REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

Principal Investigator Dr. L.N. Mutanda
Application No. 79-015 (Revised)
Title of Study Incidence of Antibiotic-resistant enterobacteria in hospital and in the Community

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)

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) Bacteriology records) — (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 Board:
   ___ Umbrella proposal - Initially submit overview (all other requirements will be submitted with individual studies)
   ___ Protocol (Required)
   ___ Abstract Summary (Required)
   ___ Statement given or read to subjects 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 Board for review.

I agree to obtain approval of the Review Board on the Use of Human Subjects for any changes involving the rights and welfare of subjects before making such change.

Dr. L.N. Mutanda
Principal Investigator

_____
Trainee

79-015 (Revised)
Recd 8/1/80

SECTION I - RESEARCH PROTOCOL

(1) Title: INCIDENCE OF ANTIBIOTIC-RESISTANT ENTEROBACTERIA IN HOSPITAL AND IN THE COMMUNITY.

(2) Principal Investigator: Dr. L.N. Mutanda

(3) Starting Date: 1st January 1980

(4) Completion Date: End of December, 1980

(5) Total Direct Cost:

(6) Availability of Funds:

(a) Scientific Director's Remarks:-

(b) Controller's Remarks:-

(7) Abstract Summary: A research project aimed at reviewing bacteriology records to determine antibiotic-resistance pattern of enterobacteria is proposed. The project hopes also to determine the role of nosocomial infection in antibiotic-resistance of enterobacteriae, and the incidence of antibiotic-resistant faecal E.coli, environmental E.coli, Vibrio and Aeromonas in surface pond and/or river waters in Dacca. Transfer of R. Plasmids from some of the enterobacteria to recipient E.coli (K12) is among areas to be investigated.

(8) Reviews: a) Research involving human subjects _____

b) Research Review Committee: _____

c) BMRC: _____

d) Director: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

The goals of the proposed research are to provide a scientific basis for the development of our hospital or national policies concerning the use of antibiotics and to provide base-line data of the incidence of resistant enterobacteria in human diarrhoeal infections and in surface river waters.

2. Background:

The appearance of antibiotic-resistant strains of bacteria is closely linked to antibiotic use for the treatment of human infection. Resistance may appear rapidly or slowly, depending on the organism concerned, the volume and type of antibiotic used and the method of application.

Widespread outbreaks due to drug-resistant enterobacteria have been reported in many countries. *S. dysenteriae* type 1 outbreak has occurred in South America (Faich *et al.* 1972), in Bangladesh (Rahman *et al.* 1975) and in England (Moss and Darling, 1977). Infections with a multi-resistant strains of *S. sonnei* have been a persistent problem. For example, in the USA, 104 students and 14 staff members were involved in a waterborne outbreak due to a resistant strain in a school in 1972 (Baine *et al.* 1975). A high prevalence of antibiotic-resistance in *S. sonnei* and *S. flexneri* in Bangladesh was reported by Huq (1979) and

Rahman, in the annual report of 1977. During 1972-1973, an outbreak due to a multi-resistant S. typhi occurred in Mexico (Anderson and Smith, 1972). Resistance to the tetracyclines of S. typhimurium became evident in 1964 (Anderson, 1968). Studies of cultures from the United Kingdom and a number of other countries revealed that drug-resistant clones of S. typhimurium and other salmonellae were causing widespread outbreaks of infection, some on an international scale (Anderson, 1977). Nosocomial infection has reportedly featured prominently in the spread of multi-resistant strains of S. typhimurium in South America, Zaire and the Mediterranean region.

Antibiotic-resistant strains of E. coli are prevalent in the general population of healthy individuals. In the United Kingdom, about 15% of strains are resistant to ampicillin and 20% to streptomycin and/or tetracyclines (Otoya, 1974). Multi-resistant strains were also shown to be frequent in some countries: 12% of the isolates in Sweden in 1971 were resistant to ampicillin, chloramphenicol and tetracycline (Lincoln, 1971), whilst in Bangladesh, the strains which were resistant were 32 out of 75 (43%) tested (Ann. report. 1977). A few studies that have been carried out on animal strains show a very high prevalence of resistance, particularly to the tetracyclines (91% in pigs) and to the streptomycin (94% in calves) (Huber, 1974). The use of antibiotics as feed additives for growth promotion has been increminated in the rapid emergence and spread of drug-resistant enterobacteria.

