

# ANNUAL REPORT 2000-2001

**National Institute of Cholera and Enteric Diseases**

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## PREFACE

Like the of previous years, National Institute of Cholera and Enteric Diseases, Kolkata continued to pursue its research goal enthusiastically on different aspects of diarrhoeal diseases, organised workshops, training programmes for manpower development, assisted the various State Governments in conducting investigations on diarrhoeal disease outbreaks including cholera outbreaks, suggested control measures and provided referral services to several laboratories in different parts of the country. During the year under report the library of the Institute has been modernised further with the setting up of Radio Frequency (RF) connection for unlimited, uninterrupted internet access. The LAN was also extended further to give nodes for each individual scientists which allows them and their students e-mail and internet access round the clock throughout the year from their laboratory. This infrastructural upgradation of the library has benefited not only the scientists and research scholars of the Institute but also the scientific community of the Kolkata in general.

Community based study documented that zinc supplementation had beneficial effect on reduction of diarrhoeal morbidity in children. Clinical study also showed that zinc supplementation had beneficial effect on clinical course of dehydrating acute diarrhoea in malnourished children. These and other studies strongly suggest that zinc supplementation may be used as a public health tool for prevention and treatment of acute diarrhoea. Superiority of azithromycin as compared to erythromycin for the treatment of cholera in children was also documented in a clinical study. Studies on *Vibrio cholerae* showed that a large percentage of *V.cholerae* O1 strains isolated from Kolkata were resistant to quinolones.

Kolkata is endemic for shigellosis, multi-drug resistant *Shigella flexneri* being the predominant serotypes isolated. A study to determine immunoregulatory function of porin of *Shigella dysenteriae* type 1 showed that porin mediated release of mediators NO and IL-1 by

macrophages are important in view of its possible role in conferring immunity against shigellosis.

The collaborative project entitled "Prevention of Emerging Diarrhoeal Diseases" with Japanese International Cooperative Agency (JICA) is progressing satisfactorily. This collaboration has lent invaluable support to research in molecular biology for bacterial, viral and parasitic enteric pathogens. A number of scientists and technicians of this Institute have been trained in advanced Japanese laboratories. Support from CSIR, DBT, WHO, UNICEF and other national and international funding agencies is also gratefully acknowledged.

The guidance, support and cooperation received from the office of the Director General, ICMR and members of the Scientific Advisory Committee are gratefully acknowledged. The dedicated and sincere efforts of our scientists, technical and administrative staff and research fellows in enhancing the activities of this Institute deserve my sincere and heartfelt appreciation.

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**Dr. S.K. Bhattacharya**  
Director

<b>Index</b>		<b>Pg.No.</b>
<b>1.</b>	<b>Study on Hospital Surveillancedy</b>	1
1.1.	Hospital based surveillance system for diarrhoeal diseases	2
<b>2.</b>	<b>Community Based Studies</b>	4
2.1.	Impact of zinc supplementation in reducing diarrhoeal morbidity amongst under five children : a community based intervention study	5
2.2.	Impact of lactation counselling on exclusive breast feeding among rural mothers : An operational research	7
2.3.	A rural community based longitudinal study on diarrhoeagenic <i>Escherichia coli</i> amongst children below 5 years	9
2.4.	Impact of zinc supplementation on incidence of diarrhoea and growth pattern among low birth weight infants of an urban slum	10
2.5.	A case control study on risk factors of dehydrating diarrhoea in children	10
<b>3.</b>	<b>Clinical Studies</b>	11
3.1	Impact of supplementation of zinc, zinc and vitamin A and combination of micronutrients and vitamins on acute watery diarrhoeal in mild to moderately malnourished children	12
3.2	Efficacy and safety of a sucrose based hypoosmolar ORS solution in adults and older children with cholera : a clinical trial	13
3.3	Evaluation of comparative efficacy of Erythromycin and Azithromycin in the treatment of cholera in children	14
		15
<b>4.</b>	<b>Studies on <i>Vibrio cholerae</i> o1 and O139</b>	16
4.1.	Emergence of Fluoroquinolone Resistant Strains of <i>Vibrio cholerae</i> O1 Biotype ElTor among Hospitalized Patients with Cholera in Calcutta	18
4.2.	Emergence of <i>Vibrio cholerae</i> O1 Biotype ElTor serotype Inaba from the prevailing O1 Ogawa serotype strains in India	22
4.3.	Characterization of rough strains of <i>Vibrio cholerae</i> isolated from diarrhea patients	23
4.4.	Virulence Genes in Environmental Strains of <i>Vibrio cholerae</i>	26
4.5.	Study the role of plasmids in adherence mechanism of <i>V.cholerae</i> non-O1, non-O139	27
4.6.	Studies on structure function relationship of <i>Vibrio cholerae</i> haemolysin	27
4.7.	Isolation of N-acetyl-D-glucosamine specific hemagglutinin receptor of <i>Vibrio cholerae</i> O1 in chicken erythrocyte membranes	29
4.8.	Vibriocidal studies on the status of vibriocidal serum bricidal antibody level against <i>Vibrio cholerae</i> O1 and serogroup O139 in Calcutta community	29
4.9.	Sequential studies of <i>Vibrio cholerae</i> O139 and some of its defined virulence phenotypes on intestinal pathology in rabbit ileal loop model	30
4.10.	Morphological studies on the flagella of <i>Vibrio cholerae</i>	31

<b>5. Studies on <i>Vibrio cholerae</i> phages</b>	32
5.1. Further application of phage typing scheme for discriminating strains of <i>V.cholerae</i> O139	32
5.2. Nationwide screening of phage types of <i>V.cholerae</i> 01 biotype Eltor	32
5.3. Molecular analysis of <i>Vibrio cholerae</i> bacteriophages: cloning and sequencing of phage DNA	33
5.4. Morphological and genomic characterization of vibriophages by electron microscopy	35
<b>6. Studies on <i>Shigella</i> species</b>	36
6.1. Antigenic recognition of <i>Shigella dysenteriae</i> outer membrane proteins using human convalescent sera and to evaluate their role in cell-mediated immune response in Shigellosis	37
6.2. Immunoregulatory functions of porin of <i>Shigella dysenteriae</i> type 1	38
6.3. Genetic studies on virulence mechanisms of <i>Shigella dysenteriae</i> 1 in relation to vaccine development	39
6.4. Molecular characterization of multi-drug resistant <i>Shigella flexneri</i> in Calcutta	40
<b>7. Studies on <i>Escherichia coli</i></b>	41
7.1. Studies to determine the role of enteroaggregative <i>Escherichia coli</i> (EAaggEC) for causing diarrhoea with reference to its virulence properties	41
7.2. Search for Shiga toxin producing <i>Escherichia coli</i> including O157: H7 strains in animals, animal products and hospitalised acute diarrhea cases in Calcutta	42
7.3. Studies on the binding of <i>Escherichia coli</i> heat-stable enterotoxin to the intestinal epithelial cells and brush border membranes of different animals	44
<b>8. Studies on other bacterial pathogens</b>	45
8.1. Differentiation of <i>Salmonella typhi</i> by molecular methods	45
8.2. Regulation of expression of bacteriophage MB78 genes : A molecular approach	47
8.3. Mode of action of <i>Yersinia enterocolitica</i> heat stable enterotoxin (YSTa) in rat intestinal epithelial cell	48
8.4. Search for virulence traits and determination of the mechanism of pathogenicity of <i>Klebsiella pneumoniae</i> isolated from childhood diarrhoea cases	51
8.5. Correlation of histology with genotypes of <i>Helicobacter pylori</i> isolated from cases of peptic ulcer, non-ulcer dyspepsia, gastric carcinoma and lymphoma	51
<b>9. Studies on <i>E.histolytica</i></b>	52
9.1. Studies on electron dense granules of <i>E.histolytica</i> containing collagenase activity	54
9.2. Studies on multiple genes in <i>Entamoeba histolytica</i> during human collagen type I and Ca <sup>2+</sup> interaction : Use of mRNA differential display	54
<b>10. Studies on rotavirus</b>	55
	58

10.1.	Molecular characterization of the VP7 genes of rotavirus isolated from the clinical samples, Calcutta, India.	58
10.2.	Detection and molecular characterization of rotaviruses	59
<b>11.</b>	<b>Investigations of outbreaks of diarrhoeal diseases</b>	60
11.1.	Investigation of outbreak of acute diarrhea in the brickfield area of Akra Fatak Calcutta	61
11.2.	Visit to Bhuj, Gujarat to investigate any impending Diarrhoeal disease outbreak following recent earthquake disaster	64
11.3.	Investigation of <i>V.cholerae</i> O139 outbreak at Baishnabghata, Patuli, Kolkata	67
11.4.	Concomitant Infection of Enterotoxigenic <i>Escherichia coli</i> in an Outbreak of Cholera Caused by <i>Vibrio cholerae</i> O1 and O139 in Ahmedabad	69
<b>12.</b>	<b>Report of the National HIV Reference Centre</b>	69
<b>13.</b>	<b>JICA-NICED project for prevention of emerging diarrhoeal diseases</b>	69
<b>14.</b>	<b>Activities of training and extension division</b>	70
14.1.	NACO Expert Group Meeting on Quality Assurance of HIV Testing	70
14.2.	JICA-NICED Domestic Training Programme on "Molecular Epidemiology of Diarrhoeal Diseases with Special Reference to Cholerae"	71
14.3.	Other Training and Teaching Programmes	70
14.4.	Guest lectures	70
14.5.	Training of W.H.O. Fellow	80
14.6.	Foreign scientist received training in the Institute	81
15.	Scientific Conference /seminars/meeting attended by the scientists/ research fellows <i>Training received by the scientists in abroad</i>	82
16.	Award/honour received by the scientists	85
17.	Other activities by the scientists	90
18.	Committee of the institute	
19.	List of publications	
20.	Staff list	

# STUDY ON HOSPITAL SURVEILLANCE

## Section highlights

A total of 1249 diarrhoea cases were included in the systematic hospital based surveillance (every 5th hospitalised patient on two randomly selected days per week). Of them 91.95% had watery diarrhoea, 4.1% had bloody diarrhoea and 3.95% had mucoid diarrhoea. Enteropathogens isolated from these cases were : *Vibrio cholerae* O1 - 8.1%, rotavirus - 8.1%, *Entamoeba histolytica* - 5.0%, *Vibrio parahaemolyticus* - 3.6%, *Giardia lamblia* - 3.4%, *V.cholerae* non O1 non O139 - 2.6%, *V.cholerae* O139 - 2.1%, *Shigella* spp. -2.0%.

## 1. STUDY ON HOSPITAL SURVEILLANCE

### 1.1. Hospital based surveillance system for diarrhoeal diseases

Name of the investigators :

Principal investigator : S.K. Bhattacharya  
 Co-ordinator : S.K. Niyogi  
 Co-investigator : D. Dutta, M.K. Bhattacharya, G.B. Nair, P. Das, S. Dutta,  
 P. Dutta, P.G. Sengupta, T.N. Naik, D.R. Saha K.  
 Rajendran and B. Manna

In the present study, a systemic sample (i.e. every 5th patient on 2 randomly selected days per week) of all patients with diarrhoea or dysentery with or without other complaints attending the casualty of Infectious Diseases Hospital, Calcutta were enrolled in the surveillance programme. During the period from April 1, 2000 to March 31, 2001, a total of 1243 diarrhoea patients were enrolled in this surveillance system. Of the 1243 patients, 27.5% were upto 5 years of age. Majority of cases belonged to urban population (87.9%). The ratio of male and female patients was 5:4. Majority of the patients were Hindu (75%).

Clinically 91.95% patients presented with acute watery diarrhoea, 4.1% cases with bloody diarrhoea and 3.95% cases with mucoid diarrhoea. Vomiting was predominant feature in 72.9% cases. Prior to hospital admission 46.1% patients received medicines, 50.06% received ORS (among which 45.5% consumed WHO ORS), 27.9% received HAF (home available fluid) and 1.1% received intravenous fluid. On admission, dehydration was present in 91.07% cases and it was of severe degree in 41.3% cases. Patients received tetracycline, furazolidone and norfloxacin in 63%, 4.8% and 25.1% cases respectively. Duration of hospital stay in majority (98.6%) cases was less than 48 hours. Death occurred in 0.48% cases.

During the period under study a total of 1046 faecal specimens were collected and analyzed for detection of enteropathogens using standard microbiological technique. The isolation rate of different enteropathogens are depicted in Table-1.

#### *Antimicrobial susceptibility:*

All the *Vibrio cholerae* O1 strains isolated during 2000-2001 were found highly resistant to ampicillin, co-trimoxazole, furazolidone, nalidixic acid, streptomycin and reduced susceptibility to ciprofloxacin and neomycin. However, *V.cholerae* O139 strains were found sensitive to co-trimoxazole and nalidixic acid. *V.cholerae* non-O1, non-O139 strains, on the other hand, were found sensitive to chloramphenicol, gentamycin, norfloxacin and tetracycline. *V.parahaemolyticus* strains isolated during 2000-2001 were found susceptible to chloramphenicol and co-trimoxazole. Majority of the strains exhibited reduced susceptibility to ciprofloxacin, norfloxacin and neomycin. Few strains of *V.parahaemolyticus* also exhibited reduced susceptibility to tetracycline.

All the *Shigella* strains were uniformly resistant to co-trimoxazole whereas number of strains showed varying degree of resistance against tetracycline (78%), ampicillin (72%), furazolidone (55.5%), gentamycin (50%), chloramphenicol (50%) and nalidixic acid (44%). The strains were found susceptible to norfloxacin and ciprofloxacin.

Table-1. Enteropathogens detected.

Enteropathogens	Number identified	Number tested	Percentage
<b>Bacteria</b>			
<i>Vibrio cholerae</i> O1	85	1046	8.1
<i>V.cholerae</i> O139	22	1046	2.1
<i>V.cholerae</i> non-O1 non-O139	27	1046	2.6
<i>V.parahaemolyticus</i>	38	1046	3.6
<i>Shigella</i> spp.	21	1046	2.0
Non-typhoidal <i>Salmonella</i> spp	2	1046	0.2
Diarrhoeagenic <i>Escherichia coli</i>	946		
<b>Virus</b>			
Rotavirus	33	409	8.1
<b>Protozoa and Helminth</b>			
<i>E.histolytica</i>	19	374	5.0
<i>Giardia lamblia</i>	13	374	3.4
Cryptosporidia	7	374	1.8
<i>A.lumbricoides</i>	35	374	9.3
<i>H.nana</i>	4	374	1.06
<i>T.trichura</i>	9	374	2.4
<i>T.hominis</i>	14	374	3.7

# COMMUNITY BASED STUDIES

## Section highlights

- o Daily dose of 10 mg and weekly single dose of 50 mg zinc supplementation showed significant reduction in diarrhoeal morbidity among the children in rural community as compared to control population. However, no difference in morbidity was observed when daily and single weekly dose supplemented groups were compared.
- o Feeding pattern of rural infants in West Bengal showed that 43.7% were exclusively breastfed, 9.8% were predominantly breast fed and 44.0% infants received complementary feeding. 2.4% of infants did not receive breast feeding.
- o In a longitudinal community based study, diarrhoeagenic *E.coli* was detected from 60.4% stool samples. These *E.coli* strains were further characterised as enteropathogenic *E.coli* (17.8%), enterotoxigenic *E.coli* (12.4%) and enteroaggregative *E.coli* (30.2%).

## **2. COMMUNITY BASED STUDIES**

### **2.1 Impact of zinc supplementation in reducing diarrhoeal morbidity amongst under five children : a community based intervention study**

Investigators:

D.N. Gupta, P.G. Sengupta, S.K. Mondal, S. Ghosh, K. Sarkar, B. Manna, D. Sur, A. Pandey, K. Rajendran

Zinc is an important trace element and essential for the body. Though required in small amounts they are responsible for many important functions of the body in relation to metabolism, enzyme regulation, growth and development and immunity. It has been suggested that deficiency of zinc causes diarrhoea and there may be a cause & effect relationship of diarrhoea and zinc deficiency. Till today diarrhea is an important cause of morbidity and mortality in developing countries of the world. The study was therefore proposed to determine role of zinc supplementation in reducing diarrhoeal morbidity amongst a cohort of rural children.

The study design was a randomized double blind community based intervention study. Four villages having around 7000 populations in the existing rural field area was selected. A total of 280 children between 6-41 months were enrolled in the study. Children were randomly allocated into three groups. One group received a daily dose of 10 mg. Zinc for five days in a week, second group received 50 mg zinc once weekly and four days placebo. The third group received placebo for five days. Zinc was supplemented for 16 weeks period from November 1999 to February 2000. M/S Geenco Biologicals Pvt.Ltd.Salt Lake City Prepared the supplemented product. Diarrhoeal episodes were detected by weekly visit during this period. After entering and analysing the data in the computer decoding was done December 2000. All the groups were comparable in all aspects at the base line (Table-2). A total of 80 diarrhoeal episodes were detected among 280 subjects in all three groups. The resident surveillance workers detected diarrhoeal episodes through weekly surveillance. The groups were compared with each other as regards to the outcome variable (incidence of diarrhea). Proportion of children suffered from diarrhoea during the period was significantly lower in the zinc-supplemented group than the placebo group ( $P=0.004$ , RR 0.52, CI 0.33-0.82). Over all incidence of diarrhoea amongst study children was 1.01 episodes/child/year. Incidence of diarrhoea in the zinc-supplemented group was 0.68 ep./ch/yr.& that of placebo group was 1.67 ep/ch/yr.(RR 0.41 & 95% CI 0.26-0.63). Incidence of diarrhoea <8 days duration was also found to be significantly lower in the supplemented group, but diarrhoeal incidence with a duration of more than a week did not show any difference between study and control group. However when compared between daily and weekly zinc supplemented groups no difference was observed. There were no adverse reactions in any of the supplemented groups. The study indicated zinc is effective in reducing diarrhoeal morbidity when administered either daily or in a weekly schedule.

Table-2. Baseline information of families having a study child (6-41 months)

Information	Group 1	Group 2	Group 3
No.of children	95	91	94
No.of families	92	88	87
Total population	399	363	373
Average person/family	4.33	4.12	4.28
Housing condition			
a. kuccha	47.8%	48.9%	52.9%
b. others (mixed)	52.1%	51.1%	47.1%
Latrine facility			
Sanitary	91.3%	85.2%	83.9%
Family income/month			
a. upto Rs.2000	83.7%	79.5%	80.4%
b. Rs.2001-5000	14.1%	18.2%	14.9%
c. above Rs.5000	2.2%	2.3%	4.6%
Occupation of Head			
a. daily labour	54.3%	56.8%	52.9%
b. others	45.6%	43.2%	47.1%
Literacy of father			
a. illiterate	28.8%	28.4%	27.6%
b. literate	71.7%	71.6%	72.4%
Literacy of mother			
a. illiterate	45.7%	56.8%	56.3%
b. literate	54.3%	43.2%	43.7%
Nutritional status			
wt/age (below normal)	45/83 (54.2%)	49/81 (60.5%)	37/75 (49.3%)

## **2.2. Impact of lactation counselling on exclusive breast feeding and safe weaning in prevention of diarrhoea in rural infants: An operational research**

Name of Investigators

S.K. Mondal, D.N. Gupta, S. Ghosh, K. Sarkar, D. Sur, B. Manna, A. Pandey, K. Rajendran

Exclusive breast feeding of babies for the first 6 months of life protects infants from diarrhoea. Breast fed babies are less likely to have severe episodes of diarrhoea and diarrhoea related deaths compared to non-breast fed babies. However, there is very little information about the exact duration of exclusive breast feeding in babies in this region. Studies in rural West Bengal showed that around 75% of infants are switched over from exclusive breast feeding to other feeding modes by 4 months of age. Promotion of breast feeding has been started in big hospitals. However, there is complete lack of follow up of the mothers at home level. Very little effort has been made for promotion of breast feeding at the community level.

