ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 14/11/83
26/11/83

pal Investigator E. Carniel
:ation No. 83-041
of Study Comparative studies of DNA hybridization techniques and routine biochemical methods for detecting Shigella and ...

Trainee Investigator (if any)
Supporting Agency (if Non-ICDDR,B)

Project status:
(X) 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).

ource of Population:
) Ill subjects (Yes) No
) Non-ill subjects Yes (No)
) Minors or persons under guardianship (Yes) No

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)

s 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 NA Yes No
Confidential handling of data (Yes) No
Compensation &/or treatment where there are risks or privacy is involved in any particular procedure NA 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).
   X Protocol (Required)
   X 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.

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

ncipal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

1. Title : Comparative studies of a DNA hybridization technique and routine bacteriologic methods for detecting Shigellae and enteroinvasive Escherichia coli in stools.

2. Principal Investigator : Elisabeth Carniel

   Co-Investigator : Dr G. Poddar

3. Starting Date : December 1, 1983

4. Completion Date : May 31, 1984

5. Total Direct Cost : $ 1,967.00

6. Scientific Programme Head :

This protocol has been approved by the Disease Transmission Working Group.

Signature of Scientific Programme Head : K. M. S. Aziz

Date : 27/11/83

7. Abstract Summary

A DNA hybridization method for detecting Shigellae and enteroinvasive Escherichia coli in stools has been described at the "Institut Pasteur de Paris", service des enterobacteries. Dr. Sansonetti, Catherine Boileau, helene d'Hauteville. It is based upon the high degree of homology shared by the virulence plasmids present in all pathogenic strains. This method proved highly specific and sensitive and should be particularly useful for characterization of atypical isolates and for large scale epidemiological studies in endemic areas. Our work will

consist in improving this method on a large scale by obtaining stool samples from ICDDR,B patients. This technique will be partly carried out at the ICDDR,B and Institut Pasteur de Paris. The hybridization results will be compared with the bacterial ones obtained at the ICDDR,B. Therefore, this will be a collaborative project between the two institutes.

8. Reviews :

a. Ethical Review Committee : _____

b. Research Review Committee : _____

c. Director : _____

_____

A. INTRODUCTION

1. Objectives :

The objective of this study is to confirm the efficacy of the DNA hybridization technique with stools specimens of suspected enteroinvasive Escherichia coli (EIEC) and Shigella cases which be assessed on a large scale in Bangladesh where shigellosis is highly endemic. This way enable us to develop a quick and easy diagnostic test for these diseases and to establish the technique at the ICDDR,B.

2. Background :

Diarrhoeal diseases are the main cause of death throughout the developing world. Shigellae represents about 12% of the causative agents of diarrhoea in Bangladesh. The pathogenicity of Shigellae relates to their ability to invade the colonic mucosa and cause tissue damage. Therefore, the ability of these bacteria to penetrate into host cells is a critical step of enteroinvasiveness. It is now possible to identify these microorganisms through a hybridization technique.

The technique was first demonstrated and applied by Moseley et al. They examined enterotoxigenic Escherichia coli (ETEC) that produced (1) both heat labile (LT) and heat stable (ST) toxin (2) LT only and (3) ST only. ETEC was identified with the Y-1 adrenal cell and suckling mouse assays. They found that all were homologous with radiolabled fragments of DNA encoding LT or ST of porcin or human origin. ETEC in stool samples of patient with diarrhoea from whom ETEC was isolated and ETEC inoculated water containing other species of bacteria were positive

with the hybridization technique. The authors concluded that the DNA hybridization assay is useful for characterizing and identifying environmental source of ETEC.

Sansonetti et al have demonstrated that in the four Shigella species as well as in EIEC, a plasmid ranging between 120 and 140 M dal is necessary for the penetration step. All these virulence plasmids share a high degree of homology which reflects their common origin. They used a 27 Kb BAM HI restriction fragment of Shigella flexneri serotype 5 virulence plasmid as probe. This probe 32_p labeled could detect Shigellae and EIEC. A prospective study including 300 Shigella isolates that were sent to the "Centre de reference des Shigelles" showed a sensitivity of the method of 99.7% and a specificity of 100%. The probe also produced positive signals on some of the bacteriologically negative stools. Shigella positive stools were reconstituted by incorporating serial dilutions of strains M 90T to the feces of healthy subjects. Standard bacteriological method detected 10^4-10^5 C.F.U. of Shigella as compared to the hybridization technique which detected 10^3 C.F.U. This represented a 10-10^2 improvement in sensitivity which might explain positive signals on bacteriologically negative diarrhoeal stools.

3. Rationale :

In Bangladesh, shigellosis is highly endemic and sometimes causes death. EIEC is also an important cause of diarrhoea and it would be useful to have a quick and easy diagnostic test for them.

The DNA hybridization method for Shigellae and EIEC is a more sensitive technique than classical bacteriological methods. It compensates for the lack of a specific enrichment medium for Shigella and also for the lack of an efficient means of detection and identification of EIEC. The DNA hybridization method could replace in the near future costly and time consuming tests such as cell culture, invasion and KERATO-conjunctivities in guinea pigs. This technique is also useful to identify isolates that either have atypical biological patterns or cannot be agglutinated by reference sera (e.g. Shigella boydii serotype 5).