Sewage and surface waters contribute to the distribution and circulation of resistant organisms. They represent a natural medium in which R-plasmid transfer can occur under certain physical, chemical or biological conditions. The waters contain resistant bacteria from human and animal wastes, and can be regarded as a source of all plasmid types, which circulate and are selected under appropriate environmental conditions (Smith, 1970; Richmond, 1972). The Escherichia coli group, including the faecal coli are sensitive and specific indicators of organic contamination, whether human or animal. They are indicators of faecal contamination, and the frequency with which they prove resistant to antibiotics will indicate how frequently they are resistant in the human flora. The Aeromonas group are aquatic bacteria. Their abundance and the frequency of resistant strains represent ecological changes. The increasing frequency of antibiotic-resistant faecal E. coli in surface waters also reflects ecological changes between man and his environment. The percentage of resistant bacteria in waste waters varies greatly depending on a multiplicity of factors, from as little as 0.1% to as much as 100%. Resistance to ampicillin seems to be most frequent, especially in Aeromonas species. Resistance to the tetracyclines is more frequent in faecal E. coli than in other enterobacteria. In surface waters, the frequency of occurrence of resistant strains varies from 31% to 73% for at least one antibiotic, being highest for streptomycin.

Transfer of resistance plasmids (R. factors) in Gram-negative bacilli takes place by direct contact between donor and recipient cells - that is, by conjugation. The antibiotic drugs against which plasmid-

mediated resistance has been detected in the enterobacteria are: penicillin and related antibiotics; chloramphenicol; tetracyclines; aminoglycosides; sulfonamides and trimethoprim. Resistance plasmids have been encountered in all species of enterobacteria. It is now apparent that resistance transfer takes place in vivo in the intestine, but such transfer must also occur outside the human or animal host - probably in sewage or other highly contaminated surface waters.

Thus, to ascertain whether a country has a problem of drug resistance, appropriate clinical specimens from nosocomial and enteric infections and from surface waters must be cultured regularly and the bacteria isolated be tested for drug resistance. Systematic collection and analysis of these data could provide an epidemiological basis for clinical and administrative guidance in the prevention and control of spread.

3. Rationale

Information concerning the drug-resistance patterns of the prevailing pathogenic bacteria and the appearance of new resistance characteristics is of the most value for a proper selection of antimicrobial agents for therapeutic purposes. Unawareness of local drug-resistance patterns in pathogens will foster misuse and often overuse of antibiotics, with all their harmful consequences. Knowledge of the incidence of antibiotic resistant enterobacteria will guide clinicians in choosing the most suitable antimicrobial agent, and will preclude the blind use of multiple antibiotics.

B. SPECIFIC AIMS

The monitoring of V. cholera and shigella antibacterial resistance is a function of this laboratory. However, little is known about the degree of antibiotic-resistance of salmonella and faecal E. coli and whether the resistant E. coli or shigella can transfer the resistance gene to the recipient E. coli (K12). Investigations of the following factors are thus important:

1. A laboratory records review of the drug-resistance pattern of salmonella.

2. A study of the role of nosocomial infection in antibiotic resistance of enterobacteria.

3. Determination of the incidence of antibiotic-resistant faecal E. coli, other environmental E. coli vibrio and Aeromonas in surface waters.

4. Determination of the number or percentage of E. coli, shigella and vibrio capable of transferring drug-resistance gene to recipient E. coli.

C. METHODS OF PROCEDURE

1. Laboratory records investigation

The bacteriology records of the International Centre for Diarrhoeal Disease Research (ICDDR) of 1970 (before the liberation war), those of 1974 (after the war) and the current ones will be

reviewed to find out the antibiotic-resistance patterns of enterobacteria during respective years.