The study was initiated to define the time of weaning and nature of poor weaning practices during first phase of the study followed by development of a message to the mothers regarding exclusive breast feeding of their babies for first 6 months and then to start safe weaning practices in the second phase of study. The objectives of the study are to determine the impact of lactation counselling on promotion of exclusive breast feeding during first 6 month, to find out the effect of educating mothers for continued breast feeding, introduction of safe weaning practices after 6 months of age and to identify the constraints, if any in counselling the mothers.

Methodology

The study will be an operational research for promotion of breast feeding in the community in collaboration with Govt. Of West Bengal. Uday Narayanpur Block of Howrah District have been selected as study area. The study will be conducted in two phases.

First Phase - The first phase of the project will be a formative study. In this study feeding practices particularly for first six months of life followed by weaning practices of mothers will be observed and analyzed. This will enable us to identify the areas for behavioral changes of mothers in relation to feeding practices of their young children. Approximately 600 mothers having children below 1 year of age will be covered.

Second phase - After identifying the areas where behavioral changes are needed, mothers will be counselled at the community level on the basis of the findings of the formative research. This counselling will be done involving the health infrastructure available at the peripheral level so that ultimately this can be integrated in the health programme.

Results of survey

A survey was conducted amongst 597 mothers of Uday Narayanpur block. A specially designed proforma was prepared in local language. Local volunteers were selected and trained. The survey was conducted under direct supervision. The data were entered subsequently in computer after translating into English.

A total of 597 mothers, having a child within one year and living in 59 villages of the

block, were surveyed. Average family size was 6.13 person with monthly income Rupees 1829/=. Electricity was available in 161(27.0) families, while 143 (24.0) families had Television, Radio was found in 333(55.8) families. Five hundred and four (84.4) families used Tube well water for drinking purpose while 572(95.8) families utilized pond water for domestic uses. Sanitary latrine although available in 188(31.0) families only 2 (0.3) families used it for disposal of children's faeces, majority of them disposed it in surface water sources. Only 117 (19.6) mothers used soap and water after ablution of their babies although 390(65.3) of them were literate. Agriculture was the main occupation for 251 (43.0) families.

Prevalence of breast feeding of infants below 6 months infants was as follows :

Exclusive breast feeding 143(43.7), Predominant breast feeding 32(9.8), Complementary feeding 144(44.0), and no breast feeding 8 (2.4). However, except only 5 babies who were predominantly breast fed, all infants of 6-12 months of age were on Complementary feeding.

Illnesses of the children during preceding one month was recorded and compiled in respect to their feeding pattern. Out of 327 infants below 6 months of age, 122 suffered from any illness. Amongst them 34 were exclusively breast fed compared to 88 in children with other feeding modes.

Second phase of the study will be conducted after training of peripheral level health workers, who will identify and counsel mothers for exclusive breast feeding/safe weaning as required.

### **2.3. A rural community based longitudinal study on diarrhoeagenic *Escherichia coli* amongst children below 5 years**

Investigators :

S. Ghosh, T. Ramamurthy, A. Pal, S.K. Mondal, D.R. Saha, S. Dutta, K. Sarcar, D. Sur, T.N. Naik, B. Manna, A. Pandey, K. Rajendran and D.N. Gupta

*E.coli* is an important versatile enteropathogen causing childhood diarrhea, adult diarrhea, dysentery and persistent diarrhea in developing countries. This longitudinal observational study was undertaken to describe the magnitude of problem caused by this pathogen in this geographical area and to identify the risk factors i.e age, breast feeding mode and nutrition for developing *E.coli* diarrhoea.

Initial baseline information in 1546 families were updated in 4 villages with a total population around 6827. We started longitudinal weekly follow up of a cross sectional cohort of 405 children aged below 47 months. Subsequently 91 were newborns added to this cross-sectional cohort. The children have been visited once a week for detection of diarrhoea. A cohort of 91 infants have been also observed weekly to record the change of feeding practice and the occurrence of diarrhoea. A total of 80 diarrhoea was noted in different breast feeding mode. Six diarrhoea cases could be observed in infants who were Exclusively breastfed compared to 74 diarrhoea cases in babies with other feeding modes. For both groups of children weight, length and MAC were taken at 6 month's interval.

The diarrhoea cases were followed up and data regarding the onset, types of stool, clinical characteristic, duration, management and outcome were recorded in a specially designed proforma. Freshly voided stool samples were collected from diarrhoea cases and age matched controls. All data are being entered and analysed by using suitable soft-ware packages.

Diarrhoeagenic *Escherichia coli* was isolated from 64 (60.4%) stool samples out of 106 diarrhea stool samples examined and collected till March 2001. DNA-colony hybridisation assay further identified 19 (17.8%) as EPEC, 13 (12.4%) as ETEC and 32 (30.2%) as EaggEC.

#### **2.4. Impact of zinc supplementation on incidence of diarrhoea and growth pattern among low birth weight infants of an urban slum**

Investigators

D. Sur, P.G. Sengupta, S.K. Mondal, D.N. Gupta, S. Ghosh, K. Sarkar, A. Pandey, B. Manna

The project is a double blind randomised community based intervention study on a birth cohort of 100 low birth weight babies in the Tiljala slum area of Kolkata(10,000 population). Informed consent was taken from parents of the study children.

The children have been randomly allocated into either of two groups each group receiving either zinc or placebo. The mothers have been advised to administer the necessary daily dosage to her child for a total span of one year under weekly supervision of female resident workers. The resident workers are trained to carry out surveillance for diarrhoea and also manage such cases with ORS at home level.

An epidemiological team visits the area regularly for supervision, management of diarrhoea cases and anthropometric measurements (length and weight). Data entry is being done simultaneously.

The project was started in November 1999 and is scheduled to be completed by December 2001.

Total number of newborn LBWs identified	-	100
Mean birth weight (in gms)	-	2250
Total child weeks of observation	-	4014
Total episodes of diarrhoea	-	206

#### **2.5. A case control study on risk factors of dehydrating diarrhoea in children**

Name of the investigators

A. Pandey, D. Datta, M.K. Bhattacharya

After the schedule preparation data collection is in progress. Cases and control for the study are identified from the ID hospital are being followed up in the community as described in the methodology. So far 30 cases and 30 control have been covered. The study is in progress and data collection work will require one and half year more.

# CLINICAL STUDIES

## Section highlights

- o Findings of a clinical study, suggest that supplementation of zinc, zinc and vitamin A and combination of micronutrients and vitamin A as an adjunct to ORS has beneficial effects on clinical course of dehydrating acute watery diarrhoea in malnourished children. However, there is no significant difference between the supplemented groups.
- o Sucrose based hypo-osmolar ORS was found to be superior for treatment of cholera in adults and older children as compared to standard ORS.
- o In a comparative clinical trial of erythromycin and azithromycin in the treatment of cholera in children showed that azithromycin is superior to erythromycin and it can be used as an alternative to erythromycin in the treatment of cholera in children.

### 3. CLINICAL STUDIES

#### 3.1. Impact of supplementation of zinc, zinc and vitamin A and combination of micronutrients and vitamins on acute watery diarrhea in mild to moderately malnourished children

Investigators

P. Dutta, U. Mitra, A. Dutta, S.K. Niyogi, S. Dutta, B. Manna, A. De and U.K. Santra.

In a double-blind, randomised, placebo-controlled, hospital-based clinical trial, a total of 167 malnourished male children (for ease of collection of stool and urine separately), aged between 6 and 23 months, suffering from dehydrating acute watery diarrhea of less than 3 days duration and fulfilled the inclusion and exclusion criteria, were admitted to the hospital. After taking written consent from the parents they were included in the study. These children were randomized according to a random number table into four treatment groups to allocate the specific numbered bottle of syrup supplementation or only syrup (placebo).

Stool samples were collected and processed for establishment of enteropathogens. Blood samples were collected on admission and on recovery for estimation of serum zinc, micronutrients and vitamin A (serum is preserved at  $-170^{\circ}\text{C}$  for future estimation) and electrolyte (sodium, potassium, chloride and bi-carbonate) concentrations. On admission children were weighed unclothed using a weighing scale of 10 gm precision; length and mid-arm circumference were also recorded.

After selection, patients received either 20 mg of elemental Zinc (2RDA) and a single oral dose of placebo Vitamin A, or 20 mg of elemental Zinc (2RDA) and a single dose of Vitamin A, or Micronutrient combination (2RDA of all micronutrients; zinc-20 mg, iron-10 mg, copper-2 mg, selenium-40  $\mu\text{g}$ , vitamin B12-1.4  $\mu\text{g}$  and folate-100  $\mu\text{g}$ ) and a single oral dose Vitamin A, or only placebo syrup and a single dose of placebo Vitamin A. The syrup was given daily in two divided doses for a period of 14 days even after cessation of diarrhea and discharge from the hospital. Vitamin A was given on admission as per national guidelines (<1 year = 100,000 I.U. and >1 year = 200,000 I.U.) in a single oral dose. The placebo syrup and placebo vitamin A were similar in taste and appearance as the zinc and micronutrient and vitamin A syrups, and supplied in identical bottles.

All the patients received standard ORS fluid and normal hospital diet. Breast-feeding was continued in breast-fed children. Patients were followed-up until recovery or upto 5 days after hospitalization if they do not fulfil the criteria of recovery within this time period. Daily input and output records were collected. Children were also followed up on day 15 and day 30 after hospitalization.

After decoding it was observed that 41 children received syrup of zinc and placebo vitamin A, 44 children received syrup of zinc and vitamin A, 39 children received syrup of combination of micronutrients and vitamin A and 43 children received both placebo syrup and vitamin A. All the four groups were comparable with regards to various initial characteristics. When the outcome variables (duration of diarrhea, percentage of recovery, stool output, intake of ORS and intake of other fluids) of all the four groups were compared it was observed that the results of the outcome variables of zinc and placebo vitamin A group, zinc and vitamin A group and micronutrient combination and vitamin A group were statistically significantly different to that of the placebo group. However, there is no statistical significance in the above mentioned variables between the three supplemented groups.

Our findings suggest that zinc, zinc and vitamin A or combination of micronutrients and vitamin A supplementation as an adjunct therapy to ORS has beneficial effects on the clinical course of dehydrating acute watery diarrhea in malnourished children though there is no significant difference in the beneficial effects between the supplemented groups.

### **3.2. Efficacy and safety of a sucrose based hypo-osmolar ORS solution in adults and older children with cholera : a clinical trial**

Investigators

D. Dutta, M.K. Bhattacharya, S.B. Ray, G.B. Nair, D. Sarkar, A. Biswas and S.K. Bhattacharya

Oral rehydration salts solution (ORS) recommended by WHO/UNICEF is universally accepted for the treatment of dehydrating diarrhoea in all age groups. However, it cannot reduce the stool output and duration of diarrhoea. Furthermore, there has been concern about the sodium in infants and small children and the European Society of Paediatric Gastroenterology Nutrition recommends an ORS with a sodium concentration of 60 mmol/l and an osmolality between 200 and 250 mmol/l. Several recent studies have evaluated the safety and efficacy of reduced sodium and glucose based ORS solutions (hypo-osmolar). Recently a randomized clinical trial was conducted in children with acute non-cholera diarrhoea to evaluate a sucrose based hypo-osmolar ORS which showed that a sucrose based hypo-osmolar ORS is highly absorption efficient as compared to WHO/UNICEF ORS (unpublished observation). This study was targeted to evaluate the efficacy of a hypo-osmolar ORS solution containing appropriate amount of sucrose in place of glucose.

A total of 60 adult male patients with a history of acute watery diarrhoea with severe dehydration were included in the study. After initial rehydration with intravenous fluid therapy - patients were randomized to receive either the sucrose-based hypoosmolar oral rehydration solution or standard oral rehydration solution; in addition - they also received 300 mg of doxycycline capsule at the time of inclusion. After rehydration, body weight was recorded and serum sodium level was estimated. The age group of the patients was between 14-55 years. No hyponatraemia was observed in the present series. All the cases recovered successfully.

The study is in progress.

### **3.3. Evaluation of comparative efficacy of erythromycin and azithromycin in the**

## **treatment of cholera in children**

### Investigators

M.K. Bhattacharya, D. Dutta, S.B. Ray, G.B. Nair, A. Chatterjee, K. Chatterjee and A. Biswas

Male patients with acute watery diarrhoea with <24 hrs. duration with moderate or severe dehydration aged 2-10 years were enrolled for the study. After fulfillment of inclusion and exclusion criteria eighty male child with a history of severe dehydrating diarrhoea were included in the present study. The age range of the cases was 3 years to 9 years. All patients received either erythromycin or azithromycin along with placebo.

A total of 80 patients were included in the study. Out of 80, 56 patients were positive for *V.cholerae*. Among 56 patients, 29 received azythromycin and 27 received erythromycin. On admission both the groups were comparable with respect to age, body weight, number of stool frequency, pre-admission duration of diarrhoea and vomiting. In response to therapy, as a whole and among the positive cases, total ORS consumption, total stool output, total duration of diarrhoea and total duration of diarrhoea after treatment were significantly less among the patients who received azythromycin. So, azythromycin is superior to erythromycin and can be used as an alternative to erythromycin in the treatment of cholera in children.

## STUDIES ON *VIBRIO CHOLERAE* O1 AND O139

### Section highlights

- o During the year 2000, *Vibrio cholerae* O1 strains isolated from Kolkata were resistant to nalidixic acid (75%), ciprofloxacin (18.7%) and norfloxacin (25%) but none of the isolated strains of *V.cholerae* O139 strains were resistant to these drugs.
- o Another study demonstrated that *V.cholerae* O1 Inaba predominating until 1989 have emerged in different parts of the country in 1988 to 1999 which have evolved from prevailing *V.cholerae* O1 Ogawa ElTor biotype.
- o Rough strains of *Vibrio cholerae* isolated as sole pathogen from clinically diagnosed cholera patients harboured *ctxA*, *ctxB*, *tcpA*, *tagA*, *int*, *aldA*, *toxT*, *LJ*, *RJ* and *toxR* genes similar to that of *V.cholerae* O1 serogroup.
- o Some of the environmental strains of *V.cholerae* non O1/non O139 isolated from water, sediment and plankton contain virulence associated genes which are the potential reservoir of virulence gene in the environment.
- o The study on *V.cholerae* hemolysin examines the contribution of surface hydrophobicity, oligomerization and receptor - binding domains in the membrane permeabilization process. Two oligomeric forms representing prepore assembly and the oligomeric pore have been identified.
- o A 30 kDa glycoprotein that specifically binds to the N-acetyl D-glucosamine specific hemagglutinin of *V.cholerae* O1 was isolated from chicken erythrocyte membrane by solubilization with lithium-di-iodosalicylate. The glycoprotein inhibited specifically hemagglutinating activity of intact bacteria.

## 4. STUDIES ON *VIBRIO CHOLERAE* O1 AND O139

### 4.1. Emergence of Fluoroquinolone Resistant Strains of *Vibrio cholerae* O1 Biotype El Tor among Hospitalized Patients with Cholera in Calcutta

Investigators :

P. Garg, S. Sinha, R. Chakraborty, S.K. Bhattacharya, G.B. Nair, T. Ramamurthy

Ciprofloxacin and norfloxacin are the second generation fluoroquinolone broad-spectrum antimicrobial agents and possess excellent activity against *Vibrio cholerae* O1 and O139 serogroups. Clinical studies have shown that these drugs are effective in the treatment for cholera in adult and children. For the past 11 years, we have been monitoring the incidence of antibiotic susceptibility, and genotypic changes of *V.cholerae* isolated from cholera patients admitted in the Infectious Diseases Hospital (IDH), Calcutta.

We have reported considerable increase in fluoroquinolone resistance among *V.cholerae* strains belonging to non-O1, non-O139 serogroups during 1996. All the *V.cholerae* strains of O1 serogroup isolated till 1994 are susceptible to ciprofloxacin as detected by standard disk diffusion technique. From 1995, we have recorded progressive increase in ciprofloxacin and norfloxacin resistance among *V.cholerae* O1 strains with highest occurrence of 38.8% in 1999 and 25% in 2000 respectively (Table). To our knowledge, this is the first description on such high incidence of fluoroquinolone resistance among toxigenic *V.cholerae* O1. The minimal inhibitory concentration (MIC) value for ciprofloxacin resistant *V.cholerae* strains ranged between 9 µg/ml and >32 µg/ml and that for norfloxacin between 192 µg/ml and >256 µg/ml when tested with the E-test strips. The incidence of nalidixic acid resistance among *V.cholerae* O1 was low before 1993 (<10%) and peaked during subsequent years (1999-100%) as shown in the Table. Possibly, ciprofloxacin resistance might have emerged in direct response to the selective pressure exerted by nalidixic acid coupled with disproportionate use of fluoroquinolones in the clinical settings. It is worth to mention here that increase in the incidence of nalidixic acid resistant strains of *V.cholerae* O1 (probably with single mutation in the *gyrA* and/or other related genes) portended further increase in the incidence of strains with clinically significant resistance to fluoroquinolones (with two or more mutations in the *gyrA* gene). We are in the process of identifying the "mutational hot spots" in the quinolone-resistance-determining region (QRDR).

Interesting observation in the present finding is the low incidence of quinolone resistance among the O139 serogroup. One possible elucidation that can be derived from the nalidixic acid resistance data is the low frequency of its occurrence among *V.cholerae* O139 strains (Table-3) and therefore, the frequency of double mutations, a prerequisite for fluoroquinolones resistance is low as reflected by resistance data of O139 serogroup towards fluoroquinolones.

In this study we have encountered high incidence of *V.cholerae* O1 strains resistance to ciprofloxacin than to norfloxacin, which is generally less potent compared to ciprofloxacin. The possible explanation for this counter intuitive results could be that i) since ciprofloxacin is in extensive use for all the bacterial infections in this region, conditions of high selective pressure would have forced the mutant *V.cholerae* strains to multiply and establish themselves as the dominant population; continued selective pressure favored these progeny to have further mutations ii) ciprofloxacin and norfloxacin break points are not comparable at least for *V.cholerae* strains. (The MIC 50, MIC 90 and MIC range test are need to be performed for both

the drugs to prove this hypothesis) and iii) accumulation kinetics for ciprofloxacin and norfloxacin might be different among *V.cholerae* O1 strains to make stable mutants.

Emergence of fluoroquinolone resistance among *V.cholerae* will certainly complicate the therapeutic use of these drugs and attention must be paid to this trend of resistance. Fortunately, *V.cholerae* O1 and O139 strains are susceptible to tetracycline, which is an effective drug for treatment of cholera patients in the IDH, Calcutta.

