

93-022
8/6/93

APPLICATION FOR PROJECT GRANT

1. PRINCIPAL INVESTIGATOR: Dr Anowar Hossain
- CO-PRINCIPAL INVESTIGATOR: Prof R. Bradley Sack
2. CO-INVESTIGATORS: To be named (seven co-investigators will be selected, one from each of the SAARC countries) ✓
3. TITLE: SURVEILLANCE OF ANTIBIOTIC RESISTANCE AMONG SHIGELLAE IN THE SAARC REGION: A MULTI CENTRE STUDY
4. STARTING DATE: As soon as Funds are available
5. DATE OF COMPLETION: 36 months after starting
6. TOTAL BUDGET: US\$ 192,390.00
(remuneration of co-principal, seven co-investigators and consultants has not been included)
7. FUNDING SOURCE:
8. SCIENTIFIC PROGRAM HEAD: Prof. R. Bradley Sack
Associate Director
Laboratory Sciences Division

R. Bradley Sack MD.

UNICEF

1642516.00
12,122.00
10,000.00
2000

ENTERED 21 JUN 1998

REF
QW 138.5. JA1
H829A
1993
ABSTRACT

Shigellosis is the most serious of all invasive diarrhoeas and is a major public health problem in Bangladesh and other countries of the SAARC region. Clinical treatment failures may occur because of rapid development of multiple antibiotic resistance by the *Shigella* strains in the region. This suggests the need for regular updated information on antibiotic sensitivity patterns of the organisms in order to formulate a policy to promote the rational use of drugs in the management of shigellosis cases. This protocol will establish an intermittent active surveillance of systematically selected, clinically suspected cases of shigellosis in order to determine the isolation rates of *Shigella*, their species identification and their antimicrobial resistance patterns using uniform standard techniques in all participating laboratories of the SAARC region. This information will help to formulate policies on rational use of antimicrobials for the treatment of shigellosis.

The study will not interfere with routine medical care of patients provided by the medical staff; limited information on demography, clinical history and physical findings will be recorded on specific precoded forms from selected patients. A stool sample or rectal swab will be collected for bacteriologic culture for *Shigella* and the determination of their sensitivity patterns using uniform techniques. Data will be accumulated in Dhaka. A periodic report will be made available to each involved laboratory, management staff and other collaborating investigators. The study will help to establish a collaborative linkage between ICDDR,B in Bangladesh and other SAARC countries and will provide ongoing information on antibiotic resistance patterns of prevalent *Shigella* strains. The surveillance system established through this research protocol will

strengthen the laboratories of the SAARC region which may then carry on further investigations or monitoring as needed.

9. AIMS OF PROJECT

A. GENERAL AIM

To establish surveillance on a selected number of clinically suspected cases of shigellosis patients attending the participating health units of the SAARC (South Asian Association for Regional Cooperation) countries in order to determine the isolation rates of *Shigellae*, their species identification and their antimicrobial susceptibility patterns, using uniform standard techniques. This information will help in the management of shigellosis cases and in the formulation of a policy for the rational use of drugs. This will also help to standardize microbiological techniques used in the region through external quality control and transfer of technology so that data are comparable from all the laboratories involved in the study.

B. SPECIFIC AIMS:

1. To develop and implement uniform methodology for optimum isolation of *Shigella* in the SAARC region.
2. To monitor the susceptibility patterns of the isolated strains.
3. To determine inter-regional variations in antibiotic resistance of the different species of *Shigella*, particularly *S. dysenteriae* 1 and *S. flexneri*.
4. To longitudinally monitor the distribution of species which may be useful in detecting trends of emerging species and antibiotic resistance patterns.
5. To establish a collaborative linkage between ICDDR,B in Bangladesh and other countries of the SAARC region in order to establish a data

base network for information exchange and to generate new ideas of research.

6. To monitor the methods used in the various laboratories and to establish external quality controls for isolation of *Shigella*.

C. SIGNIFICANCE:

The study will help to establish a collaborative linkage of research on *Shigella* between ICDDR,B in Bangladesh and the other members of the SAARC countries and will provide ongoing information regarding the antibiotic resistance patterns of prevalent *Shigella* strains. Outbreaks in specific locations can be detected and others alerted in the region. The uniform methodology will hopefully become a routine practice in the participating laboratories if found satisfactory in isolating *Shigella*. Following the establishment of an intermittent surveillance system through this research protocol, the activity may then become a routine surveillance activity for that area, and the linkage between research protocols and basic data collection may consequently be cost-effective for further research on *Shigella* in these areas.

10. ETHICAL IMPLICATIONS:

There will be no involvement of invasive procedures in the protocol. Information will be routinely obtained from patients seeking medical care with a dysenteric syndrome. Usually stool specimens will be collected, but occasionally rectal swab specimens may be collected when stool specimens are not available. Data will be analyzed in groups and individual confidentiality of patients will be maintained. While collecting strains, biological safety procedures will be maintained through out the procedures.

11. BACKGROUND, RESEARCH PLAN and BIBLIOGRAPHY:

A. Background:

Of the diarrhoeal diseases prevalent worldwide (1), shigellosis occupies (15% of cases) a leading position (2,3). Shigellosis is a major public health problem, causing large numbers of hospital visits and admissions, heavy expenses for strained national budgets, significant work-day losses in adults and older children, and a high morbidity and mortality in younger children (1,4). Shigellosis is one of the most serious among all the diarrhoeas, due to its invasive character, systemic manifestations, severe nutritional impact, and tendency to become recurrent over prolonged periods (5). *Shigella* usually cause endemic disease (6), but may also cause outbreaks (7,8) and even pandemics (9,10).

