minimal lesions in the rabbit ileal mucosa, and was negative

in the Sereny test. Plasmid containing K-12 hybrids which had incorporated various Shigella chromosomal regions gave differential reactions in the rabbit ileal loops and in the Sereny test. Analysis of these transconjugants indicated that three regions were linked with virulent phenotypes. These included the histidine (his) region (when the genes responsible for O-antigen synthesis were cotransferred) and the (kcp) keratoconjunctivitis provocation locus (linked to the lac-gal-region). Either of these chromosomal regions was sufficient to allow invasion of the rabbit ileal mucosa. In addition to both of these regions, another Shigella chromosomal segment linked to the arginine (arg) mannitol (mtl) loci was necessary for fluid production in the rabbit ileal loop and for a positive Sereny reaction. Thus, derivatives of an E. coli K-12 strain, constructed by the stepwise conjugal transfer of a large plasmid and three chromosomal segments from S. flexneri, appears to contain the necessary determinants for full pathogenicity in a variety of laboratory models (13).

The presence of a 41-Mdal plasmid was found to be correlated with tissue invasiveness (positive Sereny test) in strains of Yersinia enterocolitica involved in an outbreak of human enteric disease. Many strains produced ST but ST synthesis was not associated with any particular plasmid. A 35-megadalton plasmid was harbored by many strains but, unlike the 41-Mdal species, the smaller plasmid was not associated with any invasive property of Y. enterocolitica (Zink et al, 1980). This may be correlated with Sansonetti's observation that a large plasmid is necessary for or involved in invasion.

Though this protocol an attempt will be made to explore whether the invasive capability of all strains of different species of Shigella is plasmid mediated. Plasmid analysis of strains isolated from asymptomatic excretors would also help to provide evidence on this aspect. Asymptomatic excretors were detected in family contacts of Shigella-infected cases in Bangladesh (Khan et al, 1980). Symptomless infection (34.7%) in all age groups has also been reported (Khan et al, 1979).

Epidemiological and clinical aspects of shigellosis have been studied at ICDDR,B but little work has been done on the pathogenicity of these organisms to date except for the reports of Aziz (1975, 1979) concerning enterotoxigenicity of S. flexneri. Studies of the haemolytic uremic syndrome and Shigella sepsis are in progress. This study aims at exploring the invasive capability of Shigellae, notably the association of plasmids with invasiveness.

Rationale :

Shigellosis is one of the main causes of morbidity and mortality in our population. Except epidemiological and clinical studies no such research has been carried out on the pathogenicity of Shigella organisms at ICDDR,B or any other Medical Research Institutions of Bangladesh. Through this research protocol we expect to achieve a better understanding on the mechanism of pathogenesis of Shigella sp. which provides a good rationale for carrying out this study. Because Shigella infection is a cause of major health problem, this has been identified as one of the priority research areas by ICDDR,B research programs. The study is also justifiable being on the priority area.

ECIFIC AIMS

To determine plasmid profiles of Shigella isolates from different clinical sources (patients, asymptomatic excretors).

To correlate the presence of large plasmid DNA (120, 140 Mdal) with the invasive properties of the organisms.

3. To correlate clinical manifestation and/or symptomatology with plasmid-mediated invasion.

4. To establish plasmid transfer technique in ICDDR,B.

METHODS OF PROCEDURE

The study will be performed using two groups of Shigella isolates :

First Group : This will comprise of routine clinical isolates. Eight to ten isolates of each serotype of the individual species will be included in the study. As all serotypes of the invididual species may not be available, as many serotypes as possible to allow examination of the species variation be included. Two isolates of Shigella will be collected daily from hospital specimens for the study. Prior to selection of Shigella strains from the Microbiology Laboratory, a clinical form (Form I) will be filled in by the clinician (Co-Investigator) responsible for all patients suspected to have shigellosis.