Table-3. Resistance to quinolone drugs among *Vibrio cholerae* O1 and O139 isolated from cholera patients admitted to Infectious Diseases Hospital, Calcutta

Year	Number of isolates examined		No. (%) strains resistance to quinolones*					
	O1	O139	Nalidixic acid		Ciprofloxacin		Norfloxacin	
			O1	O139	O1	O139	O1	O139
1989	49		3 ( 6.1)		ND		ND	
1990	59		1 ( 1.7)		ND		ND	
1991	30				ND		ND	
1992	26	10	2 ( 7.7)					
1993	20	87	1 ( 5)	2 ( 2.3)				
1994	74	40	73 (98.6)					
1995	84	42	82 (97.6)	9 (21.4)	2 ( 2.4)	1 (2.4)	3 ( 3.6)	1 (2.4)
1996	69	64	68 (98.5)	5 ( 7.8)	4 ( 5.8)	3 (4.7)		
1997	53	71	50 (94.3)	5 ( 7)	10 (18.9)	1 (1.4)	4 ( 7.5)	
1998	201	55	197 (98)	7 (12.7)	20 (10)		1 ( 0.5)	
1999	49	60	49 (100)	1 ( 1.7)	19 (38.8)	1 (1.7)	8 (16.3)	
2000	16	2	12 (75)		3 (18.7)		4 (25)	

\*Interpretative criteria are based on the MIC values with *V.cholerae* O1

ND, not done

#### 4.2. Emergence of *Vibrio cholerae* O1 Biotype ElTor serotype Inaba from the prevailing O1 Ogawa serotype strains in India

Investigators

P. Garg, R.K. Nandy, N. Roy Chowdhury, K. De, T. Ramamurthy and G.B. Nair

The disease cholera, caused by toxigenic strains of *Vibrio cholerae* belonging to the O1 or O139 serogroup, is characterized by the passing of voluminous watery stools, which rapidly leads to dehydration and, if left untreated, to death. *V.cholerae* O1 is further classified into two biotypes, classical and ElTor, and into two major serotypes, Inaba and Ogawa. With the advent of the O139 serogroup in 1992, the Inaba serotype of *V.cholerae* O1 was displaced in Calcutta and other parts of India by the O139 serogroup, and the last Inaba predominance in Calcutta was observed in 1989. The isolation of *V.cholerae* O1 belonging to the Inaba serotype became rare. *V.cholerae* O1 strains are known to interconvert between the Ogawa and Inaba forms. The frequency of conversion of Ogawa to Inaba is approximately  $10^{-5}$ , whereas conversion from Inaba to Ogawa is rare and may be strain dependent. *In vivo* seroconversion correlates well with the host immune response, and this is supported by observations with germfree mice.

In December 1998, we received a representative set of strains from Delhi, two of which were identified as *V.cholerae* O1 Inaba. In November 1999 we received a set of seven strains from Sewagram, six of which were identified as *V.cholerae* O1 Inaba and one of which was *V.cholerae* O1 Ogawa. The purity and identity of the strains were then confirmed by biochemical tests. For molecular characterization we included two Inaba strains, V2 and V13, isolated in 1989 in Calcutta, two Inaba strains from Delhi isolated in 1998, six Inaba strains and one Ogawa strain isolated in 1999 in Sewagram, and four Ogawa strains from Calcutta isolated in 1998. Our rationale for examining these strains was to determine whether the Inaba strains that reappeared recently in Delhi and Sewagram bore any resemblance to the Inaba strains last isolated in Calcutta in 1989 or whether they belonged to a new clone. The antibiotic susceptibility patterns of these strains revealed that V2 and V13, representing the Inaba strains isolated in 1989, were sensitive to nalidixic acid and streptomycin, while the Inaba strains recently isolated in Sewagram and Delhi were resistant to these antibiotics (Table 1). Overall, the antibiograms of the recent Inaba strains matched those of the prevailing O1, Ogawa strains. Resistance to cotrimoxazole, furazolidone, and streptomycin suggests the possibility of the presence of the SXT element in recently isolated Inaba strains. The widespread use of co-trimoxazole, a very popular and useful antimicrobial drug combination, may have provided an additional selective pressure for the sporadic emergence of the *V.cholerae* O1 Inaba strains in Delhi and Sewagram.

*Bgl*I ribotyping showed that the Inaba strains recently isolated in Delhi and Sewagram had a ribotype different from those of the Inaba strains isolated in 1989 (Fig 1). The ribotype patterns of the Inaba strains isolated in 1989, V2 and V13 (lanes 4 and 5), were different from the ribotype patterns shown by the *V.cholerae* reference strains SG24 (O139) (lane 1), 569B (O1 classical Inaba) (lane 2), and CO840 (O1 Ogawa ElTor) (lane 3). On the other hand, the recent Inaba strains DO182 and DO183 isolated in Delhi in 1998 (lanes 6 and 7) and the Inaba strains isolated in Sewagram in 1999, SO86 and SO90 (lanes 8 and 9), showed ribotypes similar to that of the prevailing *V.cholerae* O1 Ogawa strain CO840, which is the RIII type. The ribotypes of the recent *V.cholerae* Inaba strains indicate that these strains have molecular traits identical to those of the prevailing *V.cholerae* O1 Ogawa strains. This result also points out that the recent Inaba strains are quite different from the Inaba strains isolated in 1989, when Inaba

was the dominant serotype. Southern hybridization with the *ctxA* probe using *Hind*III-digested genomic DNA detected only one band, but of varying size, in the Inaba strains. This result indicates that the CTX prophage is located at a single site in the chromosome, as *Hind*III does not have any recognition site within the CTX prophage. The arrangement and number of copies of the CTX prophage were determined by analysis of the Southern hybridization pattern generated separately with *ctxA* and RS1 probes using other restriction enzymes which do cut within the CTX prophage but not in *ctxA*. *ctxA* RFLP patterns generated with *Bgl*II- and *Pst*I-digested genomic DNA showed a single band in all the strains (Table 1), suggesting the presence of a single copy of the CTX prophage. Southern hybridization with the RS probe was carried out to determine the organization of the RS sequences upstream and downstream of the core region. The CTX prophage genome has two regions: a 4.6-kb “core region” that includes *ctxAB* and a 2.4-kb region termed RS2. The integrated CTX prophage is frequently flanked by an element known as RS1, which is related to RS2. These related elements contain three nearly identical open reading frames (ORFs), while RS1 contains an additional ORF. The restriction endonuclease *Pst*I, which cuts within the core region, was used to digest genomic DNA, which was then hybridized with the RS probe. The RS RFLP patterns generated with *Pst*I-digested genomic DNA exhibited the presence of two bands of about 30.0 and 10.0 kb in strains V2 and V13 and of about 30.0 and 16.0 kb in strain DO183, while a single band of about 30.0 kb appeared in the remaining strains (Table 1). Therefore, strains V2, V13, and DO183 each contain at least one copy of RS1 at both sides of the core region. When the results of hybridization of *Pst*I-digested genomic DNA with the *ctxA* and RS probes were analyzed, a band common to both was observed for strains V2, V13, and DO183. The size of the common band was 10.0 kb in strains V2 and V13 and 16.0 kb in strain DO183 (Table 1). This result confirms the presence of an RS1 element downstream of the CTX prophage. The *Bgl*III restriction enzyme has a site in the RS region. As expected, when *Bgl*III-digested genomic DNA was hybridized with the RS probe, three bands were observed for V2 and four bands were observed for V13 and DO183; one of these, a 7.0-kb band, is actually the size of the CTX prophage. The presence of tandemly arranged RS1 sequences is not uncommon in toxigenic *V.cholerae*, and the size of RS1 is reported to be 2.7 kb. Since hybridization with the RS1 probe never showed the presence of a 2.7-kb band in V2, the possibility of tandemly arranged RS1 on either side of the core region can be excluded. The presence of a 2.7-kb band in V13 and DO183 indicates the possibility of tandemly arranged RS1 on either side of the core region. When the Inaba strains isolated in Sewagram and strain DO182 were digested with *Pst*I, the RFLP data showed a single band upon hybridization with both the *ctxA* and RS probes (Table-4), indicating the presence of a single copy of the CTX prophage, while *Bgl*III digestion and hybridization with the RS1 probe showed a band of 2.7 kb, indicating the presence of two tandemly arranged copies of the RS1 element in these strains. Thus, strain DO183 and the Sewagram strains have a single copy of CTX prophage with one RS1 element upstream of the prophage. This organization is very similar to that of the prevailing Ogawa strains. Figure 2 shows schematic representations; based on RFLP analysis, of the CTX prophage of the Inaba strain V2, isolated in 1989 (Fig. 2a), and of strain SO85, which is typical of the Inaba strains recently isolated in Delhi and Sewagram (Fig. 2b). Analysis of PFGE patterns showed that the Inaba strains isolated recently (Fig. 3, lanes 3 to 7) had a profile different from those of strains V2 (lane 1) and V13 (lane 2), representing the Inaba strains isolated in 1989. The recent Inaba strains (lanes 3 to 7) differed from strain CO840 (lane 8) by the presence of more than one band in the 145.5-kb region. Interestingly, O1 Ogawa strains isolated during 1998 (lanes 9 to 12) had a PFGE profile identical to that of the recently isolated Inaba strains, indicating that the Inaba strains that have emerged recently are similar to the prevailing O1 Ogawa strains. This study demonstrates that *V.cholerae* O1 Inaba, after predominating until 1989, that have emerged in different parts of the country in 1998 to

1999 could have evolved from the prevailing *V.cholerae* O1 Ogawa ElTor biotype.

Table-4. Antibigrams, ribotype patterns and sizes of restriction enzyme digested DNAs of *V.cholerae* O1 strains isolated in India

Strain no. (serotype)	Isolation yr, place	Antibiogram <sup>a</sup>	RP <sup>b</sup>	Fragment size(s) (kb) with the following probe and restriction enzyme				
				<i>ctxA</i> <i>Bgl</i> II	<i>Hind</i> I II	<i>Pst</i> I	RS <i>Bgl</i> III	<i>Pst</i> I
V2 (Inaba)	1989, Calcutta	A Co Fz N S	PD	18.5	20.0	10.0	20.0, 7.0, 3.6	30.0, 10.0
V13 (Inaba)	1989, Calcutta	A Co Fz N	PD	19.0	23.0	10.0	16.0, 7.0, 3.6, 2.7	30.0, 10.0
DO182 (Inaba)	1998, Delhi	A Co Fz Na S	RIII	14.0	21.0	6.2	16.0, 8.4, 2.7	30.0
DO183 (Inaba)	1998, Delhi	A Co Fz N Na S	RIII	23.0	23.0	16.0	16.0, 7.0, 3.6, 2.7	30.0, 16.0
SO85 (Inaba)	1999, Sewagram	A Co Fz N Na S	RIII	12.0	19.5	6.2	16.0, 8.4, 2.7	30.0
SO86 (Inaba)	1999, Sewagram	A Co Fz N Na S T	RIII	13.0	19.5	6.3	16.0, 8.4, 2.7	30.0
SO87 (Inaba)	1999, Sewagram	A Cf Co Fz N Na S	RIII	13.0	20.0	6.3	16.0, 8.4, 2.7	30.0
SO88 (Ogawa)	1999, Sewagram	A Co Fz N Na S	RIII	14.0	20.5	6.2	16.0, 8.4, 2.7	30.0
SO89 (Inaba)	1999, Sewagram	A Co Fz N S T	RIII	13.0	13.0	6.2	16.0, 8.4, 2.7	30.0
SO90 (Inaba)	1999, Sewagram	A Cf Co Fz Na S	RIII	14.0	21.0	6.3	16.0, 8.4, 2.7	30.0
SO84 (Inaba)	1999, Sewagram	A Co Fz N Na S T	ND	ND	ND	ND	ND	ND
PG81 (Ogawa)	1998, Calcutta	A Co Fz N Na	RIII	ND	26.0	5.6	18.5, 8.6, 3.1	31.0
PG117 (Ogawa)	1998, Calcutta	A C Co Fz Na S	RIII	ND	26.0	5.6	17.0, 8.6, 3.1	22.0
CO840 (Ogawa)	1995, Calcutta	A Co Fz N Na S	RIII	ND	23.0	6.0	ND	23.0

<sup>a</sup> A, Ampicillin; Cf, ciprofloxacin; Co, cotrimoxazole; Fz, furazolidone; N, neomycin; Na, nalidixic acid; S, streptomycin; T, tetracycline.

<sup>b</sup> RP, ribotype pattern; PD, previously described by Dalsgaard *et al.* (J. Clin. Microbiol. 1997, 35:1151-1156); ND, not done.

### 4.3. Characterization of rough strains of *Vibrio cholerae* isolated from diarrhea patients

#### Investigators

K. De, R.K. Nandy and G.B. Nair

*Vibrio cholerae*, the aetiologic agent of cholera, is classified on the basis of the heat stable somatic 'O' antigen into more than 193 serogroups. The antigenic specificity of the repetitive units of the polysaccharides ('O' antigen) of the lipopolysaccharide (LPS) form the basis of the *V.cholerae* serotyping scheme. The genes for the 'O' antigen are synthesized by chromosomally located 'wbe' operon. Traditionally, strains belonging to O1 serotype were responsible for epidemics and pandemics of cholera. In late 1992, *V.cholerae* strains which did not agglutinate with the then existing 'O' specific antisera emerged as the causative agent of epidemic cholera and was later designated as O139 Bengal (5). Molecular epidemiological analyses such as zymovar analysis, ribotyping and pulsed-field gel electrophoresis (PFGE) showed that *V.cholerae* O139 Bengal resembles the seventh-pandemic O1 ElTor biotype (6,7). In contrast to O1, *V.cholerae*, O139 Bengal possess a short truncated form of LPS and are characterized by the presence of a capsular polysaccharide material (8).

In order to establish infection in the gut, vibrios express a number of co-ordinately regulated virulence factors, the most important of which are cholera toxin (CT), toxin co-regulated pilus (TCP) and the global regulatory element ToxR. Genes for cholera toxin (*ctxAB*) have earlier been shown to be located on a 4.5 kb DNA segment called 'core region', flanked by one or more copies of a direct repeat sequences (RS) of 2.4-2.7 kb in size (9). Recently, it has been demonstrated that a region about 7 kb in size comprising the core region and RS2 is not an integral part of the *V.cholerae* genome but is acquired by the site specific lysogenic conversion by a filamentous bacteriophage CTX $\phi$  (10). The genes for the expression of TCP, the important pilus colonization factor, have been shown to be present along with other putative virulence genes on a 39.5 kb DNA segment collectively known as *Vibrio cholerae* pathogenicity island (VPI) (11). Recent studies have also shown that *V.cholerae* acquired VPI by lysogenic conversion by another filamentous phage VPI $\phi$  (12).

*V.cholerae*, non-O1, non-O139 represent heterogeneous serogroups, which so far have not been recognized as having epidemic potential and are generally devoid of most of the virulence genes associated with epidemic causing serogroups (O1 and O139) of *V. cholerae*. However, association of non-O1, non-O139 *V.cholerae* with sporadic cases of diarrhoea has been reported (13). By biochemical tests, *V.cholerae* strains belonging to O1 and O139 serogroups are indistinguishable from their non-O1, non-O139 counterparts except for their property to be agglutinated by specific antisera as well as their sensitivity to specific lytic phages (13,14). Smooth variants of a given species in the genus *Vibrio* are characterized by the presence of both smooth (S; 'O' antigens) and rough (R) antigens, while rough variants express only the R-antigens. The exact genetic basis for the generation of a rough variant from a corresponding smooth variant or vice versa, is yet to be clearly understood. Serological studies have shown that *V.cholerae* strains possess identical R-antigens irrespective of their 'O' antigenic types (15) which need to be absorbed for preparing 'O' specific antiserum. Organisms expressing only the R-antigen are usually indistinguishable from the corresponding S-form by colony morphology and biochemical tests but may be differentiated by agglutination with rough antiserum (14).

Much attention has been focused on studying the biochemical constituents of R-antigens of *V.cholerae* (16-19). However, very limited information is available on the characterization of the virulence genes of the rough variants of *V.cholerae*, particularly those isolated from clinical cases. Existing data on rough mutants of wild type smooth virulent strains of *V.cholerae* show that these mutants are comparatively less virulent when tested in animal models (20) which has been attributed to their severe defect in small bowel colonization properties when tested in the infant mouse model (21,22).

In this proposed research, we intend to study the characteristics of the rough clinical strains of *V.cholerae* extensively including the changes which are happening at the phenotypic and genotypic level. Epidemiological studies have shown that the appearance of the rough strains of *V.cholerae* normally precedes the period when a shift of serotype from either Inaba to Ogawa or vice-versa is observed. Therefore, detection of either O1 or O139 *wb* specific genes as well as the genes of VPI in these clinical isolates of the rough variants will be of immense significance from the epidemiological point of view.

Seven rough strains of *Vibrio cholerae* isolated as the sole infecting agent from patients with cholera-like diarrhoea were examined for the presence of the regulatory element *toxR* and certain virulence-associated genes of the CTX genetic element and *V.cholerae* Pathogenicity Island (VPI). Multiplex PCR analysis using *wb* specific genes of either O1 or O139 origin showed that six of the seven strains produced an O1 *wb* specific amplicon while the remaining isolate produced an O139 specific amplicon. While analysis of LPS profiles of smooth variants of *V.cholerae* revealed the presence of long repeated units of 'O' polysaccharide side chains, all the rough variants appeared to be devoid of the same and possessed only core oligosaccharide. PCR experiments with primers specific to the *ctxA*, *ctxB*, *tcpA*, *tagA*, *int*, *aldA*, *toxT*, *LJ*, *RJ* and *toxR* genes revealed that 6 of the 7 rough strains were positive for these genes. One strain was found to be negative for *tagA* and *RJ* indicating the presence of an altered VPI. Each of these strains showed media-dependent expression of cholera toxin (CT) and produced higher amount of toxin as compared to the reference *V.cholerae* O1 ElTor strain VC20 or O139 strain SG24 under comparable conditions. Studies on the clonality of these strains by the analysis of rRNA genes indicated their relatedness to strains of *V.cholerae* O1 ElTor or O139, isolated during the same time period.

#### 4.4. Virulence Genes in Environmental Strains of *Vibrio cholerae*

Investigators :

S. Chakraborty, A.N. Ghosh, R.K. Mitra, S. Yamasaki, G.B. Nair

*V.cholerae* is a species well defined on the basis of biochemical tests and DNA homology studies, but the species is not homogeneous with regard to its pathogenic potential. Among the 193 currently recognized somatic "O" serogroups of *V.cholerae*, only the O1 and O139 serogroups are capable of causing epidemic cholera. The other serogroups of *V.cholerae* collectively referred to as non-O1, non-O139 serogroups have not been associated with epidemic cholera. In this work, *V.cholerae* strains belonging to serogroups other than O1 and O139 were studied by a variety of phenotypic and genotypic parameters to understand the ecology of origin and distribution of virulence genes among environmental strains and to insight whether or not clinical non-O1, non-O139 strains associated with diarrhea have an environmental origin.

A total of 122 samples (44 water, 34 sediment, and 44 plankton samples) collected from three sites in Calcutta were analyzed by conventional bacteriology and by multiplex PCR assay. Two multiplex PCR assays were designed to detect five known virulence genes of *V.cholerae*, including *tcpA*-EITor, *tcpA*-Classical, *ctxA*, *ctxB*, and *sto* (gene encoding the heat-stable enterotoxin of *V.cholerae*). Five water and four plankton samples examined at different time intervals were positive for either *tcpA* or *ctxAB*; none were positive for *sto*. None of the sediment samples were positive for any of the virulence genes sought. A total of 19 strains of *V.cholerae* positive for *tcpA* and another 5 strains positive for *ctxA* and *ctxB* were isolated (Table 1). These 24 virulence gene-positive strains of *V.cholerae* were isolated after examining approximately 4,800 colonies (an average of 200 colonies per search) from either TCBS, TTGA, or LA. Of the 19 environmental *V.cholerae* strains examined, the size of the *tcpA* amplicon in 17 strains matched the size of the *tcpA* amplicon (617 bp) of the reference classical strain (*V.cholerae* O395), while in 2 strains the size of the *tcpA* amplicon (471 bp) matched that of the reference EITor strain (*V.cholerae* VC20). All 19 strains were, however, negative for the 301-bp *ctxA* and 460-bp *ctxB* amplicons, indicating that these strains did not have the genetic potential to produce CT. Furthermore, five environmental strains of *V.cholerae* which were positive for *ctxA* and *ctxB* were negative for *tcpA* with the set of primers used in this study. This is contrary to the current assumption that most CT-positive strains are also positive for TCP, since TCP is known to be the receptor for CTX $\square$  infection of *V.cholerae*. All 24 strains were positive for *toxR*, a transcriptional activator of many virulence genes in *V.cholerae*. In contrast, *toxT* was found in only three strains (SCE4, SCE5, and SCE6) positive for *tcpA* and all five strains positive for *ctxAB* (Table 1). The results of PCR assays were confirmed by Southern hybridization when representative strains positive for *tcpA* or *ctxA* were hybridized with the respective probes.