Shigella infection, microbiology and epidemiology:

Shigellae are more commonly isolated than other enteroinvasive bacterial pathogens in both urban and rural areas of the developing world (11-16). An inoculum of as few as 10-100 viable organisms can cause full-blown clinical disease after an incubation period of 1-3 days (17,18). *Shigella* cause an acute, usually febrile, gastrointestinal illness characterized by abdominal pain, cramps, rectal tenesmus with frequent passage of small bloody-mucoid stools containing inflammatory cells, (18-20). Infection due to the most virulent serotype, *Shigella dysenteriae* 1 (*S. dysenteriae* 1), usually progresses to frank dysentery as do infections caused by *Shigella flexneri* (*S. flexneri*), whereas *Shigella boydii* (*S. boydii*) and *Shigella sonnei* (*S. sonnei*) may also cause more self-limited watery diarrhoeas (18).

Shigellosis is a disease of all ages but preschool children are most vulnerable (16). Most children recover within 5-7 days but malnourished children may suffer from prolonged episodes of dysentery (21). Intestinal and systemic complications due to infection with any of the *Shigella* may cause death (22). Almost all fatal cases of shigellosis occur in developing countries and data are generally compiled from investigations of epidemics caused by *S. dysenteriae* 1 or from hospital reports, rarely from community surveillance of *Shigella* infections.

Shigellosis: a global problem:

Shigellosis is a universal disease in respect to its geographical distribution, (23) but is not a threat in the industrialized, developed world, where the disease is mostly sporadic; occasional outbreaks still occur, mostly due to *S. sonnei* and *S. flexneri* (6,18). *Shigella* are also sometimes isolated from cases of 'travelers' diarrhoea (24). The overall isolation rates of *Shigella* from patients with diarrhoea in the developed (18) and developing countries (11,16) are remarkably different (5-10% vs 12-20%). More severe forms of endemic shigellosis caused by *S. flexneri* and epidemic outbreaks due to *S. dysenteriae* 1 with high mortality (5-10%) are common in the developing countries notably in central America (25,26), Africa (27-29) and Asia (7,8,16,30,31).

In the developed countries because of the self-limited nature of shigellosis due to *S. sonnei* and of the concern about increasing antibiotic resistance, (18,23), antibiotic usage is not always considered imperative. In contrast, in the developing countries, episodes of dysentery in malnourished children may develop intestinal and systemic complications and later growth stunting, and antibiotics are clearly indicated for treatment (6,18,22). The choice of antibiotics depends on the prevalent sensitivity patterns in a given community (23). In the past

decade, *Shigella* resistant to multiple antibiotics have become an international problem, particularly in the developing countries where indiscriminate use of antibiotics is common. In endemic areas, *S. dysenteriae* 1 and *S. flexneri* have already acquired resistance against the effective, low cost, easily available drugs (16,32). In the last 20 years, ampicillin and cotrimoxazole were the drugs of choice for treatment of shigellosis throughout the world. Since the middle of the last decade, however, these two drugs are no longer the drugs of choice in many of the developing countries (18,33). Co-trimoxazole remains the drug of choice in many developed countries where the organisms are still sensitive.

Shigellosis in the SAARC region:

The South Asian Association for Regional Cooperation (SAARC) is a forum which includes Bangladesh, India, Nepal, Sri Lanka, Pakistan, Bhutan and Maldives. In the context of global economy, these countries are considered "developing" countries and the status of shigellosis in this region is similar to that stated earlier.

In the early-mid 1970's, multi-drug resistant *S. dysenteriae* 1 suddenly appeared in several epidemics in the South Asian countries, particularly Bangladesh (7,16), India (8,30), Nepal (34), Sri Lanka (35,36) and Burma (31). In between the epidemics, endemic shigellosis due to *S. flexneri* continued; *S. boydii* and *S. sonnei* were rare. The overall morbidity and case fatality rates (upto 11.0%) in those epidemics on the subcontinent were reasonably high (37). One of the most likely reasons for the high fatality may be that treatment failed because of the rapid development of multiple antibiotic resistance by *S. dysenteriae* 1 and *S. flexneri*. Irrational prescriptions by physicians and self-medication probably are

responsible for the increase in selective ecological pressure from antibiotics resulting in the increase of resistance patterns of endemic and epidemic strains in the region (38). This suggests the need for a policy to regulate the rational use of drugs in the management of shigellosis cases, and a need for regular updated information on antibiotic sensitivity patterns of the organism.

Most of the data on shigellosis in Bangladesh, come from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) which has been maintaining diarrhoeal treatment centres in rural and urban areas of Bangladesh to treat shigellosis and other diarrhoeal diseases and has longitudinal data on antimicrobial resistance among shigellae and other enteric pathogens for the last 25 years. *Shigella* isolation increased gradually from 0.6% of diarrhoea cases in 1970 to 9% in 1972, 14% in 1973 and 20% of in-patients in Dhaka hospital of this institution in 1974 (39) and that in a field hospital varied between 19.3% for 1976 and 42.12% for 1984 (16). The emergence of multiresistant epidemic strains of *S. dysenteriae* 1 was noted in 1984 (40). The predominant endemic strain now is *S. flexneri* with less numbers of other shigellae (16). During the early 1970's, all species of *Shigella* were equally sensitive to sulphathiazole, streptomycin, chloramphenicol and tetracycline. In the middle of 70's they were less effective, and ampicillin and co-trimoxazole were the drugs of choice for shigellosis, although the first ampicillin resistant case was reported in 1974 (41). Almost all species of *Shigella* particularly *S. dysenteriae* 1 and *S. flexneri*, became resistant to co-trimoxazole by 1982-83 and to ampicillin by 1984-85 (42). Nalidixic acid was introduced to treat these resistant cases in 1985 (33); surprisingly some strains of *S. dysenteriae* 1 and *S. flexneri* were found to be resistant to this drug during the same year (43). An alternative drug, pivmecillinam is now used to treat nalidixic acid resistant cases of shigellosis due to *S. dysenteriae* 1 and *S. flexneri*. *S. dysenteriae* 1 have more

quickly developed resistance to antimicrobials compared to *S. flexneri* and other shigellae.