SPECIFIC AIMS

1. To test and improve a DNA hybridization technique for detecting Shigellae and EIEC in stools on a large scale.

2. To compare the results of DNA hybridization tests with those obtained by culture.

3. If the DNA hybridization technique proves to be specific and sensitive it could be established at ICDDR,B for routine diagnosis of Shigella and EIEC infections.

METHODS AND PROCEDURE

The routine bacteriological identification is carried out with rectal swab samples from ICDDR,B's hospital patients. Along with each swab sample, a stool sample will be collected for the DNA hybridization technique. Added to the routine bacteriological identification a CN broth culture for Shigella will be carried out in the bacteriological laboratory from the swab samples.

For the DNA hybridization technique:

The stools are suspended in an appropriate volume of distilled water (to be easily spotted onto nitrocellulose filters). Bacteria were grown overnight at 37°C in peptone water (5 g/l NaCl, 20 g/l indole free peptone). One spot of the stool suspension is applied onto a nitrocellulose filter (BA85 Schleicher-Schuell-Basel-Germany) placed on the surface of a McConkey agar plate. Twenty different strains are thus spotted on a filter. After overnight growth at 37°C, bacteria are lysed and the DNA denatured as described by Moseley et al: filters are placed for 10 mm on 0,5M NaOH saturated. Whatman paper no.III. This is followed by four one minute transfers onto paper saturated with 0.1M ammonium acetate and 0.02M NaOH. Filters are removed and thoroughly air dried after a fifth ten minute transfer onto an ammonium acetate NaOH saturated paper. The nitro-cellulose filter is kept overnight at 65°C and stored at room temperature. Each spot and each filter is numbered and sent to the Institut Pasteur de Paris" centre de reference des Shigelles Dr P. Sansonetti where the DNA probe is prepared and labelled. There, filters are incubated for 4 hours at 42°C in the prehybridization solution (Formamid 50%, 5 x SSC (1 x SSC: 0.15 M NaCl, 0.015 M sodium citrate), 5 x Denhardt solution, heat denatured calf thymus DNA 100 ug/ml). They are then placed in sealed plastic bags containing 10 ml of the hybridization solution (formamide 50%, 5 x SSC, 1 x Denhardt's solution, 10% dextran sulfate, 0.02 M sodium phosphate, pH = 6,5; 100 ug/ml of calf thymus and 10^6 cpm of probe DNA, both heat denatured). Hybridization is allowed to continue overnight at 42°C. The filters are then washed as follows: 3 five minute washes in 2 x SSC and

0,1% SDS, 2 thirty-minute washes at 50°C in 0,1% x SSC and 0,1% x SDS. They are air-dried before being exposed for 6 hours at -70°C to a Kodack X-OMAT(R) film (Eastman Kodak. Rochester - NY - USA) placed between two intensification screens (Philips - France). The film is developed according to the manufacturer's instructions. Results of bacterial identification found at the ICDDR,B will be compared with those of the DNA hybridization but after hybridization results are interpreted.

D. SIGNIFICANCE

From the result of this study it will be possible to determine whether DNA hybridization technique for detecting Shigellae and EIEC in stools is a specific and sensitive method, usuable on a large scale in endemic areas. Comparable results between the two methods would enable us to develop the technique at the ICDDR,B.

E. FACILITIES REQUIRED

1. Office space is needed.
2. Personnel: 1 technician 25% time
3. Laboratory space is needed
4. Hospital support: The persons who collects the rectal swabs will also collect stool samples.
5. Bacteriological support: (a) we need the results of the routine bacteriological method (b) testing of stools using special enrichment GN broth for Shigella.
6. Logistic support : none
7. Major item of equipment : None

8. Other special requirements: None

F. COLLABORATIVE ARRANGEMENTS

There is a collaborative arrangement with the "Institut Pasteur de Paris", service des enterobacteries centre de reference des shigelles, Dr. SANSONETTI, Catherine BOILEAU, Helene D' HAUTEVILLE.

BUDGET SUMMARY

Category	Taka	US Dollar
1. Personnel Services	12,300	
2. Supplies and materials	15,500	525
3. Equipments	-	-
4. Patients hospitalization	-	-
5. Outpatient care	-	-
6. Transport	-	-
7. Travel and Transportation	-	-
8. Transportation of things	-	200
9. Rent, communication,utility	-	-
10. Printing and reproduction	2,000	-
11. Other contractual services	-	-
12. Construction, Renovation, alteration	-	-
Total	Taka 29,800	US$ 725

Total Cost: US$ 1,242 + 725

GRAND TOTAL : US$ 1,967.00

Conversion rate US$ 1.00 = Tk.24.00

ABSTRACT SUMMARY

2. Stools samples will be collected in patients with diarrhoeal diseases, no special group in this population will be used.

3. There is no physical, psychological, social or legal risk for these patients.

5. Not applicable

4. No clinical records will be used, study numbers will be used for stool samples instead of patient's name.

5. Not applicable.

6. No interview will be required.

7. If the DNA hybridization technique prove highly specific and sensitive, it should be particularly useful for characterization of atypical isolates and for large scale epidemiological studies in Bangladesh where Shigellae and enteroinvasive E. coli are endemic.

8. This study only requires the use of stool samples.