Second Group : The second group will consist of a group of Shigella strains isolated from patients and asymptomatic excretors from Nandipara (From Protocol No. 82-044 (B.C. No. 01-43-00). Those isolates will be obtained as blind samples. The symptomatic and asymptomatic status of these strains will not be disclosed until the end of the study. Clinical and demographic information will be obtained for symptomatic excretors by the field workers (Form No. 2). This group of strains will consist of 200-300 isolates, depending on the availability of strains both from symptomatic and asymptomatic sources.

Asymptomatic excretor selection :

Diarrhoea patients where only Shigella but not other diarrhoea causing bacterium has been isolated will be taken as the index case (from Protocol No. 82-044 : B.C. No. 01-43-00). Because the field workers collect stool/RS of all contacts of every diarrhoea patient on the same day, asymptomatic Shigella excretors can, thus, be identified. Any contact of a Shigella-infected index case will be interviewed and observed for signs and symptoms of diarrhoea/dysenteriae each day until the 10th day from identification of the index case, in order to cover the incubation period. If any asymptomatic excretors that were identified on the 1st day develop diarrhoea during the 10 day period of interviewing, the case would not be recorded as that of an asymptomatic excretor, but will be included in the symptomatic group.

Plasmid Analysis :

The method of Birnboim and Doly will be used to isolate plasmid DNA from all of the test strains (Table 1). Isolated plasmid DNA will be electrophoresed to obtain plasmid profiles, using the agarose gel electrophoretic method for identification and characterization of plasmid DNA, as described by Meyers et al. (1976). Samples will be electrophoresed in 0.7% agarose gel. Samples will be freshly prepared or, if stored, kept at $4^{o}C$. Repeated freezing and thawing of the DNA samples can convert DNA to both open circular and linear form. Ethidium bromide (1 ug/ml) is used to stain the gels. Plasmid DNA bands are visualized by UV transillumination.

Transfer experiments :

Transfer experiments to demonstrate transfer of invasive factors will be performed. E. coli K_{12} strain with a marker (nalidixic acid resistance) will be used as the recipient strain.

Plasmid Transfer :

Purified donor and recipient cells will be grown at 37°C in Penassay broth until mid-exponential phase; 0.2 ml each of the donor and recipient cultures will then be mixed in 10 ml of Penassay broth. After overnight incubation, the mating mixture will be centrifuged and washed in saline. Appropriate dilutions will be plated on selective medium. The transfer frequency is expressed as the ratio of the number of transconjugants per input donor cell.

It transfer is not detected by broth mating, donor and recipient bacteria will be grown together on the surface of a Trypticase soy agar (TSA : BBL Microbiology System) plate. After 24 and 48 hours incubation, mating mixtures will be harvested, and appropriate dilutions plated onto the selective medium. Transconjugants will always be purified twice by single colony isolation onto selective medium and will then be tested for invasive characteristics by the Sereny Test. To ensure optimal conjugal transmission, separate matings of each donor and recipient combination will be conducted both in liquid medium and on solid medium at one of four different temperatures (20, 32, 37 and 42°C).

Culturing the mating mixture on nalidixic acid (30 µg/ml) incorporated in MacConkeys agar will allow only the recipient E. coli K_{12} to grow. Twenty

colonies from 10^{-4} dilutions will be screened for plasmids, to detect transfer of the 120 Mdal plasmid DNA potentially associated with invasive properties. All test samples will be subjected to the Sereny Test to assess invasive properties.

All recipient strains, after the transfer experiment has been completed, will be subjected to the Sereny Test to confirm transfer of plasmid DNA encoding invasion from donor cells (test samples - Shigella isolates). Plasmid transfer is a new and rather sophisticated technique. Close collaboration with a laboratory routinely employing these methods will be valuable. It will be very useful for microbiologist to go to such a laboratory to learn the methods and standardize the procedures.