There have been several epidemiologic reports from different studies in India on outbreaks of dysentery due to *S. dysenteriae* 1 (8,30,44,45). A common feature of those outbreaks was the multiple antimicrobial resistance to tetracycline, chloramphenicol, ampicillin and co-trimoxazole. A study in southern India (46) between the period of 1976-86 surprisingly reported a contrasting antimicrobial resistance of *S. dysenteriae* 1 in two of its outbreaks. one in 1976-78 by ampicillin resistant-co-trimoxazole susceptible strains and the second in 1982-83 by ampicillin susceptible-co-trimoxazole resistant strains. The overall isolation rate of *Shigella* spp. was 16.6% with a predominance of *S. flexneri* with less frequent isolation of *S. boydii* and *S. sonnei* (46). Similar results were reported in other studies (47-50). Very little information, mostly in abstract form, is available from other countries of the SAARC region such as Nepal, Sri Lanka, Pakistan, Maldives and Bhutan. It appears from these reports that shigellosis is endemic in these countries and often causes outbreaks. There was an outbreak of multidrug resistant *S. dysenteriae* 1 in 1985 in Nepal (34) and a simultaneous outbreak of *S. dysenteriae* 1 and *S. flexneri* in 1986 in Sri Lanka (35,36). In between the epidemics, *S. flexneri* was predominant and endemic. The overall isolation rates ranged from 10.5% to 38% (51-55). It is reported that diarrhoeal disease is common in Pakistan with high mortality (56,57), but studies on under 5 children (56,58) showed that *Shigella* spp. was isolated only from 5-5.8% cases of dysentery with *S. flexneri* as the predominant species followed by *S. dysenteriae* 1. Most of the *Shigella* strains isolated in those countries were resistant to several antibiotics. Ampicillin and co-trimoxazole were drugs of choice for treatment of dysentery, but today, resistance to these

antibiotics in generally high. No information is available from Bhutan and Maldives.

Isolation and identification of *Shigella*:

The different rates of isolation of *Shigella* reported in different studies are often a reflection of the differences in capability and methods used by the laboratories involved. Simple and uniform laboratory procedures are needed for all laboratories dealing with isolation, identification and characterization of enteric pathogens; the *Shigella* group, however, presents special problems.

Shigella are extremely fastidious bacteria, and optimal recovery requires a short time interval between sampling and cultivation, and transporting faecal specimens in adequate transport media, if there is delay. A portion of stool containing blood and mucus is collected and selectively cultured. Specimens should be collected during the acute stage of illness before antibiotics are given.

A combination of both mildly and a highly selective media for primary culture of *Shigella* yields the best isolation rates. The optimum media depends on the experience of the bacteriologist, the expenses involved, and the *Shigella* species that are prevalent in the area (59). Several comparative studies on MacConkey agar (MCA), Hektoen enteric agar (HEA), Salmonella-Shigella agar (SSA), Xyline-Lysine-Deoxycholate agar (XLD), Deoxycholate citrate agar (DCA), Teknaf enteric agar (TEA) were reported from different geographic locations (60,61). Further literature review showed that the use of MCA as a mildly selective medium is uniformly accepted, but the use of highly selective media like SSA, XLD, HEA and DCA is variable and depends on the choice of individual laboratories. One study in Bangladesh (61) showed that MCA was superior to SSA in isolating *S. dysenteriae* 1, but SSA was significantly better for *S. flexneri* and other

Shigella. Several other studies showed that MCA and SSA is a better combination (62-65). The SSA agar does not need autoclaving, and could be prepared easily in a small field laboratory. Some authors prefer HEA and XLD (66,67); however, HEA is more expensive and not economical for routine diagnostic use in the poorer countries. Unfortunately, XLD cannot be stored for long periods intact even at 4°C. The ICDDR,B Clinical Lab has been using a combination of MCA and SSA for more than two decades with satisfactory isolation of various species of *Shigella* particularly *S. dysenteriae* 1 and *S. flexneri*.

Other methods: Microscopic finding of leukocytes and red blood cells in the stool are used to differentiate invasive from non-invasive diarrhoea (68,69). Coagglutination tests and ELISA assays are also used to detect *Shigella* antigens in stool (70,71).

Antibiotic Sensitivity Patterns:

Oral rehydration therapy has succeeded in averting deaths due to dehydration, but has little effect on deaths due to invasive diarrhoeas (72). To decrease the severity of the disease and the excretion of pathogens the use of effective antimicrobials are essential as life saving measures (18). In the early part of the antibiotic era, there was essentially no resistance among the species of *Shigella*. Today, antibiotic resistance is very common and is becoming a major problem. Resistance patterns vary by species and by geographical locations (38). Much of the antibiotic resistance occurring today is probably due to the selective pressure of heavy antibiotic usage and this type of resistance is usually plasmid mediated (38). Antimicrobial susceptibility patterns provide useful and simple laboratory markers for comparing strains in epidemiological studies and can be used as a guide to formulate national policies on rational use of drugs in treating shigellosis.

B. RESEARCH PLAN:

1. *Study place:*

The study will be conducted in ICDDR,B in Bangladesh and other participating health units of the members of SAARC countries: India, Nepal, Pakistan, Bhutan, Sri Lanka and Maldives.

Which institutions?

2. *Study period:*

The study will be an intermittent surveillance and the period will extend initially for a period of 3 years from the starting date of the protocol and will start as soon as funding is available. However, selection of participating laboratories of the SAARC countries and orientation and training of their selected staff may require some additional time.