Analysis of *Bgl*I restriction patterns of conserved rRNA genes (ribotype) in the environmental strains revealed clonal diversity, and 10 different ribotypes (A through J) (Fig. 1). The distribution of ribotypes among the strains belonging to different serogroups is shown in Table 1. Two strains, SCE4 and SCE5, which belonged to different serogroups (O8 and O11, respectively) belonged to a single ribotype (A). Another O8 strain belonged to a different ribotype (J). Four strains belonging to the O35 serogroup shared two different ribotypes (B and G). Strains belonging to serogroup O42 shared ribotypes C and D. A toxigenic strain, SCE223, shared the same ribotype with a nontoxigenic strain, SCE228. Ribotyping was performed to determine whether strains of *V.cholerae* isolated from a given APW (pH 8.5) enrichment broth of a particular sample were siblings. For example, strains of *V.cholerae* with different serogroups (O27 and O35) as well as different ribotypes (B and G) were isolated from an APW (pH 8.5) enrichment of plankton samples collected from a freshwater pond in Calcutta on 8 January 1998. Similarly, APW enrichment of plankton samples collected from a freshwater lake on 7 July 1997 yielded three strains with two different serogroups (O8 and O11) but a single ribotype (A). We also isolated two strains of *V.cholerae* from an enrichment culture of a sample of water from a fish farm taken on 10 February 1998 which had the same serogroup (O42) and ribotype.

Among several putative colonization factors of *V.cholerae*, TCP has been shown to be essential for colonization in the infant mouse model as well as in human volunteers. To determine whether the 617-bp *tcpA* amplicon was the amplified DNA and the reported sequence of classical *tcpA*, the nucleotide sequence data of the *tcpA*-like amplicon of strain SCE5 (*V.cholerae* serogroup O11), obtained from two sequencing reactions of two independent amplicons, yielded readable sequences of 597 bases, with 97.7% identity to the *tcpA* gene sequence of the classical *V.cholerae* O1. Notably, only 14 bases differed. Furthermore, it was found that the derived amino acid sequence of TcpA in environmental strains had an identity of 98.5% when compared with the reported amino acid sequence of TcpA that is found in classical strains of *V.cholerae* O1, with differences discernible only at positions 104, 144, and 154. Homology between the environmental *V.cholerae* TcpA amino acid sequence and *V.cholerae* EITor TcpA sequence was 80.4%, with 39 of the deduced TcpA residues of the environmental strain (SCE5) differing from those of TcpA of the *V.cholerae* EITor biotype. The close similarity of most of the *tcpA* genes found in environmental strains of *V.cholerae* to the classical *V.cholerae* *tcpA* is interesting, despite the fact that the current cholera pandemic is caused by the EITor biotype. Recent epidemiological data from Bangladesh, where classical *V.cholerae* existed until 1991, show the absence of this biotype. However, the data obtained in this study indicate that a *tcpA* gene similar to the classical type is present in environmental non-O1, non-O139 *V.cholerae* strains. An environmental reservoir of *tcpA* genes of the classical type strongly suggests the possibility of a reemergence of the classical biotype via gene transfer events in the environment. The classical biotype transiently reemerged in Bangladesh in 1983 as the predominant epidemic strain, about 10 years after its apparent replacement by the EITor biotype.

Strains SCE188, -200, and -201 expressed CT in both AKI and YEP media, used for optimal production of CT from EITor and classical *V.cholerae*, respectively. However, the amount of CT antigen produced by SCE188 and SCE201 was higher in YEP than in AKI, while the yield of CT from SCE200 was the same whether grown in YEP or AKI. Despite possessing DNA fragments with sequences very similar to that of CT genes, two of the environmental isolates, SCE223 and SCE354, did not produce detectable amounts of CT when grown in either YEP or AKI. Passage of strain SCE188 in a rabbit ileum resulted in positive fluid accumulation and isolation of strains that produced twofold more CT than the wild type. This result (Table 2) suggests that selection for strains producing larger amounts of CT in the rabbit ileum occurs in

both environmental and epidemic strains.

The occurrence and distribution of selected virulence-associated genes in the non-O1, non-O139, environmental strains of *V.cholerae* that had been isolated in Calcutta, India, were demonstrated. These strains constitute a potential reservoir of virulence genes in the environment. What is most exciting is that molecular characterization of microbial ecosystems provides useful information about the ecology of *V.cholerae*, a bacterium autochthonous to riverine, coastal, and estuarine ecosystems but at the same time, pathogenic for humans. Environmental studies of *V.cholerae* have been done with the expectation that *V.cholerae* strains possessing the entire complement of virulence genes would be isolated. Now it is concluded that virulence genes are dispersed among environmental strains of *V.cholerae* and may be ferried about, given the fact that most of the virulence genes that were studied are located on mobile elements. Indeed, the potential for “mixing and matching” of genes in the environment or in the human intestine, leading to new pathogenic variants, must now be addressed. Ribotypes of the strains isolated in this study were shared by strains belonging to more than one serogroup, and conversely, a particular serogroup comprised more than one ribotype. Toxigenic strains and nontoxigenic strains belonging to an identical ribotype were also detected, further supporting the hypothesis of gene transfer among vibrios in the environment. Further studies on the ecology and evolution of *V.cholerae* will surely provide new insights into the epidemiology of cholera.

#### 4.5. Study the role of plasmids in adherence mechanism of *V.cholerae* non-O1, non-O139

Name of investigators

A. Pal, P. Garg, T. Ramamurthy, S.K. Bhattacharya, G.B. Nair and M.K. Chakraborty

In the previous studies it has been shown that there has been a rise in the incidence of *V.cholerae* non-O1 non-O139 among hospitalized patients admitted to Infectious Diseases Hospital in Calcutta from Feb 1996. We conducted investigations to address the virulence mechanism of these strains. These strains did not possess any of the known toxin genes as reported for *V.cholerae*. Bacterial adherence and colonization are the key steps, which help enterotoxigenic pathogens to establish infection in the gut. Adherence studies were done using HeLa cell with twenty strains of *V.cholerae* non-O1. non-O139 isolated as sole pathogen. Only 4 strains showed adherence with both HeLa cells and glass surface. Plasmid isolation by Kado and Liu revealed that 9 strains contained plasmids. Ten strains showed clump formation in Muller Hilton broth. Our results showed that there is no correlation between HeLa cell adherence, presence of plasmids and clump formation. Further studies were carried out to study the enterotoxicity of the strains in an in-vitro rat and rabbit intestinal model. (Ussing's Chamber). Addition of the supernatant of PL-1 resulted in the rise in intestinal short circuit current ( $I_{sc}$ ) of  $90 \mu\text{A}/\text{cm}^2$  and the change in voltage was 1.3 mV in only rabbit intestine but no change was observed in rat intestinal tissue. In PL-21 the  $I_{sc}$  was  $90 \mu\text{A}/\text{cm}^2$  and change in voltage being 0.7 mV and the rise in rat intestinal tissue was  $46 \mu\text{A}/\text{cm}^2$ . When compared to HB101 the  $I_{sc}$  was  $8 \mu\text{A}/\text{cm}^2$  and change in voltage was 0.1 mV. The  $I_{sc}$  was significantly higher in both PL-1 and PL-21 when compared to the negative control HB101. As majority of the *V.cholerae* non O1. non O139 strains produce hemolysin we also tested different concentrations of purified hemolysin tested in an in vitro rat intestinal model. The purified hemolysin was 65 kDa protein with a hemolytic titer of 20 ng/ml in rabbit erythrocyte. 12  $\mu\text{g}/\text{ml}$  of this purified hemolysin is required to elicit a positive response ie cause a rise in intestinal short circuit current of  $40 \mu\text{A}/\text{cm}^2$  when applied to the mucosal side of rat distal ileum. The major enterotoxic factor responsible for the

manifestation of diarrhoea during *V.cholerae* O1 and *V.cholerae* O139 infection is cholera toxin which disrupts ion transport by intestinal epithelial cells. However *V.cholerae* strains lacking genes for cholera toxin are still capable of producing diarrhoea in many individuals which imply that a number of additional toxins may be associated with the pathogenesis of cholera. Hemolysin produced by *V.cholerae* non O1. non O139 causes a rise in intestinal short circuit current resulting in change in ion transport leading to diarrhoea. These results suggest that the hemolysin is an enterotoxic factor in *V.cholerae*. Further studies are on to neutralize the effect of culture supernatants of PL-21 with antibody against hemolysin and study the nature of the enterotoxins produced by *V.cholerae* non O1. non O139.

#### **4.6. Studies on structure function relationship of *Vibrio cholerae* haemolysin**

Investigator : K.K. Banerjee

*Vibrio cholerae* hemolysin (HlyA) is an extracellular pore-forming toxin. The fully active 65 kDa monomeric protein, HlyA-65 and a 50 kDa proteolytic variant, HlyA-50 form pentameric diffusion channels in the biomembranes and lipid vesicles. In this study, we found that HlyA-50 in contrast to HlyA-65 which binds to immobilized  $\beta_1$ -galactosyl-terminated glycoconjugates and lipids, had no affinity for immobilized lipid, carbohydrate and erythrocyte membrane. Absence of a functional receptor-binding domain in HlyA-50 suggested that binding of the toxin to cell surface molecules was not an essential prerequisite for insertion of the protein in lipid bilayer. However, HlyA-50 had 100-fold lower specific hemolytic activity than its precursor having the receptor-binding domain suggesting that affinity for membrane components played an accessory rather than an essential role in membrane permeabilization. Two functionally distinct forms of oligomers of HlyA were the oligomer characterized : one that formed spontaneously during storage was hemolytically active and the other extracted from membrane permeabilized by the toxin was lytically inactive. The two forms had identical stoichiometry and similar global hydrophobicity. It remains to be seen if the hemolytically active oligomer represented the prelytic assembly that is postulated to precede insertion of the toxin.

#### **4.7. Isolation of N-acetyl-D-glucosamine specific hemagglutinin receptor of *Vibrio cholerae* O1 in chicken erythrocyte membranes**

Investigator : D. Sasmal

Previously an N-acetyl-D-glucosamine specific cell-associated hemagglutinin (HA)/lectin had been purified from a strain of *Vibrio cholerae* O1 and the role of this purified HA as an adhesin molecule of *Vibrio cholerae* O1 had also been established. The receptor(s) involved in this interaction has not been identified.

The project was undertaken to identify and characterize the receptor(s) in chicken erythrocyte membranes to further establish the lectin-like properties of the purified HA/adhesin.

Erythrocytes were collected from white Leghorn chicken in presence of normal saline with anticoagulant. RBCS were washed thrice with normal saline and packed cells were freeze dried. The dried cells were divided into two parts. One was used to study the receptor as glycolipid and another as glycoprotein.

As reported earlier the freeze dried chicken erythrocytes were extracted continuously with

chloroform-methanol (2:1) in a Soxhlet apparatus for 24 h, followed by extraction with chloroform-methanol (1:9) for another 24 h. The total lipid extract (CM extract) was subjected to mild alkaline hydrolysis in 0.1 M KOH in methanol-water. The vessel was then flashed with nitrogen and left with magnetic stirring in the dark for 12 h at room temperature. The alkaline hydrolysis was stopped by slow addition of 2 N HCl. Chloroform and water was then added to get chloroform-methanol-water phase (3:4:3 by volume). The aqueous phase was taken for evaporation.

Hemagglutination inhibition assay was performed with this aqueous phase after concentrating the extract. No satisfactory inhibition in hemagglutination was achieved in the presence of crude glycolipid extract.

In an alternative method to study the receptor as glycoprotein the freeze dried RBC were subjected to hypotonic saline to prepare RBC ghost membrane. Red cell membranes were suspended in 0.3 M lithium di-iodosalicylate and 0.05 M tris (hydroxymethyl aminomethane) hydrochloride pH 7.5 at a concentration of approximately 25 mg of membrane protein per ml and stirred at room temperature for 15 min. The two volumes of distilled water were then added and the turbid suspension was stirred for an additional 10 min at 4°C, the supernatant, which contained most of the membrane proteins, was decanted and mixed with an equal volume of freshly prepared 50% phenol in water. The mixture was stirred at 4°C for 15 min and centrifuged at 4000 x g for 1 h at 4°C in a swinging bucket rotor. The centrifuged material separated into two phases; the upper aqueous phase contained most of the soluble glycoprotein which was then separated and dialysed against distilled water for 36 h at 4°C with several changes.

Lithium di-iodosalicylate was used to extract glycoproteins in water soluble and biologically active form from red cell membranes. SDS-PAGE of this glycoprotein exhibited a prominent protein band of 30 kDa region. Hemagglutinating activity of *V.cholerae* O1 cells with chicken erythrocytes was studied in the presence or absence of this partially purified glycoprotein. It was observed that HA activity of *V.cholerae* O1 cells was significantly inhibited when the bacterial cells were pretreated with this glycoprotein indicating the neutralisation of the bacterial hemagglutinin by the glycoprotein. It is also interesting that pretreatment of the glycoprotein with N-acetyl-D-glucosamine specific HA before addition of bacterial cells completely nullified its (bacterial) hemagglutinating ability and thereby establishing the specificity of this glycoprotein towards N-acetyl-D-glucosamine specific HA. Initial observations, as stated above, suggest the exciting possibility of developing a rational approach towards an immunoprophylaxis of cholera.

#### **4.8. Vibriocidal studies on the status of vibriocidal serum bricidal antibody level against *Vibrio cholerae* O1 and serogroup O139 in Calcutta community**

Investigator : A.K. Sinha

At the initial phase of the study, a total of 34 blood samples have been collected from 18 selected households according to the hospital surveillance data in the year 1999, of which 13 cases from high prevalent and rest of them 21 cases from low prevalent areas, their ages varied from 6-12 years and >12 years without any history of diarrhoeal episode within last one month.

It was observed that all the sera samples (13) from high prevalent area, vibriocidal antibody level was >1280 folds against the strain V-3 of *V.cholerae* O1. whereas, antibody titre against *V.cholerae* O139 strain (MO10-T4) was upto 320 folds in 3 out of the 13 samples (15.3%). Age group among three samples, one was >12 years and rest of are not yet found any antibody titre against O139.

Vibriocidal antibody titre was also recorded in low prevalent area against both *V.cholerae* O1 and O139 strain. It was found less than 640 folds against O1, in 6 of the 21 samples and their ages varies from 6-8 years, at 160 folds antibody titre was found rest of the samples, of which 4 are at the age of >12 years. Antibody titre against O139 strain was found only in 3 of 21 samples and age group varied from 6-12 years.

The above results reflected that the baseline antibody level against O1 and O139 serotype persisted beyond are (1) month. Though the number of samples are very low due to most of them did not turn up to our response. Further studies are in progress.

#### **4.9. Sequential studies of *Vibrio cholerae* O139 and some of its defined virulence phenotypes on intestinal pathology in rabbit ileal loop model**

Investigators

D.R. Saha, A.N. Ghosh and G.B.Nair

The study was undertaken to determine the nature and location of lesion in the rabbit intestine caused by *Vibrio cholerae* O139 and two other non O1 non O139 strains at different time intervals using histologic and ultrastructural methodology. The strains were introduced in the rabbit at scheduled time interval after proper preparation of the animal. The rabbits were sacrificed timely, tissues were taken, fixed and processed separately for observation under light and electron microscope.

All these strains were examined with appropriate controls. Mild inflammatory cell infiltrations were observed with all the three strains, but. *V.cholerae* O139 Bengal appeared to be more virulent. Further work with a new non O1 non O139 strain is in progress.

Along with *V.cholerae* O139, *V.cholerae* O1 and non O1 non O139 strains were studied in rabbit ileal loop model. In the 18 hour study period, tissue necrosis and haemorrhage was marked with all the strains. Further study with these strains in ileal loop after challenging in a shorter interval of time is in progress.

#### 4.10. Morphological studies on the flagella of *Vibrio cholerae*

Investigators

A. Sen and A.N. Ghosh

The project was undertaken to study the morphological aspects of the flagella of different *Vibrio cholerae* strains. Bacteria were grown in Nutrient Broth at 37°C with shaking and on Nutrient Agar plates overnight at 37°C. Pioloform-coated copper grids stabilized with a layer of carbon were used for specimen support. Five microlitre of bacteria grown in broth culture was deposited directly on grids. Bacteria grown on Nutrient Agar plates were re-suspended in 50 mM Tris-HCl (pH 7.4), 10 mM MgCl<sub>2</sub> and 5 µl was deposited on grids. Samples were stained with one of the following stains: (a) 2% uranyl acetate (pH unadjusted) (b) 3% phosphotungstic acid (pH 7.0) (c) 1% ammonium molybdate (pH 4.0). Specimen preparation was carried out at room temperature (20°C).

The following strains were studied: *Vibrio cholerae* O1 (MAK 757), *Vibrio cholerae* O26 (NRT 36) and *Vibrio splendidus*. Negative staining was observed with all the stains used but maximum contrast was obtained with uranyl acetate (2%) and was subsequently used for staining all the strains. Both the strains of *Vibrio cholerae* viz. MAK757 and NRT36 have single polar flagellum. But the contour length and the straight (end-to-end) length of the flagella are slightly different for different growth media as well as for different strains as shown in Table-5.

Table-5. Contour length and straight (end-to-end) length of various strains of *Vibrio cholerae*

Strains	Growth medium	Contour length ± S.D.(µm)	Straight length ± S.D.(µm)	Ratio of Contour length/straight length
<i>Vibrio cholerae</i> O1 (MAK 757)	Nutrient Agar	3.33 ± 0.37	2.64 ± 0.26	1.26
	Nutrient Broth	4.36 ± 0.51	3.53 ± 0.40	1.23
<i>Vibrio cholerae</i> O26 (NRT 36)	Nutrient Agar	4.86 ± 0.43	3.91 ± 0.31	1.24
	Nutrient Broth	3.81 ± 0.36	3.10 ± 0.30	1.23

However, it is interesting to note that the ratio of the contour length to straight length is a constant quantity irrespective of the strains and growth conditions. The flagella of *Vibrio splendidus* are different from that of *Vibrio cholerae*. *Vibrio splendidus* has a bundle of flagella when grown in Nutrient Broth at 37°C as shown in Fig A. Further studies on the flagella are in progress.

## **STUDIES ON *VIBRIO CHOLERAE* PHAGES**

### **Section highlights**

- o A total of 350 *V.cholerae* O139 strains (including epidemic and endemic strains) were screened for phage typing. Majority these strains belonged to type I.
- o Six hundred forty three strains of *V.cholerae* O1 ElTor were received from different parts of our country. Three hundred fourteen representative strains were phage typed and all of these strains were grouped under type 2 of conventional phage typing scheme.
- o Attempt is being made to determine the size of DNA of *V.cholerae* O1 ElTor phage N4.
- o ElTor vibriophage M4 and DNA of this bacteriophage were electron-micrographed.

