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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 4.1.83

Principal Investigator Dr Carol Tacket

Trainee Investigator (if any)

Application No. 83-005(P)

Supporting Agency (if Non-ICDDR,B)

Title of Study Correlation of Plasmid Profile with Clinical & Epidemiologic Features of Shigellosis.

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).

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)
✓ 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.

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

Carol O Tacket
Principal Investigator

Trainee

83-005(P)
27/1/83.

SECTION I - RESEARCH PROTOCOL

1. Title : Correlation of Plasmid Profile with Clinical and Epidemiologic Features of Shigellosis.

2. Principal Investigator : Dr. Carol Tacket

   Co-Investigator : Dr. Nigar Shahid, Dr. M.I. Huq and Mr. A.R.M.A. Alim

3. Starting Date : January 3, 1983

4. Completion Date : February 25, 1983

5. Total Direct Cost : US$ 2998/=

6. Scientific Program Head :

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

Signature of the Scientific Program Head : [signature]

Date : 18/1/1983

7. Abstract Summary:

Shigellosis is a common cause of morbidity and mortality among patients treated at ICDDR,B. Shigellae commonly contain plasmid mediating such phenotypic characteristics as antimicrobial resistance and invasiveness. The presence of one or more plasmids may correlate with clinical and epidemiologic characterstics of illness. The purpose of this study is to identify plasmid patterns among a sample of Shigellae isolated at ICDDR,B. The plasmid profiles will be correlated with clinical and epidemiologic data available through ongong Surveillance Activity.

8. Reviews:

a. Ethical Review Committee: _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

a. To examine the plasmid profiles of (1) a random sample of *Shigella* isolates from patients with shigellosis seen at ICDDR,B and (2) the plasmid profiles of *Shigella* isolates from patients with shigellosis admitted to the medical ward.

b. To correlate clinical and epidemiologic features of shigellosis with plasmid profile using available surveillance data and hospital case records.

2. Background:

Shigella species are the second most common pathogen isolated from patients over 2-year-old seen at ICDDR,B; illness is associated with a case fatality rate of 0.7%(1) in 4% systematic random samples of all patients seen at ICDDR,B and 17% in hospitalized patients(2). In rural Bangladesh dysentery is the most common cause of diarrhoea-related death (3). Patients with *Shigella* infections are not homogenous and certain strain-related properties such as plasmid profile may distinguish groups of patients.

Shigella commonly contain plasmids, extra-chromosomal circles of DNA that may be transferred from one bacterium to another. The genes coded on plasmids may mediate invasiveness (4,5), antimicrobial

resistance, or other characteristics. ICDDR,B has recently begun to extract plasmid DNA using an alkaline-denuaturation proceddre requiring only small amounts of broth culture (6). This procedure been used to detect even large molecular mass plasmids in enteric pathogens.

As an epidemiological tool, plasmid profiles have been used as a marker to identify single clones of bacteria. This has facilitated studies of secondary spread of illness among contacts, spread of illness through community due to a single strain, and identification of vehicles in common source outbreaks (7,8). At CDC we have used plasmid profile analysis to study the relatedness of Shigella and other enteric pathogens and used these data as an epidemiologic tool in the investigation of several outbreaks of Shigella and Salmonella infections. We have examined a large group of Shigella strains and found that the plasmids visualized are a good marker for a single clone, making it possible to trace transmission of disease and identify common sources (7).

3. Another epidemiologic application of plasmid analysis might be to identify strains associated with more severe disease, those affecting a certain age group, those with ahigher mortality rate, or other groups. Since plasmids carry genes coding for clinically important phenomena such as invasiveness and antimicrobial resistance, the plasmid profile may be expected to correlate with certain clinical or epidemiologic features of shigellosis. The findings of this investigation will describe plasmids associated with endemic shigellosis in Dhaka and examine clinical and epidemiologic features associated with the presence of certain plasmids or groups of plasmids.

B. SPECIFIC AIMS

1. To examine plasmid profiles of a random sample of all Shigella isolates (including flexneri, dysenteriae, sonnei, and boydii) from patients treated at ICDDR,B.

2. To examine plasmid profiles of Shigella isolates from all patients admitted to the medical ward at ICDDR,B.

3. To study clinical and epidemiologic features of shigellosis using available surveillance data and determine whether the presence of certain plasmids correlates with these descriptive features.

C. MATERIALS AND METHODS

All available Shigella isolates from "surveillance patients" (a 4% random sample) who presented to ICDDR,B for treatment in 1982 will be examined. This will include isolates of Sh. flexneri, Sh. dysenteriae, Sh. sonnei, and Sh. boydii from approximately 150 patients. In addition, available isolates from a cohort of all patients with shigellosis admitted to the medical ward in the last quarter of 1982 will be studied.

DNA from each isolate will be extracted using a modified version of the Birnboin-Doly technique (5). Plasmid DNA will be separated on an agarose gel by electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light.

Clinical and epidemiologic data (available through ongoing Surveillance Activity) which correspond to each isolate will be obtained. These data include age and sex of patient, time of onset, duration of symptoms before presenting to ICDDR,B, degree of dehydration, nutritional status, dietary habits, nature of diarrhoea, method of treatment, and outcome. Patients with similar plasmid profiles will be grouped and these characteristics compared.