3. *Study population:*

The study subjects will be selected from the patients attending participating health units with clinically suspected case of dysentery (as per definition). Physical examinations and treatment will be provided by the physicians as usual, and only limited information on demography, medical history and physical findings will be recorded on prescribed precoded forms from the selected patients and/or accompanying guardians. A stool sample or rectal swab will be collected from these patients for bacteriologic culture for *Shigella*.

4. *Case definition:*

A patient will be defined as a clinically suspected case of dysentery or shigellosis if he/she has abdominal pain, cramps, rectal tenesmus and fever

with frequent passage of small bloody-mucoid stools with or without vomiting. A patient will be defined as a case of bacteriologically proven shigellosis when at least one stool or rectal swab culture is positive for *Shigella*.

5. *Frequency of specimen collection:*

Specimens and data will be collected four times in a year, i.e. on January, April, July and October, which means that specimens will be collected for a total of twelve months in a three-year period.

6. *Number of patients and positive strains:*

The number of patients will be calculated on the basis of the approximate prevalence rates of *Shigella* in a given locality, in order to obtain approximately 20 positive isolates of *Shigella* per centre per surveillance month. Therefore the actual number of patients can not be stated at the moment for any particular centre. However, screening of about 40-45 cases of suspected dysentery should yield 20 positive strains of *Shigella* in each centre each surveillance month. From seven such centres in the SAARC countries, there will be 280-315 (apprx. 300) patients for screening per surveillance month which is expected to provide 140 positive isolates in that month. Following this scheme, 1,200 (Approx.) patients will be screened per year which will yield 560 positive strains and in a 3-year period there will be 3600 patients and 1680 positive strains respectively.

7. *Sample collection:*

Stool samples will be collected in physically and chemically clean containers. In case of non-availability of stool specimens, a rectal swab will be collected using cotton tipped sterile swabs. All samples will be

transported quickly to the laboratory preferably within 2 h of collection; otherwise they will be transported in Buffered Glycerol Saline(BGS) holding media. Specimens will be collected from patients as per the following inclusion and exclusion criteria.

Inclusion criteria:

- Clinically suspicious cases conforming to the clinical definition of dysentery with the characteristic appearance of the stool.
- All age groups of patients.
- Patients who have not had any antimicrobials for the current episode of bloody-mucoid diarrhoea.
- Patients who have had antibiotics for 3-5 days but in whom there is no clinical improvement.
- Patients who are willing and cooperative to provide samples.

Exclusion criteria:

- Patients whose symptoms do not conform to the definition of clinical dysentery.
- Patients who have had antibiotics and are showing significant clinical improvement.
- Patients or guardians who are not cooperative.

8. Microbiological techniques:

Isolation and identification:

Faecal samples or rectal swabs (RS) will be cultured for *Shigella* following standard methods (73). Stool or RS specimens will be inoculated onto MCA and SSA

plates: The specimens transported in BGS will be subcultured onto MCA and SSA plates as soon as possible after arrival in the Laboratory. All of these plates will be incubated overnight at 35-37°C and screened the next morning by an experienced bacteriologist or technologist for characteristic Non-lactose fermenting (NLF) colonies. The NLF colonies are then picked for biochemical screening using KIA (Kliglers' iron agar) and MIU (Motility, indole and urea) media as the primary battery of tests. The colonies giving biochemical reactions typical for *Shigella* are then tested serologically by agglutination using group specific polyvalent and type specific *Shigella* antisera. Organisms giving biochemical reaction typical of *Shigella* but not agglutinating with specific antisera will be heated by boiling for 30-60 mins, centrifuged and retested for agglutination with *Shigella* antisera.

The characteristic biochemical reactions and a stepwise working sheet or flow chart are enclosed (appendix enclosed).

Antimicrobial susceptibility tests:

Of the standard disks diffusion methods for antimicrobial susceptibility testing, the technique of Kirby-Bauer (74) is most widely practiced and will be used in this study.

Colonies from KIA (those identified as *Shigella*) are inoculated in 2.0 ml T₁N₁ broth and incubated at 35-37°C for 4 h. The turbidity of the broth is then adjusted to 0.5 McFarland standard using physiological saline. A Mueller-Hinton agar plate is then evenly inoculated with the microbial suspension using a sterile cotton swab so as to yield a confluent growth, and seven selected antimicrobial-impregnated disks, namely, tetracycline, ampicillin, cotrimoxazole, nalidixic acid, pivmecillinam, ciprofloxacin and one oral

cephalosporin (2nd/3rd generation) are then placed onto the surface of the inoculated plate, equidistant from each other. The disks are then gently pressed down with a needle or forceps to ensure complete contact with the agar surface. The plates are incubated overnight at 35-37°C. The plates are screened the next day for the inhibition zones produced around each disk. The zone sizes are measured in millimeters and the results interpreted as sensitive, intermediate or resistant as per the manufacturer's instructions provided.

Quality control of susceptibility tests:

Since the susceptibility tests are very sensitive to small variations in media formulation, inoculum size, incubation period, temperature and other factors, the following control strains will be assayed for comparison: *Staphylococcus aureus* ATCC # 25923, *Escherichia coli* ATCC # 25922 and *Pseudomonas aeruginosa* ATCC # 27853. These control strains will be treated as specimens and subjected to the same methods described for the test strains.

Stock of isolates:

As soon as the identification and antimicrobial susceptibility tests are completed, all positive strains of *Shigella* will be saved and stocked in T1N1 soft agar by stabbing and storing at room temperature. Further study of these strains will be under taken (see below).

Overall quality control for Shigella:

Ten percent of the *Shigella* strains per centre per month (10% of 20 positive strains = 2) will be saved, stocked and sent to Dhaka every 3 months (3 x 2 = 6) for quality control tests in order to determine the accuracy of diagnosis and the sensitivity patterns reported. From seven laboratories altogether, there will be

42 strains ($6 \times 7 = 42$) for quality control testing in Dhaka every 3-months. Also two known *Shigella* strains with code numbers will be sent from Dhaka to each of the seven participating laboratory as unknowns for their identification and sensitivity testing during the same period. The results of the identification and sensitivity testing will be sent to Dhaka immediately, where they can be confirmed; and feedback will then be provided to the Laboratory concerned.