## 5. STUDIES ON *VIBRIO CHOLERAE* PHAGES

### 5.1. Further application of phage typing scheme for discriminating strains of *V.cholerae* O139

Investigators

B.L. Sarkar, A. Sengupta, S.K. Niyogi and G.B. Nair

The newly developed scheme is routinely in use for discriminating strains of *V.cholerae* O139 received from different parts of the country. This year, a total of 350 strains of *V.cholerae* O139 were included in phage typing study. Recent outbreaks strains were also included in this study. It was observed that all strains were found to be type able. Majority of the strains could be grouped under type 1. Out of 350, 9 strains were clustered in a new type which do not fall under 10 distinct types. The other types encountered were type 8 and type 10.

A comparative study was performed between the phage typing and other molecular typing systems like Rapid amplified polymorphic DNA (RAPD) and Pulsed Field Gel Electrophoresis (PFGE). RAPD could not differentiate the strains belonging to different phage types. PFGE could further discriminate strains of *V.cholerae* of different phage types. It was concluded that PFGE was much more sensitive compared to conventional phage typing system.

### 5.2. Nationwide screening of phage types of *V.cholerae* 01 biotype Eltor

Investigatgors

B.L. Sarkar, A.Sengupta

In this reference laboratory, during the course of the study, a total of 643 strains of *V.cholerae* received from different parts of the country for serotyping, biotyping and phage typing. Of these, 314 (48.8%) representative strains of *V.cholerae* 01 biotype Eltor were included for phage typing study (Table). All of the strains except one belonged to serotype ogawa. These strains were grouped under type 2 with conventional scheme of Basu and Mukerjee. Using the new scheme, all these strains were found to be typable and could be clustered into a number of distinct types of which majority were grouped under type 27 (87.2%) followed by type 26 (3.8%), type 24 (2.2%) and type 7 (2.2%) respectively. It was reflected that type 27 was the predominant phage type circulating in our country.

### 5.3. Molecular analysis of *Vibrio cholerae* bacteriophages: cloning and sequencing of phage DNA

Investigators

B.L. Sarkar and M. Chakravorty

This study is being carried out with EITor O1 cholera phage N-4. Efforts have been made to determine the size of the DNA by digesting with different enzymes. Genomic length was calculated by adding the size of different fragments obtained after enzymatic digestion taking into consideration the bands which contained more than one kind of fragments of similar length

(doublet, triplet etc.). The length seems to be approximately 30 kb. In order to construct the physical map of the phage DNA, twenty two restriction enzymes were tested. Unfortunately, out of those 22 enzymes DNA could be digested with only four, Hind III, ACC I, Hae III and EcoRV. Since the phage DNA can not be digested by a large number of enzymes, it may be assumed that the DNA is highly methylated. This however, has posed a serious problem in constructing a physical map of the phage genome. Since, Hind III, the site for which is present in most of the commonly used vectors, could digest the DNA an attempt was made to determine the sites of this enzyme on the phage DNA by digesting the DNA partially. From the pattern of partial digestion, it could be guessed that Hind III A and B fragments are present next to each other. Similarly, Hind III D and E are also contiguous fragments. Further information could not be obtained since majority of the bands are of small size. At present, the only alternative is to carry out random cloning of the Hind III fragments, sequencing small fragments and complete sequencing by “Chromosome walking”.

#### 5.4. Morphological and genomic characterization of vibriophages by electron microscopy

Investigator

A.N. Ghosh

The project was undertaken to characterize different vibriophages used in the phage-typing scheme of *V.cholerae* by electron microscopy.

Negative staining technique of electron microscopy was employed to study the morphology of vibriophages. Magnification of the electron microscope, Philips 420T, was calibrated using beef catalase crystal. Morphology of 15 different phages of *V.cholerae* biotype eltor was determined. Out of these 15 phages, morphology of 13 phages viz. Group I-V of Basu and Mukerjee, N1-N5 and L1, S5, S20 was of similar type. All of them has isometric head and short non-contractile tail. The dimensions of head and tail of these phages were, however, different from one another. Vibriophages D10 and M4 are morphologically distinct from these 13 phages. Both D10 and M4 have isometric heads but the tails are very long and contractile. Also M4 has a much bigger head than D10.

The DNA was isolated from bacteriophage by the phenol-chloroform extraction method. Kleinschmidt's basic protein monolayer technique was employed to study the DNA of vibriophages in the electron microscope. pBR322 DNA was used as an internal marker. The nature and length of the genome of different vibriophages are summarized in Table-6.

Table-6. The nature and length of the genome of different vibriophages.

<b>Bacteriophage</b>	<b>Genome</b>	<b>Length in kb</b>
Group I (eltor)	Double-stranded linear DNA	43
Group II (eltor)	Double-stranded linear DNA	38.5
D10	Double-stranded linear DNA	32
N4	Double-stranded linear DNA	40.4
S20	Double-stranded linear DNA	50

Partial denaturation map of the DNA of group II, D10, N4 and S20 phages was

constructed using alkali and in presence of formaldehyde. It was inferred from the partial denaturation map that DNA of group II, D10 and N4 phage is nonpermuted. Also the presence of cohesive ends in D10 DNA and terminal redundancy in N4 DNA was proved. It was also shown with the help of partial denaturation mapping that during packaging of DNA inside D10 phage head the GC-rich end of the DNA is packaged last which implies that during infection the GC-rich end of the DNA enters first into the host bacterium.

The partial denaturation map of S20 DNA is, however, circularly permuted. Also the permutation is not restricted to any particular segment of the DNA but it is random. Thus although phage S20 is morphologically similar to group II and N4, the genome of S20 is distinctly different from the genome of group II and N4 phage which have nonpermuted DNA.

The length of the DNA of bacteriophage M4 was also determined. However, phenol-extracted DNA was not suitable for the determination of its length as this resulted in much broken DNA. Single-step release method of Kleinschmidt was used for this purpose. In this method osmotic shock is used to rupture the protein coat of bacteriophage and the released DNA is spread simultaneously using a basic protein. As less handling of the DNA is involved more intact DNA is seen with this method particularly if the DNA is very long. The length of the DNA was computed to be 120 kb when compared with the length of pBR322 DNA.

Further studies are in progress.

# STUDIES ON *SHIGELLA* SPECIES

## Section highlights

- o An experiment showed that IFN- $\Gamma$  activated macrophage and fibroblast cells had the capacity to arrest *Shigella* replication and spread as compared to that in nonactivated cells.
- o Study to determine immunoregulatory function of porin of *Shigella dysenteriae* type 1 show that porin mediated release of mediators NO and 1L-I by macrophages is important in view of its possible role in conferring immunity against shigellosis.
- o In view to develop vaccine against shigellosis a study was carried out where a plasmid containing *rfb* and *rfc* genes of *Salmonella typhimurium* had been transferred to an invasive strain of *Shigella dysenteriae* type 1 by triparental cross. Stable transconjugate exhibited cross activity with both *S.dysenteriae* 1 and *S.typhimurium* but did not secrete 60 kDa IPaH protein. A 68 kDa surface layer (S-layer) protein was isolated from *S.dysenteriae* 1 which will be examined for possible role to pathogenicity.
- o Multidrug resistant *Shigella flexneri* was the predominant serotype isolated from Kolkata and its suburbs. 22 plasmids ranging from 3.0 to 105 kb were revealed from these strain but most prevalent was 105 kb (86.7%) 12-14 DNA fragments were identified amongst these strains by PFGE.

## 6. STUDIES ON *SHIGELLA* SPECIES

### 6.1. Antigenic recognition of *Shigella dysenteriae* outer membrane proteins using human convalescent sera and to evaluate their role in cell-mediated immune response in Shigellosis

Name of the investigator

A.K. Sinha

The study was undertaken with two primary objectives, namely, to find out the major immunodominant components after antigenic recognition of the outer membrane proteins (OMPs) from *S.dysenteriae* 1 and to evaluate their role in cell-mediated immunity in shigellosis.

Earlier, we have reported the effect of gamma interferon (IFN- $\gamma$ ) activation of mouse macrophage cell line (J-774) on intracellular shigella survival was evaluated by in-vitro study.  $1.25-1.5 \times 10^6$  CFU of bacteria was used to infect confluent J-774 cells. After 15 min of infection, the bacteria was replaced with DMEM supplemented with FBS (10%) and Gentamycin (50  $\mu$ g/ml). Gentamycin killed the extracellular bacteria only but did not affect the intracellular ones. 100 U/ml of IFN- $\gamma$  was used for activation of the macrophage cell line. It was observed that at the onset of the first hour after infection, and at 15 min intervals thereafter, lower number of intracellular shigella was recovered from cells activated with IFN- $\gamma$  as compared to cells which were not activated. In non-activated cells, a significant increase in the number of intracellular shigella was observed ( $P < 0.05$ ) between 3-5 hrs post infection, demonstrating the ability of shigella to replicate with in these cells. The above observation indicate that IFN- $\gamma$  have some role to eradicate interacellular shigella.

The role of IFN- $\gamma$  in induction of *Shigella* killing by fibroblast (rat, lung L2 cell-line) was evaluated before establishing the IFN- $\gamma$  is essential in host resistance against *Shigella* infection.  $5.0 \times 10^5$  CFU of bacteria was used to infect confluent monolayer of fibroblast cells in petridish (35 mm). After 15 min of centrifugation, the bacterial suspension was replaced with MEM and supplemented with FBS and gentamycin. IFN- $\gamma$  was used to a final concentration of 100 U/ml. Quantitation of viable *Shigella* with in the fibroblast cell lines was carried out after 15 hrs of infection (as *Shigella* is not known to induce apoptosis after infection of fibroblast cells). The formation of plaques by *Shigella* in fibroblast monolayers was then performed.

Cells were assessed for the formation of bacterial plaques sizes at 24 and 48 hrs after infection. It was observed that both the cells types for example, macrophages and fibroblast, activated by IFN- $\gamma$  eradicated the intracellular *Shigella*. while, the non-activated macrophages fostered *Shigella* replication and non-activated fibroblast cell fostered both *Shigella* replication and intracellular spread. The results suggest that IFN- $\gamma$  possess the capacity to arrest *Shigella* replication and spreading to some extent. Further studies are in progress.

## 6.2. Immunoregulatory functions of porin of *Shigella dysenteriae* type 1

Name of the investigator

T. Biswas

The ability of *Shigella dysenteriae* type 1 porin to induce the release of nitric oxide (NO) and interleukin-1 ((IL-1) from peritoneal macrophages of mouse and to regulate lipopolysaccharide (LPS) and gamma interferon (IFN- $\gamma$ ) mediated release of the two proinflammatory mediators was investigated. Porin released nitrite when added to macrophage cultures. A maximum of 3.2-fold nitrite release by macrophages was observed with 100 ng/ml of porin. The nitrite release of LPS was enhanced significantly by lower concentrations of porin, whereas the effect of IFN- $\gamma$  was enhanced by porin at higher concentrations. Polysaccharide (PS) moiety of LPS stimulated the nitrite release of elicited macrophages by 1.6-fold compared to untreated control. It also enhanced the stimulatory effect of 1 and 10 ng/ml of porin by 1.3-fold. Lipid A (LPA) moiety of LPS did not release nitrite, nor did it increase the porin mediated nitrite production. Porin treated 24 h-old macrophage culture supernatants were applied for ConA activated thymocyte proliferation as a measure for determination of IL-1 release. Sixty percent depletion of thymocyte proliferation was observed when the porin treated macrophage supernatants were absorbed with anti-IL-1 antibody. A maximum of 5.5-fold increase of thymocyte proliferation over control was found with 1 and 10 ng/ml of porin. One or 10 ng/ml of porin and LPS augmented the thymocyte growth, 1.5-fold beyond that obtained by porin and 1.8-/1.7-fold more than that obtained by LPS, alone. Similarly, porin and IFN- $\gamma$  co-stimulated the cell growth also. PS enhanced the thymocyte proliferation by 5-fold. It also enhanced the thymocyte growth by co-stimulating 1.4-fold the effect observed by 1 or 10 ng/ml of porin alone. LPA could not participate in the cell proliferating activity nor did it enhance the stimulatory effect of porin. Therefore, both nitrite release and thymocyte proliferation by LPS could be substituted by PS only.

The study of porin mediated release of mediators by macrophages is important in view of its possible role in conferring immunity to shigellosis. The findings raise the possibility that the strongly antigenic porin of *S.dysenteriae* type 1 may prove to be useful as immunostimulator or as adjuvant. However, although NO plays a critical role in effector functions of macrophages such as cytotoxicity towards tumor cells and microorganisms, the releases of NO and IL-1 are among the variety of proinflammatory mediators implicated in the pathogenesis of tissue destruction. This could be correlated with the strong inflammatory reaction of the colonic mucosa during *Shigella* invasion. Therefore, the release of proinflammatory cytokines and not of anti-inflammatory cytokines, may require the suppression of the mediators during protection studies with porin. The tight association of porin and LPS on outer membrane of the bacteria would simply mean to co-signal and trigger a range of host cell populations to synthesize cytokines and thus tell the body that it has been invaded.

### 6.3. Genetic studies on virulence mechanisms of *Shigella dysenteriae* 1 in relation to vaccine development

Investigators

R. Kumar and S. Roy

The most important *Shigella* strain which should be targeted for vaccine development are *S.dysenteriae* 1, *S.flexneri*, *S.sonnei*. A major problem in the assessment of *Shigella* vaccine efficacy is the absence of correlates of protection. The limits of our understanding of the pathogenesis and the immune defense mechanisms against shigellosis have important consequences on the efforts to develop anti-dysentery vaccines. An ideal vaccine should be easy to administer, well tolerated, render long-term protection after a single dose, multivalent, directed against the most representative *Shigella* serotypes causing epidemic and endemic infection. Live attenuated vector strain expressing one or more of the presently known critical surface components such as LPS, a few selected Ipa antigens (IpaBCH) and perhaps the B subunit of the Shiga toxin may be considered for anti-dysentery vaccines. Cell wall is an important structural component of prokaryotic organisms and essential for many aspects of their life. Particularly, the diverse structures of the outermost boundary layers strongly reflect adaptations of organisms for specific ecological and environmental conditions. One of the most common surface structures on bacteria are monomolecular crystalline arrays of proteinaceous subunits termed surface layers or S-layers. S-layers are metabolically expensive products and must provide the organisms with different functions. Chemical analyses of isolated S-layers showed that they are mostly composed of a single glycoprotein species with apparent molecular weights of 40,000 to 200,000. Sometimes S-layers play an important role in pathogenicity.

A plasmid, pPR1347 carrying both the *rfb* gene cluster and the *rfc* gene of *Salmonella typhimurium* has been transferred to an invasive *Shigella dysenteriae* 1 strain by triparental cross. Stable transconjugants (hybrid) exhibited cross reactivity with both *S.dysenteriae* 1 and *S.typhimurium* antisera. A 60 kDa IpaH protein was not secreted into the culture supernatant by the transconjugants. Ligated ileal loops were prepared in rabbit which were inoculated with  $10^8$  CFU/ml each with hybrid and invasive *S.dysenteriae* 1 strains. After 18 hrs, the fluid accumulation (FA) ratio was 0.4 and 1.6 for hybrid and invasive strains of *S.dysenteriae* 1, respectively. Rabbit intestinal mucosa infected with hybrid *S.dysenteriae* 1 strain showed the presence of villi tips and un-ruptured intestinal mucosa whereas total necrosis of intestinal mucosa and villi was observed in *S.dysenteriae* I - infected region.

We have observed that *Shigella* spp. often become non-invasive during prolonged cultivation or laboratory storage at room temperature. However, the plasmid profiles of the invasive and non-invasive strains are unaltered. We would like to examine whether the invasive property of virulent *Shigella* spp. directly or indirectly related with S-layers whose attachment involves components of the outer membrane (e.g. lipopolysaccharides). We have isolated a S-layer negative ( $S^-$ ) mutants of *S.dysenteriae* 1. Tryptic soy agar containing 30  $\mu$ g of congo red/ml was used to differentiate  $S^+$  and  $S^-$  strains. All the  $S^-$  mutants were selected by growth at 30°C. Tryptic soy broth stationary phase cultures were harvested by centrifugation at 4000 x g for 10 min at 0°C and suspended in sterile saline. Indicated amounts of cells ( $10^8$  cell/ml) were incubated in 3 ml of saline containing 15  $\mu$ g of congo red/ml. After 5 min incubation at room temperature, the cells and bound congo red were removed by centrifugation at 27,000 x g for 5 min. The unbound congo red in supernatant fluid was quantified spectrophotometrically at 480 nm. Examination of fresh isolates ( $S^+$ ) and mutants ( $S^-$ ) strains by electron microscope is under progress.

#### 6.4. Molecular characterization of multi-drug resistant *Shigella flexneri* in Calcutta

Investigators

S.K. Niyogi, G.B. Nair, P. Dutta, D. Dutta and S. Yamasaki

Shigellosis is a major public health problem in developing countries. Increased incidence of antibiotic resistance in *Shigella* spp. constitutes a major concern. High frequency of resistance of *Shigella flexneri* to many of the first line antimicrobial agents (multi drug resistant) have been reported in recent years from Calcutta. Most of the conventional typing methods are based on the phenotypic properties of the micro-organisms and offer little strain discriminatory information. The objective of this study is to analyze clonal relationships among isolates of multi-drug resistant *Shigella flexneri* using different molecular typing methods to determine changes at the genetic level and to understand their implications in the epidemiology of the disease.

Presently *Shigella flexneri* is the predominant serotype followed by *Shigella boydii* and *Shigella sonnei* isolated from diarrhoea cases. No *Shigella dysenteriae* was detected. This picture reflecting the endemicity of *Shigella* in this part of the country. *Shigella* strains are resistant cotrimoxazole, tetracycline, ampicillin and furazolidone and sensitive to gentamicin, cefuroxime, nalidixic acid and fluoroquinolones. About 73% of the isolated strains of *Shigella* spp are multidrug resistant. To analyze clonal relationship among isolates of multidrug resistant *Shigella flexneri* strains, molecular typing methods to determine changes at the genetic level and to understand their implications in the epidemiology of the diarrhoea were undertaken.

Plasmid analysis revealed 22 different plasmids ranging in size from 3.0 to 105 kb. The most prevalent was 105 kb plasmid (86.4%). Digestion of chromosomal DNA with the restriction endonuclease XbaI produced clearly resolveable restriction endonuclease analysis pattern (REA) after PFGE. Different REA pattern was identified amongst the isolated strains consisting of 12-14 DNA fragments. *Shigella flexneri* strains originated from different clones.

# STUDIES ON *ESCHERICHIA COLI*

## Section highlights

- o To study the virulence attributes of enteroaggregative *Escherichia coli*, 5 strains isolated from diarrhoea cases and 3 strains isolated from controls were tested which showed that all the strains were negative for LT, ST, VT, *hly*, *eae* genes and also non-invasive to HeLa cells. Strains were agglutinated with human Gr 'O' RBCs. All the strains had identical OMP profile with a major band at 38,000 kDa region and possessed 60 MDa heavy plasmid. These strains caused remarkable rise in intracellular Ca<sup>++</sup> level in HeLa cells.
- o Twenty strains of shiga toxin producing *Escherichia coli* (STEC) were isolated from dairy cattle, beef samples and stool samples at Kolkata. Of these 20 strains, 15 belonged to O157:H7 serotype. All these O157:H7 strains produce shiga toxin and possessed *eae*, *hly* A genes and also possessed *stx1* or *stx2* only or both *stx1* and *stx2* genes. All these strains contain 60 KDa heavy plasmid.