Correlation of clinical data with Plasmid profile:

Clinical data sheet will be maintained for individual patients both from cases reported to our Dhaka hospital and from cases identified at field. An attempt to correlate significant clinical menifestations with the plasmid profile of individual Shigella species, if not with the overall shigella organisms will be made after a reasonable amountof data is available. The patients would be graded according to the severity of infection into 3 groups:

a) Patients with mild or no constitutional symptoms.

b) Patients with moderate constitutional symptoms eg. high fever/ headache/malaise.

c) Patients having severe constitutional symptoms or complications eg. convulsions/hemolytic uremic syndroms etc.

Plasmid profiles of Shigella isolates will also be correlated with degree

of severity. Data sheets will be maintained in such a way so that computer can be used for data analysis if necessary.

SIGNIGICANCE

Being a cuase of major health problem, Shigella need be studied more extensively form different aspects, more intensively on each aspects. This study would cover microbiological and genetic aspect intensively. Significant results may be obtained from this type of basic studies.

FACILITIES REQUIRED

1. Office space: Already provided
2. Laboratory Space: Already provided.
3. Hospital Resources: Shigella infected patients.
4. Animal Resources: Guinea-pig.
5. Logistic Support: Yes
6. Equipment: Gel tank, and film for plasmid work.
7. Other Requirements; None

COLLABORATIVE ARRANGEMENTS

Dr. K. Wachsmuth, Molecular Biology Laboratory, Biotechnology Branch Division of Bacterial Diseases, Centre for Infectious Diseases. Centre for Disease Control, Atlanta, Georgia, U.S.A. and Dr. C. Tacket Centre for Disease Control. Atlanta, Georgia, U.S.A. have agreed to collaborate. Dr. Rita R. Colwell, Prof. of Microbiology. University of Maryland has also communicated that she is willing to this collaboration and will arrange facilities for the PI to work on plasmid transfer

technique in her laboratory. She has also informed that Dr. Y. Kopecko, who is currently working specifically on the involvement of large plasmid in the invasive ability of Shigella sp., will be working in her laboratory for sometime. Dr. Kopecko's adivse and help will be available if collaborative arrangements are made.

Table 1.

Modifed Birnboim Procedure: Isolation of Plasmid DNA

Cells grown overnight in L broth or BHIB (on roller at 37°C)
0.5 ml into 1.5 ml microcentrifuge tube
Spin for 15 seconds.

Aspirate supernatant and resuspend pellet in 100 µl Soln. I
Hold at 0.C 30 minutes.

Add 200 µl Soln. II, vortex gently. The solution should clear after 5 minutes at 0.C

Add 150 µl Soln. III, invert gently. Chromosomal DNA will clump and precipitate

Hold at 0.C for 60 minutes (minimum)
Spin 5 minutes

Transfer supernatant (approx. 400 ml) to fresh tube

Add 1 ml cold ethanol (-20°C), mix well
Hold at -70°C for 30 minutes

Spin 5 minutes.

Aspirate supernatant and resuspend pellet in 100 µl 0.1M NaAc, 0.05 M tris, HCI, pH 8.0

Add 200 µl cold ethanol, mix, well
Hold at -70°C 30 minutes or -20°C overnight.

Spin 2 minutes

Aspirate supernatant, air dry

Dissolve DNA in 25 µl TE, add 6 µl stop mix

Solution I - 4 mg/ml lysozyme, 50 mM glucose, 10 mM EDTA, 25 mM Tris HCl, pH 8.0
Prepare fresh daily, hold at 0.C

Solution II - 0.2 N NaOH, 1% SDS
Store at room temperature (stable approx. 1 week)

Solution III - 3 M NaAc (pH 4.8)
Dissolve anhydrous NaAc in minimal volume water
Titrate to pH 4.8 with glacial acetic acid
Bring to full volume

FORM I

Studies on the pathogenicity of different Shigella species

Clinical data sheet for hospitalized cases

Identification No. / / / / / / 1-5

Date: / / / / / / / 6-11

Age: / / / / / / / 12-17

Sex: Male = 1, Female 2 / / 18

History:

Duration of diarrhoea (hrs) / / / / 19-21

Frequency of motion (in last 24 hrs) / / / 22-23

Character of stool:

Watery = 1, Mucoid = 2
Mucoid bloody = 3 / / 24

Vomiting:

No.1, yes = 2 / / 25

Abdominal pain;

No = 1, yes = 2. / / 26

Fever:

No = 1, yes = 2 / / 27

Rectal prolapse

No = 1, yes = 2 / / 28

Convulsion

No = 1, yes = 2 / / 29

Urine:

Normal =1, scanty = 2, Not urinated within last 12 hours = 3 / / 30

General Examination:

Nutrition: Normal = 1, Marasmas = 2, Kwashiorkor =3, Marasmic Kwashaki =4 / / 31

Temp: /oF / / 32

Jandice: No.=1,yes = 2 / / 33

Dehydrational status:

No.=1,mild=2, Moderate=3, Severe=4 / / 34

Stock:

No=1,Hypovolaemic=2,Septic=3 / / 35

Rashes: No=1,pitacheal=2 / / 36

Chest Infection:

No=1,Brouchitis=2,Br.Pneumonia=3
Lobar pneumonia=4 / / 37

Abdomen:

Distansion:

Absent=1,Mild=2,Marked=3 / / 38

Tenderness: No=1,yes=2 / / 39

Liver:

Not palp = 1, palp=2 /___/40

Spleen;

Not palp=1,palp=2 /___/ 41

Bowel sound

Normal=1, sluggish=2,Abset=3 /___/ 42

Consciousness:

Alert=1,Drowsy=2,Semi-consciousness=3,
Coma=4. /___/43

Convulsion:

Absent=1,present=2 /___/44

INVESTIGATION:

R/S

Stool M/E

pH: /___/45

Acid=1,Alkaline=2,

Pus cell/H.P.F /___/___/___/ 46-48

R.B.C./H.P.F /___/___/___/ 49-51

Macrophage /H.P.F. /___/___/ 52-53

C.B.C:

Hct % /___/___/ 54-55

T/C /mm^3 /___/___/___/___/___/___/56-61

D/C /___/___/___/___/___/___/62-67

P% /___/___/ 68-69

Platelet/cmm /___/___/ 70-71

RBC fragmantation % /___/___/ 72-73

Band % /___/___/ 74-75

Chemistry:

Na^+ (mmol/l) /___/___/___/ 76-78

K+ (m mol/l) /___/___/ 79-80

Cl (m mol/l) /___/___/___/ 81-83

TCO_2 /___/___/ 84-85

Glucose (m mol/l) /___/___/ 86-87

Urea (m mol/l) /___/___/ 88-89

Creatinine (u mol/l) /___/___/___/ 90-92

Protein (G/l) /___/___/ 93-94

P/3..

Culture (Blood):

Sh. dysenteriae 1 = 1 /___/ 95

Sh. dysenteriae 2 = 2

Sh. dysenteriae (3-10) = 3

Sh. flexneri (1-6, x&y) = 4

Sh. sonnei (phase 1&2) = 5

Sh. boydii (1-6) = 6

Sh. boydii (7-11) = 7

Sh. boydii (12-15) = 8

Hospital stay:

Outcome

Cured/Disch = 1, Death = 2 /___/___/ 96-97

Culture (stool; R/S):

Sh. dysentieriae 1 = 1

Sh. dysenteriae 2 = 2

Sh. dysenteriae (3-10) = 3

Sh. flexneri (1-6, x&y) = 4

Sh. sonnei (phase 1&2) = 5

Sh. boydii (1-6) = 6

Sh. boydii (7-11) = 7

Sh. boydii (12-15) = 8 /___/98

CLINICAL DATA SHEET FOR SYMPTOMATIC CASES OF FIELD STUDY FORM 2

Identification No. / / / / / / 1-5

Date: / / / / / / / 6-11

Age: / / / / / / / 12-17

Sex: Male 1; Female = 2. / / 18

History:

Duration of Diarrhoea(hrs) / / / / 19-21

Consistency of stool: Watery=1; Mucoid=2, Bloody Mucoid=3. / / 22

Frequency of motions (last 24 hours) / / / 23-24

Vomiting: No.=1; Yes = 2. / / 25

Abdominal pain: No.=1; Yes = 2. / / 26

Abdominal discomfort: No.=1; Yes=2. / / 27

Tenesmus: No.=1; Yes=2. / / 28

Abdominal distention: No.=1; Yes=2. / / 29

Rectal Prolapse: No.=1; Yes=2. / / 30

Fever: Present 1; Absent=2. / / 31

Chill/Rigor: No.=1; Yes=2. / / 32

Rashes: Present=1; Absent=2. / / 33

Cough: Present=1; Absent=2. / / 34

Convlusion: No.=1; Yes=2. / / 35

Urine: Normal=1; Scanty=2; Not Urinated within last 12 hrs.=3. / / 36

Dehydration: None=1; Mild=2; Moderate=3; Severe=4. / / 37

Hospitalization: No.=1; Yes=2. / / 38

Specimen: Stool=1; R/S=2. / / 39

Cont'd /2.....

Shigella dysenteriae 1 = 1	/ /	40
S. dysenteriae 2 = 2	/ /	41
S. dysenteriae (3-10) = 3	/ /	42
S. flexneri (1-6, x & y) = 4	/ /	43
S. sonnei (phase 1 & 2) = 5	/ /	44
S. boydii (1-6) = 6	/ /	45
S. boydii (7-11) = 7	/ /	46
S. boydii (12-15) = 8	/ /	47

References

1. Sansonetti, P.J., D.J. Kopecko and S.B. Formal, 1982. Involvement of a plasmid in the invasive ability of Shigella flexneri. Infect. Immun. 35(3):852-860.

2. Sansonetti, P.J., D.J. Kopecko and S.B. Formal, 1981. Shigella sonnei plasmids: evidence that a large plasmid is necessary for virulence. Infect. Immun. 34:75-83.

3. Kopecko, D.J., O. Washington and S.B. Formal, 1980. Genetic and physical evidence for plasmid control of Shigella sonnei form 1 cell surface antigen. Infect. Immun. 29:207-214.

4. Aziz, K.M.S., 1975. Initial Studies on the toxin of Shigella dysenteriae type 1 and Shigella flexneri. Proceedings of the 10th meeting of the Scientific Review and Technical Advisory Committee of Cholera Research Laboratory, 1975:15-22.

5. Aziz, K.M.S. and K. Alam, 1979. Comparative studies of Crude Culture filtrates of Shigella dysenteriae Type 1 and Shigella flexneri, 1979. Bangladesh J. Microbiol: 1 (1):11-15.

6. Birnboim, H.C. and J. Doly, 1979. A rapid alkaline entraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research, 7 (6):1513-1523.

7. Khan, M.U. and M. Shahidulla, 1980. Contrasting epidemiology of Shigellae dysenteriae and Shigellae flexneri, Dhaka. Trans. Roy. Soc. Trop. Med. and Hyg., 74(4):528-533.

8. Khan, M.U., G.T. Curlin and M.I. Huq, 1979. Epidemiology of Shigella dysenteriae Type 1 infections, in Dhaka urban area. Trop. Geogr. Med., 31:213-227.

9. Sereny, B., 1955. Experimental Shigella conjunctivitis. Acta. Sci. Hang., 2:293-296.

10. Zink, D.L., J.C. Feeley, J.G. Wells, C. Wanderzant, J.C. Vickery, W.D. Rool and G.A.O. Donovan, 1980. Plasmid mediated tissue invasiveness in Yersinia enterocolitica. Nature (London) 283:224-226.

11. Meyers, J.A., D. Sanchez, L.P. Elwell and S. Falkow, 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid Deoxyribonucleic acid. J. Bacteriol. 124(3):1529-1537.

12. Elwell, L.P. and P.L. Shipley, 1980. Plasmid mediated factors associated with virulence of bacteria to animals. Ann. Rev. Microbiol., 34:465-496.