A work plan of the quality control scheme will be provided.

9. Data management:

Data collection:

Data will be collected on precoded questionnaire forms which will include the following entries: limited demographic information, short medical history and the results of laboratory investigations on the identification and sensitivity patterns of the *Shigella* isolates.

Data processing:

All coded data will be entered into the computer, at each respective laboratory at a suitable time, in order to have complete data results each surveillance month.

Data accumulation:

Data accumulated on computer diskettes will be sent to Dhaka after every surveillance month along with a computer print out of data for that month.

Data analysis:

All of the data accumulated in computer diskettes will be processed by a data management officer (50% time) in Dhaka and a print out of the

processed data will be made available each surveillance month. The results will be illustrated in tables, graphs and figures as necessary, with appropriate use of statistical tools. A periodic report will be issued to each involved laboratory, management staff and other collaborating investigators.

10. FACILITIES REQUIRED:

The study will be carried out in the clinical lab of ICDDR,B and in the designated laboratories of the participating countries. Basic laboratory facilities and the instruments for carrying out various tests for isolation and identification of *Shigella* are available at the clinical Laboratory in ICDDR,B; the facilities in the other countries will need to be explored. Specific reagents and chemicals will have to be purchased. Existing personnel in each participating laboratory may be utilized by providing orientation and training. Only a Lab-cum-data entry technician will be required additionally at ICDDR,B. A computer (PC) along with a printer will be needed in Clinical Laboratory of ICDDR,B for data management.

11. COLLABORATIVE ARRANGEMENTS:

The protocol will be carried out under a collaborative arrangement between ICDDR,B in Bangladesh and the laboratories of the members of the SAARC countries as appropriate and/or designated by UNICEF (United Nations Children's Emergency Fund) where similar basic laboratory facilities are expected to be available. However, there will be a need by Principal/Co-principal Investigator(s) to visit those laboratories initially to investigate the facilities (equipment & supplies) and technical capabilities available in each laboratory designated and arrange for orientation and training in ICDDR,B Dhaka for one staff or co-investigator from each participating laboratory.

SPECIFIC TASK OF EACH INVESTIGATORS:

The functions of each investigators involved in the protocol has been shown in the appendix (enclosed). The functions of other staffs will be specified when actual work will be started.

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BUDGET

BUDGET FOR YEAR 1

A. Personnel

A.1 For ICDDR,B

Position	Level	No.	% time	Rate/M (US \$)	Total 12 M (US \$)
Principal Investigator	NO-B	1	10	1,050.00	1,260.00
Co-Principal Investigator	P-6	1	05	-	- *
Co-Investigator	-	1	10	-	- *
Data Management Officer	GS-5	1	25	388.00	1,167.00
Lab-cum-data entry tech.	GS-4	1	100	314.00	3,768.00
Lab Attendant	GS-1	1	25	186.00	558.00

Sub-total = 6,753.00

A.2 For each SAARC Laboratory

Position	Level	No.	% time	Rate/M (US \$)	Total 12 M (US \$)
Co-Investigator	-	1X6	-	-	-
Lab-cum-data entry Tech.	GS-4	1X6	100	314.00	22,608.00
Lab Attendants	GS-1	1X6	25	186.00	3,348.00

Sub-total 25,956.00

TOTAL FOR A = 32,709.00

* The rate and total amount for these two positions have not been calculated

B. Office equipment (for ICDDR,B)

Name of equipment	Specificat	No.	Rate/Unit	Total
Personal Computer	IBM compat	1	2,140.00	2,140.00
Printer	EPSON	1	1,145.00	1,145.00
Voltage stabilizer/UPS		1	850.00	850.00

Sub-total = US 4,135.00

C. Lab Equipment & materials (includes all SAARC Labs including ICDDR,B)

C.1 Lab media and glasswares

Lab supplies	No./4 month/Year	Rate/Unit	Total
Glasswares	2X4X7 = 56 pkt	1.10	62.00
Stool container	75X4X7 = 02 pkt	225.00	450.00
Swab stick	75X4X7 = 07 Pkt	15.00	105.00
Microbiological Media			
Buffered Glycerol Saline	30X4X7 = each	0.35	294.00
MacConkey agar	70X4X7 = each	0.75	1,470.00
Salmonella-Shigella agar	70X4X7 = each	0.75	1,470.00
Kligler Iron agar	70X4X7 = each	0.20	392.00
Motility, Indole, Urea	70X4X7 = each	0.16	314.00
Mueller Hinton agar	25X4X7 = each	0.65	455.00
T1N1 Broth	25X4X7 = each	0.15	105.00
Misc.			500.00

Sub-total = US\$ 5,617.00

C.2 Shigella Antisera *

Name of item	No.	Rate/Unit	Total
S. dysenteriae 1	2x7	50.00	700.00
S. dysenteriae 2	1x7	50.00	350.00
S. dysenteriae 3-10	1x7	50.00	350.00
S. flexneri	3x7	50.00	1,050.00
S. boydii 1-6	1x7	50.00	350.00
S. boydii 7-11	1x7	50.00	350.00
S. boydii 12-15	1x7	50.00	350.00
S. sonnei	1x7	50.00	350.00
Sub-total = US\$			3,850.00

C.3 Antibiotic Disks

Name of item	50 disks/	No.	Rate/Unit	Total
Tetracycline	do	2x7	5.00	70.00
Ampicillin	do	2x7	5.00	70.00
Cotrimoxazole	do	2x7	5.00	70.00
Nalidixic Acid	do	2x7	5.00	70.00
Pivmecillinam	do	2x7	5.00	70.00
Ciprofloxacin	do	2x7	5.00	70.00
Cephalosporin	do	2x7	5.00	70.00
Sub-total = US\$				490.00