## 7. STUDIES ON *ESCHERICHIA COLI*

### 7.1. Studies to determine the role of enteroaggregative *Escherichia coli* (EAggEC) for causing diarrhoea with reference to its virulence properties

Investigators

S. Dutta, G.B. Nair, M.R. Saha, S.K. Niyogi, P. Dutta

The objective of the study was to study the difference in virulent attributes of EAggEC strains isolated from both diarrhoea cases and control children in a hospital based case control study conducted in the year 1996-97. Five sole isolates of EAggEC from cases of acute diarrhea and three isolates from controls were subjected to test for production of conventional toxins, haemolysin, haemagglutinin, secretary protein profiles and outer membrane protein profiles respectively. Plasmid profiles of those isolates were also tested. Attempts were made to compare the results of these two groups of isolates. It was seen that all identified EaggEC strains showed negative result, when tested for LT, ST, VT genes by PCR method. Salt aggregation tests (SAT) performed with all strains using various molar concentration of ammonium sulphate. Almost all strains showed agglutination (++++) with 1.0(M) and lower conc. of ammonium sulphate, indicating their adhesive properties. Almost 60% strains were positive for EAST. All strains were negative for *hly* and *eae* genes. All strains were found noninvasive, when tested by Hela cell invasion assay. Almost 80% strains showed mannose resistant haemagglutinin activity with human Gr "O" RBCs. Total secreted protein content and outer membrane protein content of the strains were examined by running SDS-PAGE. The results were compared and analysed. Surprisingly it was observed that strains both from cases and controls had identical OMP profile with major band at 38000 K Da region. The strains were also tested for their plasmid profiles and plasmid encoded toxin (PET) by available probes and primers. All strains were found to possess a 60 MDa heavy plasmid, which was reportedly responsible for their adherence properties. Further studies are in progress.

### 7.2. Search for Shiga toxin producing *Escherichia coli* including O157: H7 strains in animals, animal products and hospitalised acute diarrhea cases in Calcutta

Investigators

S. Dutta, S. Ghosh, P.G. Sengupta, P. Dutta

The objectives of the study were to determine if dairy cattle are potential sources of shiga toxin producing *Escherichia coli* (STEC) including O157: H7 serotype and to ascertain the presence of STEC in foods (beef, pork, milk) marketed in India and in animal handlers residing in Calcutta. Investigations were carried out to determine the role of STEC in the causation of acute diarrhoea among hospitalized children. Attempts would be made to study the relatedness of the isolated STEC strains including O157: H7 by performing antibiotic resistance pattern, plasmid profiles and various molecular typing.

Twenty strains of STEC were isolated from dairy cattle and beef samples and clinical samples screened in this study from Calcutta. Clinical samples yielded only three STEC isolates. Of 20 strains 15 belonged to O157:H7 serotype. All O157: H7 strains were multidrug resistant and possessed important virulent factors, which included shiga toxin production and the presence

of *eae* and *hlyA* genes. Out of 20 strains 13 strains possessed Stx1 gene whereas only 1 strain showed presence of Stx2 genes, only 3 strains showed presence of both Stx1 and Stx2 genes and three strains did not have any of the toxin genes. When tested for the plasmid profiles, it was observed that all strains had a 60 MDa heavy plasmid, but overall there was no uniformity among the strains with respect to their plasmid profiles. Bacterial DNA was extracted from all O157: H7 strains, the DNA was digested with Hind III and Eco RI restriction enzymes and electrophoresed onto 1% agarose. The digested fragments were analysed. The electrophoretically separated fragments were blotted onto nylon membrane, DNA was fixed by baking at 80°C and kept at 4°C for southern hybridisation using Stx1 and Stx2 probes for RFLP typing. Attempts have been made to perform PFGE typing of the STEC strains. The PFGE profiles of the strains showed not much similarity among the strains. Further studies are in progress.

### 7.3. Studies on the binding of *Escherichia coli* heat-stable enterotoxin to the intestinal epithelial cells and brush border membranes of different animals

Investigators

M.K. Chakrabarti, A. Pal, and K.M. Hoque

The principal objective of this study was to purify and characterize the receptor for *E. coli* STa from a high density receptor system. Initially, STa was purified to homogeneity and receptor assay was done with iodinated STa. We reported earlier that binding of  $^{125}\text{I}$ -STa to the brush border membranes of rat, rabbit (*J. Diarr. Dis. Res.* 17, 28-33, 1999), hamster and guineapig (*Indian J. Med. Res.*, 113, 5-10, 2001) was specific, time and temperature dependent. A single class of receptors were present in all the tested animals and the number of receptors remained lower in hamster in comparison to rat, rabbit and guineapig. Autoradiographic demonstration of SDS-PAGE of intestinal brush border membranes showed STa binding proteins of apparent MW of 160 KDa in rat 118 KDa in guineapig, 140 and 38 KDa in rabbit and 65 KDa in hamster. We also reported that STa binds to a single class of receptors in COLO-205 human colonic carcinoma cell. Binding was specific, time and temperature dependent. STa binding protein with MW of 95 KDa was detected in this cell line (*FEMS Microbiol. Lett* 156, 79-83, 1997). STa was found to stimulate G-cyclase in COLO-205. It has been found that besides stimulating cGMP STa also involves two potential intracellular signal. It increases rapidly inositol triphosphate and cytosolic free calcium in COLO-205 cells prelabelled with myo[2- $^3\text{H}$ ] inositol resulted in a rapid rise of [ $^3\text{H}$ ] inositol triphosphate. Using fluorescent indicator, Fura 2AM, intracellular free  $\text{Ca}^{2+}$  has been found to increase 5.12 fold compared to control. Suspension of cells in calcium was chelated with EGTA. This effect was not observed with cells that were pretreated with dantrolene which suggest that the intracellular calcium rise might be due to mobilization of intracellular stores. This study demonstrated for the first time a change in cytosolic calcium in cultured human colonic cell by STa, which was accompanied by inositol tri-phosphate activation (*Biochim. Biophys. Acta*, 1403, 1-4, 1998). The involvement of protein kinase C (PKC) in the mechanism of action of STa in COLO 205 had also been shown.

During the reported period, the effect of STa in the translocation of PKC and the role of enterotoxin-induced  $[\text{Ca}^{2+}]_i$  in this translocation has been examined. To prepare cytosolic and membrane-bound PKC, COLO 205 cells were treated with or without STa and incubated at 37°C for 1 min. The reactions were stopped by addition of ice-cold BSS. Cells were washed twice, suspended in 20 mM Tris-HCl (pH 7.5) and homogenized. The homogenate was centrifuged at 100,000 x g for 1 hr and supernatant served as the source of cytosolic PKC. The pellet was then

resuspended in 20 mM Tris-HCl (pH 7.5) and homogenized briefly in presence of 1% Triton X-100, recentrifuged and resulting supernatant served as the source of membrane bound PKC. STa action on the PKC activity both in cytosol and membrane fraction was determined and it was found that most of the PKC activity in the cytosolic fraction was found at '0' time period; thereafter the enzyme activity was decreased within 1.5 min of enterotoxin treatment. During this time period, the enzyme activity was increased from  $40 \pm 2$  nM/mg protein/min to  $230 \pm 10$  nM/mg protein/min in the membrane fraction when STa-induced cytosolic rise of  $[Ca^{2+}]_i$  also reached to its maximum ( $412 \pm 5$  nM). To correlate the enhanced PKC activity with the initial rise of  $[Ca^{2+}]_i$ , the cells were preincubated in Dantrolene, a drug which inhibits the mobilization of  $Ca^{2+}$  from  $IP_3$ -sensitive intracellular stores, and then the activity of membrane-bound PKC was measured. A significant inhibition (about 60%) of kinase C activity was found ( $230 \pm 10$  nm/mg protein/min vs  $92 \pm 5$  nM/mg protein/min). However, the basal enzyme activity was not altered by Dantrolene. So, the present study has shown, STa treatment causes translocation of PKC from cytosol to membrane fraction of COLO 205 in a  $Ca^{2+}$  dependent manner.

## STUDIES ON OTHER BACTERIAL PATHOGENS

### Section highlights

- o Forty eight *Salmonella typhi* strains isolated from Kolkata during 1990-2000 showed ribotype 1 (16 strains), ribotype 2 (10 strains), ribotype 3 (6 strains) and other 16 strains were heterogenous ribotypes. Eight biotypes (A to H) were also observed among these strains with majority belonged to biotype A (30 strains) and biotype B (8 strains).
- o A study is continuing to establish the mode of action of *Yersinia enterocolitica* heat stable toxin (YSTa) in rat intestinal epithelial cell.
- o Search for pathogenicity of diarrhoeagenic strains of *Klebsiella pneumoniae* showed that aggregative adherence in HeLa cells and this adherence leads to influx of extracellular  $\text{Ca}^{2+}$ . Unrestricted passage of calcium ions across cell membrane could cause phosphorylation of proteins involved in ion transport across the membrane which could result in secretory diarrhoea.
- o One hundred twenty biopsy specimen taken from the antrum of the stomach of the patients suffering from gastritis, gastric ulcer and non-ulcer dyspepsia were processed for histopathological examination. Fifty five *Helicobacter pylori* strains, isolated from these specimens, were genotyped.

## 8. STUDIES ON OTHER BACTERIAL PATHOGENS

### 8.1. Differentiation of *Salmonella typhi* by molecular methods

Investigators

M.R. Saha, T. Ramamurthy, G.B. Nair and P. Dutta

Several epidemics of typhoid fever caused by multi-drug resistant strains of *S.typhi* have been reported from different parts of the world including India from time to time. In recent years, there have been a number of molecular methods developed to detect plasmid and chromosomal DNA. The present study was undertaken to determine spatial and temporal variation of clonal typing in Calcutta with the retrospective strains of *S.typhi* isolated since 1990 and strains of *S.typhi* of recent origin by molecular analysis.

Initially, 48 *S.typhi* isolates during the period of 1990 to 2000 showing different antimicrobial resistant patterns and phage types were included. The strains were studied by ribotyping according to the method of Altwegg et al. 1989. The restriction enzyme Pst 1 and Spe 1 used in this study were commercially obtained from (Boehringer Mannheim, USA). The strains for ribotypes following digestion by Pst 1, were separated into 48 different ribotypes. The 32 strains fell into three classes of ribotypes; most strains ribotype 1 or ribotype 2 or ribotype 3. Among the whole sets of 48 strains, 16 strains were of ribotype 1, 10 were of ribotype 2, 6 were of ribotype 3. The remaining 16 strains were of heterogenous ribotypes.

Besides the above, a biotyping system was undertaken to evaluate the ability of the strains to ferment five different sugars (dulcitol, cellobiose, D-xylose, arabinose and glycerol) and decarboxylate L-arginine. Eight biotypes (A to H) were observed among the 48 *S.typhi* strains, with biotype A (n=30) and biotype B (n=8). Both the biotypes were characterized by the ability to decarboxylate L-arginine and to utilize glycerol for biotype A and by the ability to utilize cellobiose for biotype B. Biotype C (n=4) were unable to utilize glycerol. Biotype D (n=2) strains were similar to biotype A strains except that they did not utilize glycerol and were able to ferment arabinose. Biotype E through H (n=1 each) comprised the rest of the observed profiles. The results suggest that the Calcutta epidemic was produced by multiple sources of infection. The study is in progress.

### 8.2 Regulation of expression of bacteriophage MB78 genes : A molecular approach

Investigators

M. Chakravorty, P. Dutta, P. Mallick and A.N. Ghosh

#### **Temperature sensitive mutation in the 38 kDa minor structural protein of phage MB78 results in formation of tail fiber-less particles.**

Bacteriophage MB78 isolated in our laboratory is a virulent phage of *Salmonella typhimurium*. It contains a linear, double stranded, circularly permuted and terminally redundant DNA which is 42 kb long. The DNA replicates as concatamer which is subsequently converted to full length phage DNA through headful packaging mechanism. A number of promoter containing fragments have been cloned and two strong promoters have been studied in details. A genomic

library of the phage was also constructed in M13mp11 using SalI and HindIII restriction enzymes and some of the fragments have been studied extensively. Although locations of a number of genes (ORFs) on the physical map of the genome have been determined, their functions are yet to be ascertained. Hence, conditional lethal mutants were isolated and attempt was made to determine their physical locations on the genome and the functions affected due to such mutation. Six temperature sensitive mutants were isolated. Out of those only three had single mutation as confirmed by complementation test. To determine the physical location of the temperature sensitive mutation on the phage genome the following strategy was adopted. The mutant phages were plated on LB5000 ( $r^m^+$  strain of *Salmonella typhimurium*) transformed with pUC19 carrying different genomic fragments of MB78. A large number of genomic fragments were tested. Out of the three mutants only the mutant MB78ts23 produced plaques at 40°C (non-permissive temperature) on host carrying SalIG fragment.

The SalIG fragment, a 2.047 kb fragment of bacteriophage MB78 DNA, located almost at the middle of the genome expresses a protein which moves as a 38 kDa protein in SDS-PAGE. The open reading frame of this protein has been identified. It codes for a protein with 329 amino acids. The ORF suggests that the predicted molecular weight of the protein is 36.5 kDa. The Shine-Dalgarno (SD) sequence that could be identified is only GAA lying nine nucleotides upstream of the initiation codon ATG. Down stream of the first initiation codon there is another ATG codon which is in the same reading frame. So, it is difficult to ascertain which ATG is the actual initiation codon. The gene for the 38 kDa protein (g38) could be identified as a gene for a minor structural protein of the phage which is involved in morphogenesis of the phage particles (attachment of the tail fibers to the capsid). Nucleotide sequence analysis of the mutant gene revealed that in the temperature sensitive mutant MB78ts23, the sequence GCA has been altered to GTA. As a result valine is incorporated in place of alanine. A change in 75th amino acid from alanine to valine makes the protein temperature sensitive. Electron microscopy of particles produced at non permissive temperature indicated that the particles formed are tail fiber less. Two types of particles are seen : (i) Empty capsids which are not perfect icosahedral, (ii) icosahedral particles filled with DNA (Fig 1a and b).

### **Sequencing of the phage genome**

One of the objectives of our work is to complete sequencing of the phage genome. In course of the work it was realised that some parts of the genome can not be cloned may be due to presence of lethal gene(s). Hence, sequencing was standardised using total phage DNA and custom made primers. This method provided an advantage. It solved the orientation problem of the sequences determined. Orientation of the sequence of HindIII and SalIG could be determined with the help of sequence obtained using total genome. During the last one year more than 3 kb sequence could be determined.

### **8.3. Mode of action of *Yersinia enterocolitica* heat stable enterotoxin (YSTa) in rat intestinal epithelial cell**

Investigators

M.K. Chakrabarti and A. Pal

The principal objective of this study was to evaluate the mechanism of action of heat stable enterotoxin secreted by *Yersinia enterocolitica*. For this study five enterotoxigenic strains of *Yersinia enterocolitica* were taken from the Microbiology Division of this Institute. At the initial stage of this study, attempts were made to purify YSTa. Culture filtrates of all these strains were checked for their toxin producing ability. Cells were grown in 50 ml Casamino Acid Yeast Extract (CAYE) media supplemented with glucose, pH 8.5 at room temperature for 48 hrs. YSTa activity was then assayed in suckling mice. 0.1 ml of culture filtrate was administered by gastric tubes into the stomach of 2-3 days old suckling mice with 0.01% Evan's Blue dye as marker. Mice were kept for three hrs and were then sacrificed. A ratio of intestine weight to body weight of 0.08 was considered to be a positive response. The strain, which showed higher toxin production, was used for further purification studies. The bacteria were grown in 1 L of CAYE media at room temperature for 48 hrs. Culture was centrifuged and supernatant was then passed through membrane filter. It was then fractionated by ammonium sulfate precipitation (80% saturation). After centrifugation at 15,000 x g for 30 min at 4°C the pellets were resolved in a buffer containing 0.1 M NaCl, 10 mM Tris. HCl, pH 7.2 (A) and dialysed against the same buffer. The dialysate was then applied to a DEAE sephacel column equilibrated with buffer A. The column was washed with buffer A and eluted with a linear gradient from 0.1 to 0.5 M NaCl. An aliquot of each fraction was tested for enterotoxicity in the suckling mice. Fraction was then concentrated for further purification.

### **8.4. Search for virulence traits and determination of the mechanism of pathogenicity of *Klebsiella pneumoniae* isolated from childhood diarrhoea cases**

Investigators

S.K. Niyogi, G.B. Nair, S. Dutta and A. Pal

The project was undertaken with the objective to determine if *Klebsiella pneumoniae* produce any known enteric toxin(s). *K.pneumoniae* strains isolated as a sole pathogen from acute diarrhoea cases admitted to the Dr. B.C. Roy Memorial Hospital for Children, Calcutta were included in this study.

Previously, we reported a number of *K.pneumoniae* strains exhibited aggregative adherence that was distinct from the stacked brick enteroaggregative pattern shown by *Escherichia coli*.

We studied to determine the involvement of calcium in the pathogenesis of aggregative *K.pneumoniae* strains.

Infection of cultured HeLa cells with aggregative *K.pneumoniae* strains resulted in five-fold elevation of intracellular level of free calcium ( $\text{Ca}^{2+}$ ) with maximum  $\text{Ca}^{2+}$  influx at 3 h after bacterial infection. Chelation of extracellular  $\text{Ca}^{2+}$  with [ethylenebis (oxyethylenitrile)] tetra-

acetic acid and suspension of cells in  $\text{Ca}^{2+}$  free buffer suggested that the rise of  $\text{Ca}^{2+}$  in aggregative *K.pneumoniae* infected HeLa cells was due to influx of  $\text{Ca}^{2+}$  from extracellular medium.

This study showed aggregative adherence in HeLa cells and this adherence leads to influx of extracellular  $\text{Ca}^{2+}$ . The unrestricted passage of calcium ions across cell membrane could cause phosphorylation of proteins involved in ion transport across the membrane, which could result in secretory diarrhoea.

### **8.5. Correlation of histology with genotypes of *Helicobacter pylori* isolated from cases of peptic ulcer, non-ulcer dyspepsia, gastric carcinoma and lymphoma**

Names of the investigators

D.R. Saha, S. Datta, A. Chowdhury and G.B. Nair

*Helicobacter pylori* chronically infects the gastric mucosa of most people in developing countries including India, and constitutes a major cause of gastric and duodenal ulceration, distal gastric adenocarcinoma and gastric mucosa associated lymphoid tissue (MALT) lymphoma. However, in most *H.pylori*-infected persons, colonization of gastric mucosa and the associated inflammation are tolerated for decades without causing clinical symptoms and serious gastroduodenal illness occurs in only some 10-20% of infected individuals. The heterogeneity of the clinical outcome of *H.pylori* infection is probably related to the genotypes of individual *H.pylori* strains, the characteristics of individual hosts, and environmental factors including diet, stress and history of other infections.