2. Study of nosocomial enterobacterial infections

A total of 100 children and adults (irrespective of age and sex) to be admitted on Tuesdays and Fridays of every fortnight to ICDDR Hospital will be selected. The patients to be selected will be those reporting for mild diarrhoea of 1-2 days duration, have received no therapeutic antimicrobial agents and are willing to participate in the study. Selection of patients will also be based on areas of residence to avoid bias in respect to low and high class social groups. The selected patients will stay in the ward for at least 72 hours without receiving antimicrobial drugs, except electrolyte rehydration. Rectal swabs will be taken from the patients on the first day of admission and again on the day of discharge. The swabs to be taken will be cultured for enterobacteria immediately after collection on Salmonella-Shigella and MacConkey agar plates. Culture plates will be examined for growth of both lactose and non-lactose fermenting colonies. Separate colonies (5) of each genus of Gram-negative bacilli will be transferred to agar slants, and stored away until needed for antibiotic-resistance testing. Mueller-Hinton agar medium will be used for the tests. The susceptibility of strains to selected antibiotics will be determined by measuring the zones of bacterial growth inhibition, as described by Bauer et al. 1966.

3. Sampling and examination of river waters

Samples of river and/or pond waters will be collected from varied sites in Dacca. The sites will be those from which patients already found harbouring multi-antibiotic resistant E. coli have come from. These sites are namely Lalbagh, Rly Colony, Ramna, Mirpur, Tejgaon and Malibagh. Water samples will be collected from these sites by use of plastic bottles once a fortnight. On reaching the laboratory, samples will be diluted serially tenfold down to 10^{-5} in 25% Ringers' solution and then each dilution will be seeded on 3 plates of suitable medium. Lactose agar (MacConkey) is suitable for counting faecal E. coli. This agar will be employed, and the reading of the plates done after 48 hours' incubation at $44^{o}C$. Deoxycholate citrate lactose medium will be used for studying the total E. coli (TC) other than faecal E. coli after 48 hours' incubation at $37^{o}C$. Another medium (Von Graeventiz's medium) which contains deoxyribonucleic acid, toluidine blue and ampicillin (20 µg/ml) will be used to isolate Aeromonas. Suspect colonies will be counted after 72 hours' incubation at room temperature. Additionally plates containing antibiotics will be seeded for each sample and dilution of water. Samples of water will also be cultured for vibrio using conventional methods, and the vibrio will be tested for resistance against antibiotics. Antibiotics will be used at the following final concentrations: ampicillin (20 µg/ml), streptomycin (12.5 µg/ml), kanamycin (12.5 µg/ml), chloramphenicol (12.5 µg/ml) and tetracycline (12.5 µg/ml). These

plates will be incubated at the same temperatures as those mentioned above for respective organisms. This procedure will permit determination of the concentrations of Aeromonas, faecal E. coli and TC present in the samples, and calculation of the percentages of resistant bacteria for each antibiotic in relation to the total bacteria counts. The technique requires the seeding of 45 agar plates for the analysis of a single water sample. Eight samples are intended to be collected on every Tuesday. Two samples are to be collected from Lalbagh, from 2 already located river areas and another one from a pond. One sample will be collected from a pond in Malibagh, another from a pond in Tejgaon. These ponds are frequently used by many people for swimming and bathing. Other three samples will be collected from Mirpur from areas likely to be polluted from human or animal excreta. In all 208 water samples will be collected within a period of one year.