TOTAL FOR C = 9,957.00

D. Travel and perdiem

Local (land transport)	1,000.00
International (Principal Investigator, Co-Principal Investigator)	4,000.00
Orientation and training of seven Co-investigators/staff	4,000.00
Sub-total = US\$	9,000.00

E. On going expenses

Office supplies	2,000.00
Transportation of supplies	1,000.00
Printing costs	2,000.00
Memo/Xeroxing	500.00
Telx/FAX/Postal	500.00
Sub-total = US\$	6,000.00

F. Miscellaneous 2,000.00

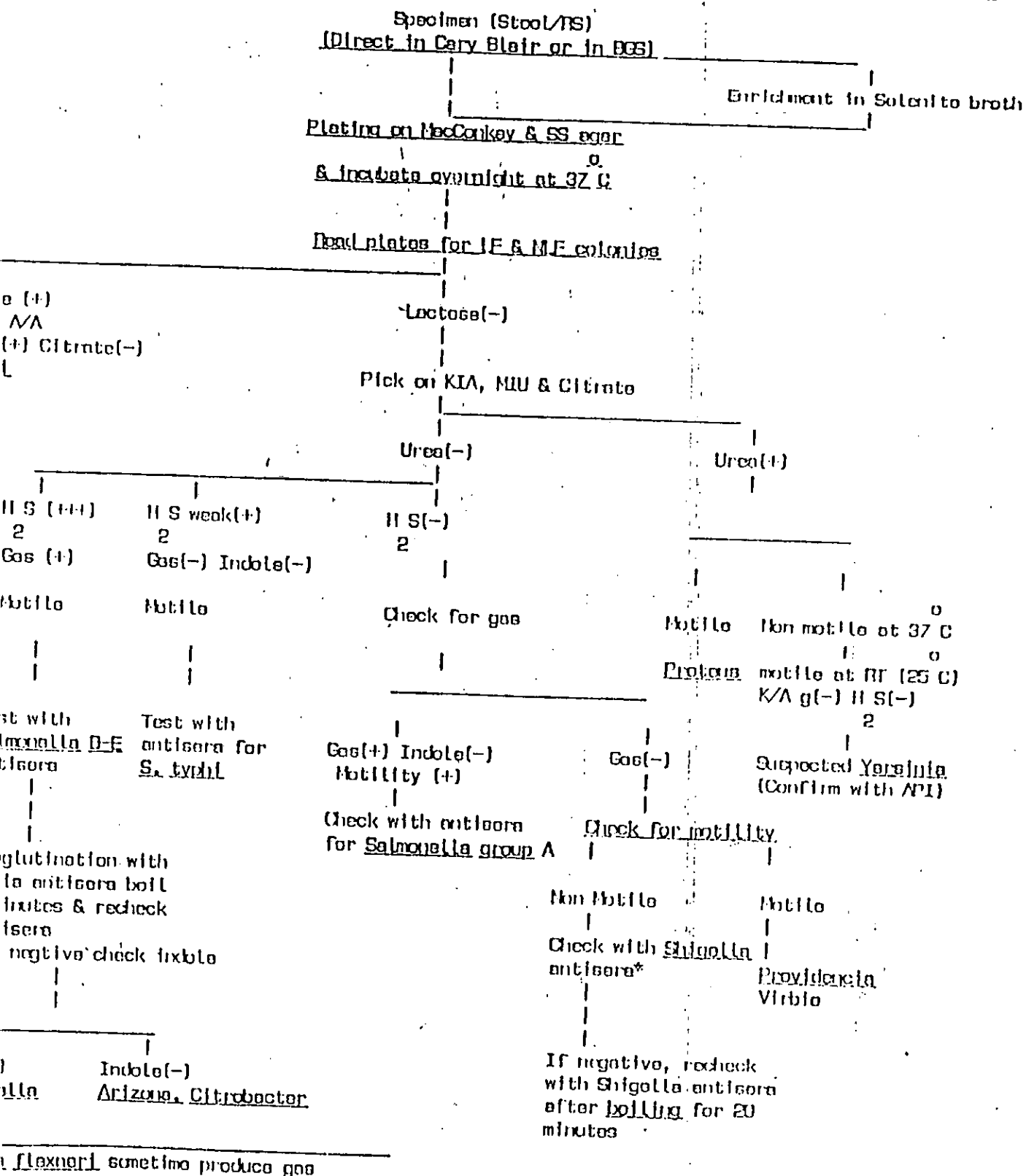
GRAND TOTAL (A+B+C+D+E+F) = 63,801.00

SUMMARY BUDGET FOR 3 YEARS

Sl.#	Budget Head	Year 1	Year 2	Year 3
A.	Personnel	32,709.00	35,980.00	38,578.00
B.	Office Equipment	4,135.00	-	-
C.	Lab Media & Supplies	9,857.00	10,953.00	12,048.00
D.	Travel and Perdiem	9,000.00	5,500.00	6,050.00
E.	On going expenses	6,000.00	6,600.00	7,260.00
F.	Miscellaneous	2,000.00	2,200.00	2,420.00
Total = US\$		63,801.00	61,233.00	67,356.00

GRAND TOTAL = US\$ 192,390.00

LOW UNIT FOR IDENTIFICATION OF CLINICALLY SIGNIFICANT MEMBERS OF THE FAMILY ENTEROBACTERIACEAE



APPENDIX B

FUNCTIONS OF PERSONNEL

INVESTIGATORS

1. Principal Investigator
Md. Anowar Hossain

Development of study design, protocol writing, implementation of the study, supervision and quality control of the study including strains and data collection, development of analysis plan, periodic report writing, final data analysis and final report writing.