The present study was undertaken to determine the association and tissue response to *H.pylori* infection in different diseased conditions and to correlate the histopathological changes with the virulence determinants of *H. pylori* in order to determine the most probable bacterial genotype associated with disease outcome. Biopsy samples for *H.pylori* culture and histological examinations were collected from the antrum and body of the stomachs of patients with gastritis, ulceration and non-ulcer dyspepsia, undergoing endoscopy at S. S. K. M. Hospital, Calcutta. Biopsies for histopathological examinations were fixed in buffered 4% formalin while those for culture were transported to the laboratory in Brucella broth containing 20% glycerol. Formalin treated tissues were processed for paraffin embedding, followed by cutting of thin sections, and staining them with haematoxyline and eosine to see the histological changes by light microscopy. Modified Giemsa stain was done to detect *H.pylori*. The bacteria was cultured using standard methods which entails smearing of single biopsy specimens on petri plates containing brain heart infusion (BHI) agar supplemented with 7% sheep blood, 0.4% IsoVitaleX, amphotericin B (8 µg/ml), trimethoprim (5 µg/ml), and vancomycin (6 µg/ml) and were incubated at 37°C in an atmosphere of 5% O<sub>2</sub>-10% CO<sub>2</sub>-85% N<sub>2</sub> for 3 to 6 days. Chromosomal DNA was prepared by the CTAB (hexadecyltrimethyl ammonium bromide) extraction method from confluent plate cultures. To determine the presence of virulence genes namely *cagA*, alleles of *vacA* and *iceA*, specific PCR was carried out in 20-µl volumes using 10 ng of DNA, 1 U of *Taq* polymerase, 10 pmol of each primer per reaction and 0.25 mM (each) deoxynucleoside triphosphate in standard PCR buffer for 30 cycles generally under the following conditions: 94°C for 40 s, 55°C for 40 s, and 72°C for a time chosen based on the size of the expected fragment (1 min/kb). The present study is in progress and so far one hundred twenty samples have been processed for histological examinations and fifty five *H.pylori* strains were cultured and their

genotypes determined. Study is in progress.

## STUDIES ON *E.HISTOLYTICA*

### Section highlights

- o Electron dense granules (EDGs), which are produced during the incubation of pathogenic *E.histolytica* with human collagen type I and  $\text{Ca}^{2+}$  were analyzed and showed that they are a complex of mainly cationic proteins, which contain numerous proteolytic activities. In order to amplify the genes encoding for collagenase of *E.histolytica* different oligoes were designed and after amplification few sequences were found to be new.
  
- o Using RNAs of 2 hr from collagen-activated and non-activated *E.histolytica* three differentially expressed bands (400-500 bp) were observed in collagen activated *E.histolytica* trophozoites compared to non-activated *E.histolytica*. The northern blot analysis using labeled cDNA probes, recognized three mRNAs in non-activated cells and of those three mRNAs two were absent in activated cells and one mRNA was over-expressed in activated cells. The sequencing of the bands are under progress.

## 9. STUDIES ON *E.HISTOLYTICA*

### 9.1. Studies on electron dense granules of *E.histolytica* containing collagenase activity

Investigators

P. Mukhopadhyay, A. Debnath, S. Sengupta, A. Akbar and P. Das

During the course of invasive intestinal amoebiasis, *E.histolytica* actively penetrates the mucosa of host intestine. But the exact mechanism and the factors by which parasite destroys the human tissue are still unclear. In recent years some progresses have been made on the molecular mechanism of pathogenesis. The major objective of the present study was to characterize the electron dense granules of *E.histolytica* in greater detail and clone the genes coding for these proteins.

Earlier, we characterized the electron dense granules (EDG) produced during the incubation of pathogenic (HM1:IMSS or HM2) *E.histolytica* with human collagen type I (purified from human placenta) and  $\text{Ca}^{2+}$ . Analysis of EDG showed 8 fold more collagenase activity than whole trophozoites. Chemical and elemental analysis of purified EDGs showed presence of inorganic phosphate, pyrophosphate and Na, Mg, S, Cl, K, Ca and Fe as measured by Scanning transmission electron microscopy. In SDS PAGE six polypeptides with apparent molecular weights of 108, 106, 104, 97, 68 and 59 kDa were found exclusively in EDG preparation. Similarly, two protease activities with apparent molecular weights of 40 and 85 kDa were detected only in EDGs.

During the year purified EDG was used in raising antibodies in rabbit. This antibody was used in immunoscreening of the cDNA library constructed from pathogenic *E. histolytica*. Inserted DNA has been released from ten plaques and they show approximate 750 bp to 1 kb. Sequencing and characterization of the inserts are under progress.

Attempts were also made to amplify the genes encoding for collagenase of *E.histolytica*. After computer search, two oligos were designed from most conserved sequences of collagenase genes of other organisms for PCR. Using *E. histolytica* genomic DNA as template and oligos, four bands (TE51, TE52, TE53, TE54) were amplified by PCR. The largest fragment (TE51) containing 585 bp has been sequenced and homology analysis shows its homology with *E. histolytica* gene (Ehvmal) encoding the catalytic peptide of a putative vacuolar proton-transporting ATPase (V-ATPase) (Accn. No. U04849). In dot blot (Fig. 1) and Southern hybridization, the fragment (585 bp) recognizes only *E. histolytica* genomic DNA and not other enteric pathogens. The second larger amplicon containing 485 bp has been sequenced and homology analysis shows its homology with *E. histolytica* chaperonin-containing TCP-1 gene, zeta subunit, (Accn. No. AF293981). In dot blot and Southern hybridization, the fragment (485 bp) recognizes only *E. histolytica* genomic DNA and not other enteric pathogens. Characterizations of other two fragments are under progress.

### 9.2. Studies on multiple genes in *Entamoeba histolytica* during human collagen type I

## and Ca<sup>2+</sup> interaction : Use of mRNA differential display

### Investigators

A. Debnath, P. Mukhopadhyay, S. Sengupta, A. Akbar and P. Das

Amoebiasis, a parasitic infection in man due to the protozoan *Entamoeba histolytica*, is an invasive enteric illness that can spread to multiple tissues, particularly mucosa and submucosa of host intestine. Because, collagen is major component of the extracellular matrix and basal lamina of human intestine, it is thought that collagenase which was detected in pathogenic *E.histolytica* is one of the important factors of tissue lysis during invasive amoebiasis. Till date no study has been conducted about the expression of collagenase genes in *E.histolytica*. In this study attempts were made to clone and sequence some of the genes that are differentially expressed during activation with human collagen type I and Ca<sup>2+</sup> and compare homology analysis with the sequences in Gen Bank.

Using RNAs of 2 hr from activated and non-activated *E.histolytica* and T<sub>12</sub>VA & PD1 and T<sub>12</sub>VA & PD2 primers, four differentially expressed bands were reproducibly observed in collagen activated *E.histolytica* trophozoites compared to non-activated *E.histolytica* (Fig. 1). These four bands were successfully excised from the sequencing gel and reamplified. Each reamplified differentially expressed band showed single band in the agarose gel in the range of 400-500 bp (Fig.2). In dot blot analysis one band showed overexpression in activated cells and this was further confirmed by northern blot analysis (Fig. 3). Of the three bands, two showed overexpression in northern blot analysis (Fig. 4&5). One band failed to show any overexpression in northern blot analysis (Fig. 6). This may be due to false reaction (artifact) or incorrect alignment of the film on the dried gel. The northern blot analysis using labeled cDNA probes, recognized three mRNAs (2.5 kb, 1.5 kb and 400 bp) in non-activated cells and of those three mRNAs two (2.5 kb and 1.5 kb) were absent in activated cells and the 400 bp mRNA was overexpressed (about 2-4 fold) in activated cells. Although single cDNA band should recognize single mRNA transcript the presence of more than one transcript in non-activated cells may be due to the presence of multiple cDNA fragments in a single cDNA band or may be due to the presence of closely related gene family or one single ORF may produce several mRNAs (post-transcriptional processing). To sort out the problem, first cloning of the PCR products and then northern with those cloned products, in combination with sequencing are under progress.

# STUDIES ON ROTAVIRUS

## Section highlights

- o The in-depth study of genomic diversity of rotaviruses revealed several variants of the G-serotype specific, outer capsid glycoprotein [G1,G2,G4,G6,G8,G9,G10 respectively], the P-genotype specific, outer capsid protein (P[8], P[4] & P[6] respectively), the non-reactive type of Group A specific, inner capsid antigen among human rotaviruses in Calcutta and the occurrence of reassortment in nature between porcine rotavirus genes and those of human rotaviruses in Manipur.
- o A combined reverse transcriptase-polymerase chain reaction (RT/PCR) was used to produce cDNA of VP7 gene of rotavirus present in the stool samples. Multiplex PCR using the type specific primers revealed the presence of G1 (49/150, 32.7%), G2 (27/150, 18%) and G4 (30/150, 20%) genotypes, 18 samples (12%) were of mixed genotype and the remaining 16 samples (10.6%) could not be typed. Comparative analysis of the full length genes of the representative strains with corresponding genotypes incorporated in the human-rhesus rotavirus tetravalent vaccine (RRV-IV) formulation demonstrates that the circulating G1, G2, and G4 genotypes do not exactly match with the vaccine strains, d, DS1, and ST3 respectively.

## 10. STUDIES ON ROTAVIRUS

### 10.1. Molecular characterization of the VP7 genes of rotavirus isolated from the clinical samples, Calcutta, India.

Investigators

S. Chakrabarti, D. Khetawat, P. Dutta

The gene coding for outer capsid protein of VP7, of rotaviruses, detected among children suffering from diarrhoea in the eastern part of India, was studied. Fecal RNAs, isolated from stool samples, were analysed for the presence of different VP7 genotypes by multiplex PCR. Full length gene, coding for VP7, was synthesized by combined reverse transcriptase-polymerase chain reaction and was used as a template for multiplex PCR. It was found that G1, G2 and G4 were the predominant strains present. The full length VP7 gene of one representative from each genotype was cloned by direct ligation into TA Cloning vector, PCR TM 2.1 (Invitrogen, USA). The clones were characterized by digestion with different restriction enzymes. For sequencing, WD33 (G1) cDNA was digested with restriction enzymes EcoRV, EcoRI, HincII, BamHI and each of the fragments were cloned into pUC18 vector. All the subclones were sequenced by Sanger's dideoxy method using the Sequenase Version 2.0 (United States Biochemical Co., USA). The overlapping regions were sequenced across and the complete nucleotide sequence of the VP7 gene of WD33 strain was obtained. The G2 (SC4) and the G4 (SC134) clones were sequenced by ABI PRISM method, in the ABI PRISM 310 Genetic Analyzer, using the M13 forward and reverse primers. The nucleotide sequence data reported have been submitted to EMBL databases and have been assigned the accession number Y18786 (WD33), AJ293718 (SC4), and AJ278217 (SC134).

A phylogenetic tree was constructed with the amino acid sequences of the known strains of G1, G2 and G4 all over the world, along with the vaccine strains of the respective genotypes, incorporated into the rhesus rotavirus tetravalent (RRV-TV) vaccine (Fig.1).

Previously, Jin *et. al* performed a comparative analysis of the deduced amino acid sequences from VP7 genes of 41 G1 rotaviruses from 12 countries and showed the existence of four distinct 'lineages' (I-IV) based on inter strain deduced amino acid homologies and patterns of amino acid substitutions. The VP7 protein of each lineage possessed distinctive amino acid residues that were found in most members of the same lineage at most positions, but were not present in most other strains, e.g., lineage I (Ile-57, Ser-68, Thr-217 and Ile-281), lineage II (Ser-38, Ala-68, Glu-97), lineage III (Ala-65, Thr-68, Glu-75, Val-76, and Ser-147), and lineage IV (Val-16, Thr-43, Thr-101, Ile-193, Tyr-235, Asp-278, Ile-326). It was found by similar analysis that the deduced amino acid sequence of the VP7 gene of WD33 did not have amino acid profile characteristic of any particular lineage. More interestingly, this strain was found to be closely clustered with the Egyptian strain, egypt-7, although the two strains differed from each other as revealed by the distance separating them (Fig.1A).

Phylogenetic analysis of the G2 strains revealed two clusters (Fig.1B). Geographical clustering was very much apparent from the phylogenetic tree. The Manipur strain clustered with the Calcutta strain, though they were isolated ten years apart (1987/88 and 1997/98 respectively). The Australian strains of 1994 clustered with the South-East Asian strains of Taiwan, Pakistan and India, but, that of 1992 and 1995 were present in a separate cluster with the Chinese and the

Japanese strains. Similarly, the Australian strains formed a separate cluster. The most important finding from the tree was the distance of the G2 vaccine strain, DS-1, from the Indian strains. DS-1, whose VP7 is incorporated into the tetravalent rhesus rotavirus vaccine formulation, seemed to be dissimilar to the circulating G2 rotavirus from India. The G2 VP7 (derived from the strain DS1) in the vaccine formulation exhibited sequence variation in at least one of the three neutralization epitope regions when compared with other G2 viruses. The local strain SC134 clustered with the Italian strain, PV5249 and the South African strain, GR846/86, but, not with the vaccine strain ST3 (Fig.1C).

From our study, it is clear that the current vaccine strains composed of the different genotypes are not similar to the circulating strains. The phylogenetic analysis clearly demonstrates the variation, albeit, only at the genetic level. The representative strains of all the three prevalent genotypes, namely, G1, G2 and G4, were clearly distinct from the vaccine strains, D, DS1, and ST3 respectively.

## 10.2. Detection and molecular characterization of rotaviruses

### Investigators

T.N. Naik, T. Krishnan, S. Das, V. Varghese, A. Sen.

A total of 159 samples which were available in adequate quantity were selected for study to determine their G-and P-types depending on the characteristics of VP7 and VP4 gene. The G-typing analysis showed that G1 (39.7%) was the most predominant genotype followed by G2 (23%) and G4 (12%) respectively, besides some mixed G-types (7.4%). However, no G3 rotavirus was detected during this study period and in 29 cases (17.8%) the G-types were untypeable, although most of the G-type specific primers (G1-4, G6, G8-12) were used in multiplex PCR. The P-typing was also done using P[4], P[6], P[8] and P[9] specific primers and analysis showed that, the most predominant P-type was P[8] (36%) followed by P[4] (25.7%) and P[6] (5.5%). A number of samples with multiple rotavirus infections with different P-types were seen in 19% of the samples and 12% of the specimens remained untypeable during this study period. The G1P[8] and G2P[4] genotypes were the most prevalent ones. The detection of G1P[4](4%), G2P[8](2.5%), G2P[6](0.6%), G4P[4](2.5%) and G4P[6](1.25%) rotavirus strains are very interesting as they are rarely been detected in human being.

Another interesting finding was the isolation of the non-reactive strains (9.5%) in ELISA for the study of VP6 (inner capsid) protein. These samples did not show any reaction towards either group/subgroup or polyclonal antisera, although they all showed characteristic group A rotavirus specific RNA migration pattern in polyacrylamide gel electrophoresis. The amplification of some structural genes (VP4, VP6, VP7) and non-structural genes (NSP1, NSP4, NSP5) were carried out using the group A rotavirus specific primers. We have successfully cloned all the PCR products in pCR 2.1 vectors and sequenced some of the genes. The phylogenetic analysis (in CLUSTAL X programme) of the sequence data revealed that the VP6 gene of the strain showed maximum homology with a SGI strain US1205 (G9P2[6]), which is very interesting. Cluster alignment showed that seven amino acids were different between these two strains suggesting that the changed amino acid must have vital role in the structural conformation of VP6 protein and for binding group/subgroup specific monoclonal and polyclonal antibody. The secondary structure of RNA and the hydrophobicity plot of these two strains were also found to be different using DNASIS software programme.

Some group A rotavirus positive in 1989 outbreak samples from Manipur were further characterized recently. We have selected some long electropherotypes isolates of SGI specificity, which are common in animal but not in human. Typing of VP4 and VP7 gene showed a very high percentage of mixed infections among those samples. Uncommon human G-types like G6, G8, G9 and G10 was also detected as part of multiple infections along with common G-types (G1-G4). The predominant P-type was P[4] followed by P[8] and P[6]. A few non-structural genes of two Manipur isolates were also sequenced and analyzed. NSP5 gene of RMC100 and NSP4 gene of RMC321 showed maximum homology to porcine strains, where NSP4 of RMC100 and NSP5 of RMC321 showed maximum homology towards human strains suggesting occurrence of reassortment between human and animal strains in nature. Moreover, association of such strains in epidemic of rotavirus diarrhoea will be extremely difficult to control with present day vaccine.

# INVESTIGATIONS OF OUTBREAKS OF DIARRHOEAL DISEASES

## Section highlights

Teams from this Institute investigated the following outbreaks of diarrhoeal diseases in different areas:

- o A localised outbreak caused by *Vibrio cholerae* O1 occurred during 4-18 April, 2000 in the brick field area of Akra Fatak, Kolkata.
- o An explosive outbreak caused by *Vibrio cholerae* O139 observed during 28th September to 12th October, 2000 at Patuli, Kolkata.
- o An epidemic caused by *V.cholerae* O1 and O139 with concomitant infection of enterotoxigenic *E.coli* occurred during the month of January, 2001 in Amhedabad, Gujrat.
- o A team visited to Bhuj, Gujrat after devastating earthquake to investigate any impending diarrhoeal disease outbreak.
- o Team visited Orissa to investigate the possible risk of developing epidemic of diarrhoeal diseases.

## **11. INVESTIGATIONS OF OUTBREAKS OF DIARRHOEAL DISEASES**

### **11.1. Investigation of outbreak of acute diarrhea in the brickfield area of Akra Fatak Calcutta**

Investigators

P. Dutta, P.G. Sengupta, D.N. Gupta, S.K. Mondal, S. Ghosh, G.N. Dey

A localised outbreak of acute diarrhea occurred between 4-18 April 2000 amongst migrant labourers working in the brick field area No. 23 and 8 of Akra Fatak Calcutta. An investigation was carried out in the area by house-to-house survey to find out the magnitude of the problem, to detect the domiciliary diarrhoea cases, treatment received and to determine the source and mode of transmission of the disease.

A total of 71 persons were affected. Attack rate was 28.9%. Highest attack rate (43.9%) was noted amongst children below 5 years of age. 24 cases(33.8%)were admitted to ID Hospital Calcutta. The single death, which occurred at the ID hospital Calcutta, was a 7 years old male child from the same brick field area and was admitted with severe dehydration and profound shock on 5th April and died on the same day.

During the survey it was noticed that the drinking water for the entire population was provided through only one tube well and the people were forced to collect and store water for drinking and domestic use. The platforms around the tube wells were found to be in broken condition with stagnation of water in surrounding area. Although both the Brick Fields are located at the bank of the river Hooghly but the workers do not utilise this flowing water. The river water, has been tunneled into the low lying excavated ditches within the brick fields for the purpose of preparation of brick. This stagnant water resulted in formation of cesspools which is used for bathing and washing of the utensils. No latrine facilities exist in this area and the people use surrounding open field for defaecation including the area surrounding the cesspool. This cesspool water is also used for ablution purpose.

The living space allotted to the workers and their families is grossly inadequate and ill-ventilated. There is extreme overcrowding facilitating transmission of infection. There is lack of proper health care facilities in terms of both manpower and logistics.

Domiciliary cases had history of watery diarrhoea with or without vomiting. All cases had dehydration. Severe dehydration was noticed in some cases. The first case was a female child aged 4 years residing in No.8 brickfield. Her illness started with profuse watery diarrhoea and vomiting on 29th March and was admitted to ID Hospital Calcutta on the same date. Most of the affected children were malnourished.

Two local persons(unqualified doctors) were the treatment providers. They treated the cases with combined antibiotics along with antiperistaltic like Lomotil. Although ORS was advised but inadequate dose with faulty instruction were given by them.