4. Tests for transferability of drug resistances

Broth cultures of donor (E. coli or shigella) and recipient (E. coli K12) strains will be grown with shaking at 37°C to exponential phase containing approximately 2×10^8 organisms per ml. For overnight crosses, donor and recipient cultures will be mixed in equal volumes, 1 ml each, and incubated overnight. Crosses will be performed at 28°C and 37°C, because a few resistance plasmids are transferred with much higher frequency at 28°C than at 37°C. After each cross 0.1 ml quantities will be plated in duplicate on MacConkey agar plates with and without suitable concentrations of the appropriate antibiotics. The plates will

be incubated overnight at 37°C and the colonies will be counted. The transfer frequency in overnight crosses will be calculated as the proportion per recipient cell. Drugs will be used at the following concentrations to select for transfer of resistance: ampicillin 100 µg/ml, chloramphenicol 20 µg/ml, streptomycin 40 µg/ml and tetracycline 20 µg/ml.

5. Data analysis

The statistical method for testing the degree of resistance in faecal E. coli, vibrio TC and Aeromonas from surface waters will be a variation of the permutation test (or randomization test) proposed by Fisher (1960). The test will basically consist of comparing the frequency of antibiotic resistance with the random frequency that will be generated by pairing the 8 water sites to the 208 water samples. One would expect a higher degree of identical patterns between river sites than pond sites. Analysis of other data is mentioned in the respective sections under Methods of Procedures.

D. SIGNIFICANCE

The data to be collected from this study will provide a scientific basis for the development of hospital and probably national policies concerning the use of antibiotics. The data can also be used to provide epidemiological material for determining the incidence of resistant bacteria in human infections in hospital and in the community. The economic benefits could also follow from prevention of the use of

antibiotics to which enterobacteria have acquired high resistance.

Since only patients with mild diarrhoea of 1-2 days duration will be admitted, this study will also throw light on the type of enterobacteria found in these patients and the percentage of patients subsequently developing severe diarrhoea during their stay in the hospital.

E. FACILITIES REQUIRED: None

F. COLLABORATIVE ARRANGEMENTS: Not required

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES			Annual Salary		Project Requirements	
Names	Position	% Effort	Taka	Dollars	Taka	Dollars
Dr. L.N. Mutanda	Investigator	20%	-	18,069	53,728	3,358
Mr. A.K.M.G.Kibryia	Research Ass't	20%	48,680		9,736	609
Ms M.N. Mansur	Research Ass't	100%	20,536		20,536	1,284
Mr. M. Rahman	Technician	50%	18,120		9,060	566
Overtime for Ms Mansur and Mr Rahman					8,788	549
				Sub-Total:	101,848	6,366

2. SUPPLIES		
Items		
Agar Plates	38,880	2436
Agar bottles and tubes	38,880	2436
Antibiotic discs	24,000	1500
Antibiotic powder	300	19
Sub-Total:	101,760	6391

3. EQUIPMENT: None

4. PATIENT HOSPITALIZATION		
300 patient days @ Tk 135/day	45,000	2,812
Sub-Total:	45,000	2,812

5. OUT PATIENT CARE: None

6. ICDDR,B TRANSPORT:		
1560 miles @ Tk 3.00 : Mileage-Dacca	4,680	402
Sub-Total:	4,680	402

7. TRAVEL AND TRANSPORTATION OF PERSONS: None

8. TRANSPORTATION OF THINGS: None

9. RENT, COMMUNICATION & UTILITIES: None

10.	PRINTING & REPRODUCTION	Tk	1,000	$ 63
	Sub-Total:	Tk	1,000	$ 63

11. OTHER CONTRACTUAL SERVICES: None

12. CONSTRUCTION, RENOVATION, ALTERATIONS: None

B. BUDGET SUMMARY

Category	Taka	Dollars
1. Personnel	101,848	6,366
2. Supplies	101,760	6,391
3. Equipment	None	
4. Hospitalization	45,000	2,812
5. Outpatients	None	
6. ICDDRB Transport	4,680	402
7. Travel Persons	None	
8. Transportation Things	None	
9. Rent/Communication	None	
10. Printing/Reproduction	1,000	63
Grand Total:	254,288	16,034

Taka Dollars

Conversion Rate: (Taka 16.00 equals $1.00)