2. Co-Principal Investigator

Prof. R. Bradley Sack

Overall review of study design, protocol writing, implementation of the study, supervision and quality control of the study including data collection, development of analysis plan, periodic report writing, final data analysis and final report writing.

3. Co-Principal Investigators

(To be named)

Collaborative and help implementation of the study, supervise strains and data collection.

APPENDIX C

Data Sheet for Surveillance of antibiotic resistance among Shigellae in the SAARC region: a multicentre study.

1. Country Name: _____
2. Patient's Name: _____
3. Identification No.: _____
(Start from 0001 to)
4. Age (in years or months if below 5 years): _____
5. Sex : _____
6. Type of diarrhoea: _____
(Watery, Mucoid, Bloody, Bloody-mucoid)
7. Date of onset: _____
8. Frequency/24 hours: _____
9. Abdominal pain (Yes/No.): _____
10. Fever (Yes/No.): _____
11. Antibiotic treatment: _____
(If any, give name & for how long)
12. Date of specimen collection: _____
13. Type of specimen (Stool or R/S): _____
14. Bacteriological Culture result : _____
15. Date of Isolation : _____
16. Results of antibiotic susceptibility test:
Strain sensitive to : _____
Strain resistant to : _____
17. Date of saving strain: _____
18. Name and Signature of
Interviewer/Technician: _____

Response to reviewers' comment

First Reviewer

Response to reviewer's comment are given according to serial number given by the reviewer.

1. Letters of agreement have not been incorporated at the moment. We shall go for such an agreement with the institutions designated by UNICEF as the protocol was the outcome of the idea thrown by UNICEF.

We are not concern with the development of microbiology laboratory, rather we want to utilize the existing facilities of the participating laboratory to see the efficacy of uniform technique in isolating Shigella, because Shigella are extremely fastidious in nature and different media formulations have different capability in isolating Shigella (as detailed in protocol, pp 11-12). Prevalence of Shigella species thus, has the chance to be shifted, because of this false variation in performance of culture media.

2. This point has already been incorporated (pp 11-12).
3. Tetracycline has been included just for monitoring any change or shifting of resistance pattern against this antimicrobial. A third generation oral cephalosporin has been included (as per suggestion, pp 16-17) in the list of antimicrobials to be tested.
4. This suggestion is accepted and modified accordingly.
5. The protocol design has been modified to an intermittent surveillance and accordingly the number and cost of personnel has been reduced (the suggestions are partially accepted, pp 26-27).

Second Reviewer

The study design has been modified according to his suggestion to an intermittent surveillance i.e. there will be a quarterly surveillance (January, April, July and October) in a year giving a total of 12 surveillance months over the 3-year period (p 14) and the budget has been modified accordingly, pp 26-27).

First Reviewer

: Review of proposal entitled "Surveillance of Antibiotic Resistance Among Shigellae in the SAARC Region: A Multicentre Study" by Drs. A. Hossain and RB Sack.

ar Brad;

Thank you for asking me for my comments and review of the above mentioned proposal yours and Dr. A. Hossain. The proposal is admirable in intent, and is clearly written. I do, however, have major concerns about the proposal as it is now designed.

The Proposed Collaboration is Currently Nebulous.

Although the authors refer to this as a collaborative proposal, they provide no specifics about the nature of the collaboration. Neither the collaborating institutions nor the collaborating investigators are listed. No statements of agreement between collaborating institutions are provided. The latter are usually a standard feature of collaborative proposals. I feel that before the proposal is considered the details of the collaboration should be specified, and letters of agreement provided.

Secondly, the implicit (and at time explicit) assumption of this proposal is that the CDC, B is the only institution in the region that is capable of performing adequate microbiologic cultures to detect enteric pathogens. For instance, on page 22, the authors state

... "There will be a need for the Principal Investigator to visit those laboratories initially to get an idea about the facilities and technical capabilities available in each laboratory designated and arrange for orientation and training in ICDDR,B Dhaka..."

The reference section makes clear, however, that there are a number of institutions in the SAARC region already quite capable of isolating *Shigella* from microbiologic culture. There are numerous laboratories in India and Sri Lanka, and lesser numbers in Pakistan, that already do a very adequate job of isolating bacterial enteric pathogens. Although the authors go to considerable length in the protocol to describe methods for isolating *Shigella* and determining antimicrobial susceptibility, these methods are routine and are common in any recent microbiology textbook, such as the ASM Manual of Clinical Microbiology. There is no reason to think that the best laboratories in other SAARC countries deviate from these standard procedures. I have visited a number of these laboratories, and they appear in no way less competent as the ICDDR,B laboratory.

There are of course many health care facilities in the SAARC region that require decent microbiology laboratories but currently lack them. A number of the medical colleges in Bangladesh for instance, do not have decent functioning microbiology laboratories. The same certainly is true of other countries. And certain countries, such as Bhutan and the Maldives, perhaps don't have any functioning enteric microbiology laboratory (the epidemiologic importance of the two latter countries is limited, however, as they account for <1% of the total population in SAARC countries).

The authors have to make a decision as to what their primary objective is - is it to collect data on the antimicrobial resistance pattern of *Shigella*, or is it to develop enteric microbiology laboratories in facilities where none currently exist? If the former (accumulating data on the prevalence of resistance) is the main objective, then this could be easily (and far less expensively) done by simply corresponding with laboratories that are currently isolating *Shigella*. Although laboratories may miss the occasional *Shigella*, I think it is more important to identify *Shigella*. If this is a serious concern, then strains could be stocked (as has been proposed) and submitted on a periodic basis for confirmation. Using already existing laboratories should not at all add additional bias to the study, as the six laboratories the authors propose to develop will in themselves be a no more representative reflection of what is happening in the region than existing labs.