13. Sansonetti, P.J., T.L. Hale, G.J. Dammin, C. Kapfer, H.H. Collins Jr., & S.B. Formal, 1983. Alterations in the pathogenicity of E. coli K-12 after transfer of plasmid and chromosomal genes from Shigella flexneri. Infect. Immun. 39(3): 1392-1402.

Abstract Summary

The presence of large molecular weight plasmids (120, 140 megadalton) has been demonstrated in Shigella flexneri and S. sonnei. Involvement of the plasmids in the invasive ability of these organisms has been experimentally shown. Therefore, we intent to study the plasmid profiles of Shigella strains belonging to different species and serotypes isolated in Bangladesh from different clinical sources (patients, asymptomatic excretors). The invasive capability of those isolates will be screened by the Sereny test. An attempt will be made to transfer the plasmid (s) to a nonvirulent recipient E. coli K_{12}, which in turn will be tested in experimental models (Sereny test) to determine whether the characteristic has been acquired. Plasmid preparations from recipient strains in transfer experiment will be electrophoresed to detect plasmid DNA incoded invasive properties. Plasmid transfer methods will established within ICDDR,B Laboratory through this protocol. Clinical data sheets will be maintained to correlate clinical manifestations with invasive capability of the organisms.

Methods of research do not create potential risk.

Consent from the subject is not needed because the study is mainly a laboratory based study.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% or No. of Days	Annual Salary	Project Requirements Taka	Dollar
Dr. S.Q. Akhtar	P. Investigator	50%	-	36,000	-
Dr. M.I. Huq	Co-Investigator	5%	-	-	2,250
Dr. A.R. Samadi	Co-Investigator	5%	-	-	2,900
Mr. K.A. Talukdar	Lab. Technician	50%	-	20,000	-
Dr. Al-Mahmood	Assoc. Scientist	10%	-	10,000	-
Dr. M.A. Salam	Med. Officer	10%	-	7,000	-
To be named	Field Worker	100%	-	33,000	-
			Sub-Total:	106,000	5,150

2. SUPPLIES AND MATERIALS

Culture	(2000 x 15)	30,000	-
Gel Tank and other excessories		-	2,500
Chemicals		-	1,500
Guinea pigs	(35 x 800)	28,000	-
Stationery, Office Supplies		15,000	-
	Sub-Total:	73,000	4,000

3. EQUIPMENT - None

4. PATIENT HOSPITALIZATION - None

5. OUTPATIENT CARE - None

6. ICDDR,B TRANSPORT - 5,000 -

7. TRAVEL AND TRANSPORTATION OF PERSONS

Air-Ticket for collaborative Scientists	- (ATL/DAC/ATL)	-	2,650
	- (DAC/ATL/DAC)	-	2,650
Exchange visit for establishment of plasmid transfer technique.			
	Sub-Total:	-	5,300

8. TRANSPORTATION OF THINGS - - 1,000

9. RENT, COMMUNICATION AND UTILITIES - 1,710
- 1,260
Sub-Total - 2,970

10. PRINTING AND REPRODUCTION - 7,000

11. OTHER CONTRACTUAL SERVICES - None

12. CONSTRUCTION, RENOVATION AND ALTERATIONS - None

B. BUDGET SUMMARY

		TAKA	DOLLAR
1.	Personnel Services	106,000.00	5,150.00
2.	Suuplies and Materials	73,000.00	4,000.00
3.	Equipment	-	-
4.	Patient Hospitalization	-	-
5.	Outpatient Care	-	-
6.	ICDDR,B Transport	5,000.00	-
7.	Trave and Transportation of Persons	-	5,300.00
8.	Transportation of Things	-	1,000.00
9.	Rent, Communication and Utilities	-	2,970.00
10.	Printing and Reproduction	7,000.00	-
11.	Other Contractual Services	-	-
12.	Construction, Renovation and Alteration	-	-
	Total: Total:	191,000.00	18,420.00

Grand Total: 27,102.00

Conversion Rate US$ 1.00=Tk. 22/-