Abstract Summary

A research project aimed at reviewing bacteriology records to determine antibiotic-resistance pattern of enterobacteria is proposed. This project hopes also to determine the role of nosocomial infection in antibiotic-resistance of the enterobacteria. For this, 100 children and adults to be admitted to hospital with mild diarrhoea will have rectal swabs taken on the days of admission and discharge after 72 hours. The swabs will be cultured for enterobacteria, and the susceptibility of these enterobacteria to selected antibiotics will be determined. The frequency with which river and/or pond water faecal *E. coli*, vibrio and the *Aeromonas* group are resistant to antibiotics will also be investigated, by isolating the organisms and testing them for antibiotic resistance. Transfer of resistance plasmid (R-factors) to a recipient *E. coli* KJ2 will be attempted as well.

Information concerning the drug-resistance patterns of the prevailing enteropathogens is of the most value for a proper selection of anti-microbial agents for therapeutic purposes. Knowledge of the incidence of antibiotic resistant enterobacteria will guide clinicians in choosing the most suitable antimicrobial agents. The economic benefits will follow from prevention of the use of the antibiotics to which enterobacteria have acquired high resistance.

This project involves no interviews, physical, psychological, social, legal or any other risks. Only review of bacteriology records and taking rectal swabs from patients are required.

PERMISSION FORM - ANTIBIOTIC RESISTANCE OF ENTEROBACTERIA

The International Centre for Diarrhoeal Disease Research is carrying out research to determine the best antibiotic for enterobacteria. We would like you to participate in a study under the direction of Dr. Labius Mutanda to determine the pattern of antibiotic resistance of enterobacteria. If you agree to participate in this study you can expect the following:

1. You will be in the hospital for 3 days.

2. We will take a rectal swab from you.

3. You will get treatment for your diarrhoea as judged by the ward doctor.

4. You do not have to participate in the study. If you decide not to enter the study or if you leave the study, you may still be treated here at the Centre for your illness. Your decision regarding the study will not jeopardise your medical care.

5. We will answer any questions you have.

If you agree to participate in this study, please sign your name here.

_____ Name

_____ Date

If you agree to allow your child to participate in the study described above, please sign your name here.

_____ Name

_____ Date

সম্মতি পত্র

(অন্ত্র রোগ সৃষ্টিকারী জীবাণুর জীবাণু বৈশিষ্ট্যের ক্ষমতা নিরূপণ হবে)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র অন্ত্র রোগ সৃষ্টিকারী জীবাণুর প্রতিষেধক নিরূপণ করিতে গবেষণা করিতে চায়। উক্ত গবেষণার ডাক্তার সেটিকে সুতরাং পর্যবেক্ষণ করিবেন। আমরা চাই আপনি এই গবেষণায় অংশ গ্রহণ করুন।

আপনি যদি এ গবেষণায় সহযোগিতা করিতে চান নিম্নে লিখিত বিষয়গুলি আপনার জানা প্রয়োজন।

১। আপনাকে তিন দিন হাসপাতালে থাকিতে হইবে।

2। আপনাকে পায়খানার একটি সোয়াব (swab) দিতে হইবে।

৩। হাসপাতালে কর্তব্যরত ডাক্তারের চিকিৎসা অনুযায়ী আপনি চিকিৎসা পাইবেন।

৪। এই গবেষণায় অংশ গ্রহণ বাধ্যতামূলক নয়। যদি আপনি ইহাতে অংশ গ্রহণ করিতে না চান বা করিবার পরও ইহা হইতে নাম কাটিতে চান তবুও আপনার চিকিৎসার ক্ষেত্রে কোন ব্যতিক্রম হইবে না।

৫। এই ব্যাপারে আপনার আরও কিছু জানার থাকিলে নিঃসংকোচে আপনি জিজ্ঞাসা করিতে পারেন।

আপনি যদি গবেষণায় অংশ নিতে রাজী থাকেন তবে নিচে স্বাক্ষর করুন।

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তারিখ ______________

যদি আপনার সন্তান বা পোষ্য কেহ এই গবেষণায় অংশগ্রহণ করিতে চান তবে নিচে স্বাক্ষর করুন।

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তারিখ ______________