If the authors' major objective is to develop laboratory capabilities, I would suggest they start closer to home by working with regional laboratories in Bangladesh. If they want to work in other countries, I would suggest that it be in collaboration with laboratories that already exist in those countries, or with the Ministries of Health.

2. Public Policy Implications.

Rather than devote so much attention in the protocol to describing standard microbiologic

methods, I would prefer to have seen the authors address the question of how they would use the results to influence policy and practice. Will this information simply be provided to the yet unnamed collaborating investigators, or will it go to all physicians? Will it go to Ministries of Health, and if so how will it be utilized? Do any nations in SAARC already have surveillance systems for tracking the resistance of *Shigella*.

3. Which Antimicrobial Agents to Evaluate.

Of the agents that the authors apparently propose to test susceptibility to, tetracycline is in common use and currently it is rarely if ever used to treat shigellosis. The test for norfloxacin and ciprofloxacin is probably superfluous, as organisms that are resistant to one will be resistant to the other. The authors might want to add a third generation oral cephalosporin, as they will probably be increasingly used to treat shigellosis.

4. Informed Consent

As all of the information that is being collected would be routinely obtained in the care of a patient with dysentery, I am not certain there is a need to obtain informed consent.

5. Budget

The budget as it is now written contains a number of costs, especially in the area of personnel, that don't appear to be justified by the work that this study would entail. For instance, the budget lists at the ICDDR,B three full time staff that will be involved with data management or office coordination - a full-time GS5 data management officer, a full time GS-4 lab-cum-data entry technician, and a full time GS-3 office assistant.

One can only wonder what they will be doing. As I read the protocol, there will be 140 data forms (20 patients from 7 centers) generated monthly. Each data form (as provided in the protocol) has 18 variables. A very generous assumption is that it takes a data-entry technician with modest skills 10 seconds to enter a single variable into a data base - so it will take 180 seconds, or 3 minutes, for each record. To enter 140 records will take 3 minutes x 140, or 420 minutes, or 7 hours. That is one day of work a month to enter all of the records. Such a workload hardly justifies three staff. The analysis of data should be very straightforward, and can easily be accomplished by one of the Principal Investigators.

The need for a full time lab attendant is also somewhat dubious. The clinical lab in Dhaka is already isolating on average 150 strains of *Shigella* a month. That is far more than

the 20 isolates this study calls for. These strains are isolated from patients admitted to the inpatient ward, from patients entered into the surveillance study, and from patients entered into clinical studies. Obtaining the antimicrobial resistance patterns on these isolates will not require an additional laboratory attendant.

Other budget items that draw attention are the personal computer (is not the ICDDR,B already overburdened with personal computers - is it simply a matter of keeping up with the Joneses?) and the travel budget that averages \$10,000 yearly. As most of the training will ostensibly be done in the first year, why is the travel budget actually greater in Study Years 2 and 3? (as I mentioned above, I think that the objectives of the study could be achieved simply by correspondence, without any, or minimal, travel required).

I realize that sometimes it is necessary to shift costs - that there is a need to support staff on a project budget even if they will be doing most of their work on other projects. This is often the case with externally funded proposals (will this project be externally funded?). But as a reviewer, the amount of personnel listed on this budget seems extreme, even given cost shifting.

In summary, I think that the major objective of this study, to obtain information on the antimicrobial resistance pattern of *Shigella* in the SAARC region, is an important one. I do think, however, that this information could be obtained by correspondence with existing laboratories at a cost of probably at most \$1-2,000 a year, rather than by the elaborate system the investigators have proposed. If the major objective of the study is to develop additional laboratories in SAARC countries, then the proposal should be rewritten from that perspective. In addition I think that any revised protocol should contain the details of the proposed collaborations.

Title: Surveillance of antibiotic resistance among Shigellae in the SAARC region : A Multi Center Study.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

Rank Score

	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design		✓	
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget		✓ Expense esp. in terms of time	
Potential value of field of knowledge		✓	

CONCLUSIONS

support the application:

- a) without qualification
- b) with qualification
 - on technical grounds
 - on level of financial support

☒
☐
☐
☐
☐

would consider intermittent

prevalence studies

which might

be done

lastly

do not support the application

Name of Referee:

Signature:

Position:

Institution

Surveillance of antibiotic resistance among Shigellae in the SAARC region: A Multi Center Study

P. I. Dr. Anowar Hossain

The investigators propose to establish an active surveillance of systematically selected suspected cases of shigellosis in order to determine the isolation rates of shigellae, the species seen, and their antimicrobial resistance patterns. The goal is to help formulate policies of rational use of antimicrobials for the treatment of shigellosis, and perhaps more importantly, since susceptibilities may continue to evolve and change with time, to establish a collaborative linkage between the center in Bangladesh and other countries in the region which currently lack the facilities and quality control to adequately investigate the problem.

The routine methods are straightforward and adequate. It was not completely clear to me if testing and susceptibility were to continue over the entire three year period or whether the entire first year was for set up and the entire third year was for analysis. In any event, my major suggestion for possible improvement is related to this and I will give that opinion. If I have misunderstood the design or time period of testing, then these comments may be less apropos. Rather than a three year or even a one year study period, it would seem that intermittent prevalence surveys would accomplish the same information with less effort. If the study is not to go on for three years, having some data from the three year period would nonetheless be of particular interest in tracking changes. If the personnel and staff can be maintained in the appropriate locations and intermittent surveillances done of perhaps one month each every six months or some other formula, this may be of just as much interest. The number of personnel and the length of time involved appears on the high side but may be necessary just to guarantee that these individuals will be around for the needed time. Under A.2. I can't follow the calculations which would extend from there being "6" individuals at the given positions for "each" SAARC laboratory. I will assume that the totals they use are correct and not worry about the arithmetic.