D. SIGNIFICANCE

This investigation may reveal associations between the presence of one or more plasmids and certain clinical and epidemiologic characteristics of shigellosis. If plasmids mediating antimicrobial resistance are identified, characteristics of infection with resistant strains will be studied. This study is an application of the established surveillance system and a new epidemiologic tool, the plasmid profile.

E. FACILITIES REQUIRED

1. Materials and equipment available in the plasmid laboratory.

2. Computerised surveillance data (already available).

3. Reference Shigella strains isolated from "surveillance patients" in 1982 and from medical inpatients from October 1-December 31, 1982 (already present in stock).

F. COLLABORATIVE ARRANGEMENTS

The study will be done in collaboration with Centers for Disease Control, Atlanta, USA.

REFERENCES

1. Stoll BJ, Glass RI, Huq MI, Khan MU, Banu H, Holt J. Epidemiologic and Clinical Features of Patients with Shigella Attending a Diarrhoeal Disease Hospital in Bangladesh. J Infect Dis 1982; 146:177-183.

2. Islam SS, Khan MU. Morbidity and Mortality in a Diarrhoea Hospital of Bangladesh, in preparation.

3. Samad A, Sheikh K, Sarder AM, Becker S, Chen LC. Demographic Surveillance System - Matlab, Volume 6. Vital Events and Migration - 1977. International Centre for Diarrhoeal Disease Research, Dhaka; Bangladesh, Scientific Report No. 18, February, 1979.

4. Sansonetti PJ, Kopecko DJ, Formal SB. Involvement of a plasmid in the Invasive Ability of Shigella flexneri. Infect Immun 1982; 35:852-35:852-860.

5. Sansonetti PJ, Kopecko DJ, Formal SB. Shigella sonnei plasmids: Evidence that a Large plasmid is Necessary for Virulence. Infect Immun 1981; 34:75-83.

6. Birnboim HO, Doly J. A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA. Nucleic Acids Res 1979; 7:1513-1523.

7. Tacket CO, Cohen ML. Use of Plasmid Profile Analysis in Two Outbreaks of Shigellosis. J Ped Inf Dis 1983; in press.

8. Taylor DN, Wachsmuth IK, Shangkuan Y-H, Schmidt EV, Barrett TJ, Schrader JS, Scherach CS, McGee HB, Feldman RA, Brenner DJ. Salmonellosis Associated with Marijuana: A Multistate Outbreak Traced by Plasmid Fingerprinting. N Engl J Med 1982; 306:1249-1253.

ABSTRACT SUMMARY

1. The investigation will involve 2 study populations (1) all patients with shigellosis in the 4% random sample used for ICDDR,B Surveillance Dacca Station, in 1982 whose isolates are stocked and (2) all patients with shigellosis admitted to the medical ward between October 1 - December 31, 1982 whose isolates are stocked. The second group was chosen because these patients have the most severe and complicated infections and should be examined as a special group.

2. No risks.

3-6 NA

7. The study will provide a practical application of a new epidemiologi al tool to further the understanding of endemic shigellosis in Dhaka.

8. The investigation requires the use of hospital case record and surveillance data.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% effort or No. of days	Annual Salary	Project Requirements Taka	Dollar
Dr C. Tacket		100%	-	-	-
Dr N. Shahid		15%	Tk85,120	3192	
Dr I Huq		5%	$ 54,700		680
Mr. ARMA Alim		15%	Tk75,980	2850	

2. SUPPLIES AND MATERIALS

Media	50
Chemicals	50

3. EQUIPMENT - None

4. PATIENT HOSPITALIZATION - None

5. OUTPATIENT CARE - None

6. ICDDR,B TRANSPORT - None

7. TRAVEL AND TRANSPORTATION OF PERSONNEL 1580

8. TRANSPORTATION OF EQUIPMENT - None

9. RENT, COMMUNICATION AND UTILITIES 1255

10. INFORMATION SERVICES (LIBRARY AND PUBLICATION)

11. PRINTING AND REPRODUCTION 4000

12. COMPUTER TIME Tk 200/hr 5 hrs 1000

13. CONSTRUCTION, RENOVATION, ALTERATIONS - None

BUDGET SUMMARY

		TAKA	US DOLLAR
1.	Personnel Services	6,042.00	680.00
2.	Supplies and Materials	-	100.00
3.	Equipment	-	-
4.	Patient Hospitalization	-	-
5.	Outpatient Care	-	-
6.	ICDDR,B Transportation	-	-
7.	Travel and Transportation of Personnel	-	1,580.00
8.	Transportation of Equipment	-	-
9.	Rent, Communication and Utilities	-	1,255.00
10.	Information Services (Library and Publication)	-	-
11.	Printing and Reproduction	400.00	-
12.	Other Contractual Servides (Computer Time)	1,000.00	-
13.	Construction, Renovation and Alterations	-	-
	TOTAL : -	7,442.00	3,615.00

Grand Total: US$ 3,953.00

Staff Commitment: US$ 955.00

Contingency Cost: US$ 2,998.00

Conversion Rate US$ 1.00 = Tk. 22.00