A total of 22 water samples were collected from different sources. These include Tube well water-2, Stored water-12, and cesspool water-8. In addition 5 rectal swabs from detected domiciliary cases of diarrhoea were collected in Cary and Blair media. The samples were

transported to NICED Calcutta and processed using standard techniques.

Two of 5 faecal samples(40%) collected from domiciliary cases were positive for *V.cholerae* O1. Of 22 water samples collected from different source. *V.cholerae* O1 were positive in 10 (45.4%) samples. The single tube well sample positive for *V.cholerae* was from brickfield No.23. Five (41.7%) of 12 stored water were also positive. The remaining 4 positive water samples were collected from 8 cesspools indicating extensive spread of *V.cholerae* in the surrounding environment. Strains were sensitive to tetracycline, norfloxacin, ciprofloxacin, gentamycin and were resistant to co-trimoxazole, nalidixic acid, streptomycin, ampicillin and furazolidone.

From clinical, epidemiological and microbiological data it is apparent that there was an outbreak of cholera localised in the brickfield No. 23 and 8. During the investigation, it became clear that every year groups of migrant labourers with their families come to these brick fields for work, during the period from winter till the end of summer. Cases of watery diarrhoea with or without vomiting with occasional death have been reported every year by the local authorities. Higher attack rate in children below five years of age clearly indicate that the area is endemic for diarrhoeal diseases including cholera.

The investigating team also carried out health education including preparation and delivery of ORS. Proper feeding practices including breast-feeding and safe storage of drinking water was suggested to families. The team also suggested the probable control measures to the authority.

## **11.2. Visit to Bhuj, Gujarat to investigate any impending Diarrhoeal disease outbreak following recent earthquake disaster**

Investigators

D.N. Gupta, S. Dutta

The team visited to Bhuj during 3-8 February 2001 and involved in investigating any impending outbreak of diarrhoeal diseases following devastating earthquake disaster at Bhuj, Gujarat. Eastern part of Kachchh district was worstly affected due to this earthquake. Most affected blocks were Bhuj, Anjar, Bachau, Gandhidham Rapar. Mundra & Mandvi blocks were moderately affected. The epicenter was at Lodai village, 30 Km from Bhuj. It was found a control room of Disease Surveillance Cell of Ministry of Health & FW, Govt. of India was operational in Patel Trust Guest House, Bhuj.

From disease surveillance report it was known, there were 187 reported diarrhea cases amongst 1,18,793 population in Bhuj town giving an attack rate of only 0.15/100 population. A Survey amongst 10850 population was conducted by two team in three villages in Bhuj and Anjar block was done and no diarrhea cases was detected. A daily visit to the makeshift hospital run by Israeli team & an open air temporary clinic run by Patel Trust Hospital, no diarrhea cases was also detected to attend or admitted in the clinic or Israeli hospital during that period.

Drinking water supply arrangement was done by Govt. and other local agencies by delivery of chlorinated & filtered water in pouches. Construction of trench latrine was started from 5 February. Decision was taken to set up a field laboratory for disease surveillance. Accordingly, a field laboratory (microbiology) was set up on 5 February by NICED team in the nearby Patel Trust Hospital and investigation center. A total of 344 water samples (drinking) from various sources were tested for OTT & 184(53%) were positive and suitable for drinking. The other 160 samples(47%) were negative which needed to be chlorinated before consumption. Proper health education on chlorination of drinking water for containment of water borne diarrhoeal diseases, was given by health workers, under guidance of public health experts. As no active diarrhoea cases was detected or reported, no diarrhoeal stool sample or rectal swab was available in the laboratory for processing.

It was observed that only sporadic cases of diarrhea have occurred during that period and not linked to each other. Number of cases was within expected limit. Proper control measures were suggested to the concerned authority to contain the spread of diarrhea if any in the affected population in future.

### **11.3. Investigation of *V.cholerae* O139 outbreak at Baishnabghata, Patuli, Kolkata**

Investigators :

P. Dutta, P.G. Sengupta, S.K. Mondal, D. Sur, T. Ramamurthy

An explosive outbreak of diarrhoeal disease caused by *V.cholerae* O139, occurred in Baishnabghata, Patuli in the Kolkata Municipal Corporation area during 28th September to 12th October, 2000. The outbreak was investigated on 4th and 16th October, 2000 by a team from National Institute of Cholera and Enteric Diseases, Kolkata. Two medical camps had been organized by local health authorities to attend to the fresh cases with their follow up. Clinical and epidemiological data was collected from patients attending the camps and also from the hospitalized cases. Discussions were held with attending doctors, paramedical staffs and local NGOs regarding treatment modalities. House to house visits were also made to detect domiciliary cases. Faecal samples were collected from fresh cases and water samples from various sources. Local health authorities were advised to campaign for control measures through the camps, health workers and NGOs.

A total of 710 cases of diarrhoea occurred with an attack rate of 7.1%, of which majority were adults. All the 6 faecal samples collected from untreated cases and 2 water samples from collecting points were positive for *V.cholerae* O139. This was the first localized outbreak of *V.cholerae* O139 after the nationwide devastating epidemic in 1992. The strains were uniformly (100%) susceptible to commonly used drugs for cholera like tetracycline, norfloxacin, ciprofloxacin and co-trimoxazole but resistant (100%) to furazolidone and ampicillin. At the recommendation of the investigating team, extensive chlorination was done of all water sources on 5th October, which resulted in a dramatic decline of the outbreak, which ultimately subsided completely by 12th October. The investigating team nevertheless made a subsequent visit on 16th October to evaluate the effect of the chlorination programme. Samples collected during the second visit did not harbour live culturable *V.cholerae* O139.

#### 11.4. Concomitant Infection of Enterotoxigenic *Escherichia coli* in an Outbreak of Cholera Caused by *Vibrio cholerae* O1 and O139 in Ahmedabad

Investigators

S. Chakraborty, T. Ramamurthy, G.B. Nair and S.K. Bhattacharya

The current investigation highlights association of three pathogens associated with a large outbreak of diarrhea in a metropolitan city of Gujarat state, India. From January 1 to 17, 2000, a total of 809 patients reported in three different hospitals, namely I.D. Hospital, V.S. General Hospital and L.G. Hospital in Ahmedabad, with acute watery diarrhea. Cases of diarrhea were reported from at least 40 wards. The total population served by these three hospitals is in the range of 800,000 to 900,000. Attack rate of this outbreak was about 0.2%. Bacteriological culture was performed on 734 of the 809 hospitalized patients. Of the 734 stool specimens tested, 72 were positive for *V.cholerae* O1, 31 for *V.cholerae* O139, and 24 for *E.coli*. All these isolates were confirmed by the National Institute of Cholera and Enteric Diseases (NICED) Calcutta and the representative isolates were selected at random to exclude any bias for the detection of different virulence genes by PCR, molecular typing and antibiotic susceptibility testing using standard techniques.

Examination of the 103 isolates of *V.cholerae* revealed that 72 (70%) were *V.cholerae* O1 Ogawa serotype ElTor biotype while 31 (30%) were identified as belonging to *V.cholerae* O139 serogroup. All the 25 representative *V.cholerae* isolates including 17 serogroup O1 and 8 serogroup O139 were positive in multiplex PCR for *ctxA* and *tcpA* of the ElTor variant. Six different serotypes of *E.coli* were encountered with O1 serotype being dominant (41.6%) followed by O146 (16.6%), while 16.6% of the isolates were untypable (ONT). In the PCR assay, 18 (75%) of the *E.coli* isolates harbored *elt* gene of which 9 (50%) belonged to the serotype O1. All the *E.coli* isolates tested did not harbor *est*, *stx1* and *stx2* genes and were negative in EA<sub>g</sub> PCR assay. None of the ETEC isolates possessed any of the 12 commonly prevalent CFAs that were examined in this study.

Antibiotic susceptibility results of 23 and 9 O1 and O139 isolates respectively, revealed that all these isolates were resistant to ampicillin, furazolidone and nalidixic acid. In addition, *V.cholerae* O1 isolates were resistant to co-trimoxazole, streptomycin, and 21.7% to chloramphenicol whereas *V.cholerae* O139 isolates were susceptible to these antibiotics. Majority of the *E.coli* isolates showed high resistance to several antibiotics including to the quinolone group of antimicrobial drugs. Ribotyping of 8 representative isolates of O1 Ogawa revealed that 7 of them (Fig. 1, lanes 2, 5, 7, 8; only representative isolates are shown) showed the previously documented RIII type while one isolate AHO94 (Fig. 1, lane 4) showed slightly different pattern as compared to RIII type which is the current prevailing type by the presence of an additional band at approximately 5.6 kb. Ribotype pattern of 5 representative *V.cholerae* O139 isolates (Fig. 1, lanes 1, 3, 6) were identical to the most commonly found BII ribotype pattern which is the ribotype currently prevailing among *V.cholerae* O139 isolates in Calcutta.

PFGE of all the 6 representative O1 Ogawa isolates (Fig. 2A, lanes 1 to 6) exhibited identical pattern and comparable to the 'H' pattern of the new clone of O1 reported in Calcutta (Fig. 2B, lane 1). PFGE analysis of the 3 *V.cholerae* O139 isolates (Fig 2A, lanes 7 to 9) showed identical patterns which differed from the PFGE patterns of O139 isolates reported during 1992 to 1997 in Calcutta. PFGE analysis was done on 8 representative *E.coli* isolates. Of these, 5

belonging to *E.coli* O1 serotype (Fig. 3, lanes 1 to 5) showed that two isolates, E14 and AV185 (Fig. 3, lanes 4 and 5) exhibited identical patterns while the remaining three (Fig. 3, lanes 1 to 3) were different from each other despite belonging to the same serotype. Two isolates of *E.coli* O146 serotype (Fig. 3, lanes 6 and 7) showed different PFGE profiles. Surprisingly, PFGE pattern of E10 isolate belonging to O1 serotype (Fig. 3, lane 3) was identical to that of the E2 isolate belonging to O146 serotype (Fig. 3, lane 7). PFGE pattern of one representative *E.coli* O untypable (ONT) (Fig. 3, lane 8) isolates was different from *E.coli* isolates belonging to either O1 or O146 serotypes.

Ahmedabad outbreak of acute diarrhea was unusual in that, two major enterotoxigenic enteropathogens namely *V.cholerae* and ETEC were involved. To our knowledge, this is the first report describing involvement of more than one enteric pathogen in an outbreak setting in India.

# **REPORT OF THE NATIONAL HIV REFERENCE CENTRE**

## **Section highlights**

Institute is recognised as one of the National HIV Reference Centre. During the year under report, a total of 1551 serum samples were screened for HIV by ELISA and spot test and confirmed by highly specific ELISA or Western Blot test. Three hundred twenty two samples were tested positive for HIV during the period April, 2000 to March 2001.

## **12. REPORT OF THE NATIONAL HIV REFERENCE CENTRE**

### **12.1. Report of HIV screening**

Investigators

S.K. Bhattacharya and T.N. Naik

The National HIV Reference Centre of the Institute is funded by National AIDS Control Organization of Ministry of Health and Family Welfare, Government of India since 1992. The activities of the reference centre includes (1) serosurveillance for HIV infection, (2) confirmation of serum samples received from different surveillance and zonal blood testing centres located in different states of Eastern India, (3) training manpower (doctors, medical laboratory technologists) for HIV surveillance and laboratory diagnosis of HIV infection as and when requested by State Health authorities, hospitals or service organization and (4) HIV kit evaluation.

Between April 2000 and March 2001 a total of 1551 serum samples were screened by highly sensitive ELISA and positive samples were confirmed by either highly specific ELISA or Western Blot.

National HIV Reference Centre  
 Division of Virology,  
 National Institute of Cholera & Enteric Diseases, Kolkata 700 010  
 Sample screened for Human Immunodeficiency Virus (HIV)  
 Antibody by ELISA and/or Confirmatory Test  
 From 1st April, 2000 to 31st March, 2001

Source of Samples	No. of Tested	No. or Positive
A. WEST BENGAL		
1. Antenatal Mother	Nil	Nil
2. Blood Donor	24	09
3. Border Security Force	10	09
4. Drug Users	Nil	Nil
5. Eastern Command Hospital	99	88
6. Foreigners	60	Nil
7. High Risk Group	04	01
8. Patients with blood diseases	Nil	Nil
9. Sex Workers	773	35
10. Heterosexual	331	10
11. Miscellaneous	52	06
<b>Sub Total</b>	<b>1353</b>	<b>158</b>
B. OTHER STATES		
1. Bihar	17	12
2. Meghalaya	03	03
3. Mizoram	19	19
4. Orissa	159	130
<b>GRAND TOTAL</b>	<b>1551</b>	<b>322</b>

**JICA/NICED PROJECT FOR  
PREVENTION OF EMERGING  
DIARRHOEAL DISEASES**

### 13. NICED PROJECT FOR PREVENTION OF EMERGING DIARRHOEAL DISEASES

This Institute entered into a collaborative project with Japan International Cooperation Agency (JICA) entitled “Prevention of Emerging Diarrhoeal Diseases” since February 1998. The aim of this project is to strengthen the research capability of this Institute and to establish rapid and accurate diagnostic, appropriate therapeutic, and effective prevention methods for the control of emerging diarrhoeal diseases with ultimate objective of reduction of diarrhoeal diseases morbidity and mortality. The three major components of the project are:

- i) Provision of sophisticated equipment
- ii) Visit of Japanese experts at NICED
- iii) Training of NICED scientists in Japan or in a third country where another JICA project is being implemented

Dr. S. Yamasaki, Division Chief, International Medical Centre of Japan was overall in charge of the project and being a specialist in molecular bacteriology visited this institute during 4.6.2000 to 30.6.2000; 24.9.2000 to 2.10.2000; 23.11.2000 to 15.12.2000 and 6.2.2001 to 20.2.2001. He had introduced several molecular techniques for rapid identification of enteric pathogen like *Vibrio cholerae*, *V. parahaemolyticus*, different types of *E. coli*, *Shigella* spp., *Salmonella typhi*, etc. These techniques have been utilized by the technical staff of the Microbiology Department.

Mr. S. Takeno, Coordinator of the project is efficiently coordinating all administrative and counterpart training programs for the NICED scientists and staffs.

Two Short term Japanese experts visited this Institute. Dr. Hisao Karazone, Microbiology and Dr. Nobomichi Koboyashi, Virologist, visited this institute during the period between 23.11.2000 to 15.12.2000; 22.1.2001 to 1.2.2001 respectively.

In the reporting year, Dr. T. Biswas, Division of Immunology and Dr. T. Krishnan, Division of Virology visited Japan for training during 28.8.2000 to 26.6.2001. Mr. S.K. Bhowmik, Technical Assistant is studying Masters course in Molecular Microbiology at the school of Medicine, Tsukuba University in Japan since April 1999 under the Japanese Government scholarship program.

The JICA-NICED domestic training programme was conducted between November 27 - December 6, 2000. Thirteen participants were attended the training programme.

Dr. Y. Takeda, Director General of the National Institute of Infectious Diseases, Japan coordinated the project from JICA Headquarter, Japan. JICA project is functioning satisfactorily.

# ACTIVITIES OF TRAINING AND EXTENSION DIVISION

## Section highlights

- o Organised two workshops
  - i) Quality assurance of HIV testing (NACO)
  - ii) Molecular epidemiology of diarrhoeal diseases with special reference to cholera (JICA)
- o Organised training for the students of the University of Kolkata
  - i) MBBS; ii) BHMS; iii) MD (Microbiology)
- o Organised training for :
  - i) M.Sc. students of Jadavpur University;
  - ii) M.Sc. student of Vidyasagar University
- o Organised seminars
  - i) Weekly Institutional seminar and seminar for distinguished guests
- o Organise training programme for three WHO Fellows of DPR Korea who visited this Institute for training on microbiological aspects on diarrhoeal diseases.
- o Dr. A. Szpilewska, Polish Scientist, received training on molecular genetics.

## **14. VITIES OF TRAINING AND EXTENSION DIVISION**

### **14.1. NACO Expert Group Meeting on Quality Assurance of HIV Testing**

WHO-NACO Expert Group Meeting on Quality Assurance of HIV Testing was held at NICED during April 14-15, 2000. The workshop was jointly organised by NICED, NACO, Govt. of India and WHO. A total of 20 experts in the field of HIV/AIDS participated and discussed the issues relating to the different diagnostic tests of HIV. The formation of nodal and reference laboratory for HIV diagnostics was discussed in detail. The function and responsibility of different centres around the country was one of the major theme of the workshop.

### **14.2. JICA-NICED Domestic Training Programme on "Molecular Epidemiology of Diarrhoeal Diseases with Special Reference to Cholerae"**

The JICA-NICED domestic training programme was conducted between November 27 - December 6, 2000. Thirteen participants were attended the training programme. The external experts and local staffs during the training programme gave fifteen lectures. For practical, eight different aspects composed of isolation and identification of *Vibrio cholerae* and *Vibrio parahaemolyticus*. *Escherichia coli* and *Shigella* spp. pulsed-field gel electrophoresis (PFGE) to determine the clonality of *V.cholerae*, simplex and multiplex polymerase chain reaction (PCR) to determine the specific genes among *V.cholerae* O1 and O139 serogroups, *V.parahaemolyticus*, diarrhoeagenic *Escherichia coli*, group-specific PCR for the detection of pandemic strains of *V.parahaemolyticus*; extraction and analysis of chromosomal DNA form *V.cholerae*; extraction and analysis of plasmid DNA containing the r-RNA probe fragment; construction of dendrogram using PFGE profiles; ribotyping of *V.cholerae* O1 and O139 serogroups were covered under this programme.

### **14.3. Other Training and Teaching Programmes**

M.B.B.S. students of Calcutta National Medical Colleges and BHMS students from the National Institute of Homeopathy visited the institute to acquaint themselves with different facets of diarrhoeal diseases and its management. BHMS students were also apprised of the present situation of AIDS in the country. Lectures on respective topics were delivered by Dr. S. Chakrabarti, Dr. P.G. Sengupta, Dr. P. Dutta, Deputy Directors (Senior Grade), Dr. D.N. Gupta, Dr. S.K. Mondal, Deputy Directors and Dr. D. Sur, Dr. U. Mitra, Senior Research Officers.

M.Sc. (Physiology and Botany) students of Vidyasagar University with special paper in Microbiology visited the institute to get oriented with the different aspects of biomedical research. Some students of M.Sc. (Physiology) of Vidyasagar University and M.Tech (Biotechnology) of Jadavpur University carried out their dissertation work at this institute under Dr. M.K. Chakrabarti, Deputy Director.

The students of M.D. (Microbiology), Calcutta University were deputed to this institute for acquiring advance knowledge in different aspects of enteric organisms.

Besides the above, weekly seminars on different aspects of diarrhoeal disease research were held regularly.

#### 14.4. Guest lectures

The following scientists delivered lectures at this institute on various topics as mentioned below :

Speaker	Date	Topics
Dr. Pranab Kumar Das, Department of Pathology/Dermatology, University of Amsterdam, Netherlands	02.01.2001	Molecular immunopathology of intra cellular infection: leprosy as a model
Dr. Sumit Majumder, Los Angeles, USA	04.01.2001	Highly active anti retroviral therapy in AIDS
Dr. N. Kobayashi, Supporo Medical University, School of Medicine, Sapparo, Japan	25.01.2001	Reassortment of Rotavirus RNA segments
Dr. N. Kobayashi, Supporo Medical University, School of Medicine, Sapparo, Japan	30.01.2001	Evaluation and dissemination of methicillin resistance gene in staphylococci
Dr. H. Kojima, Shizuoka General Hospital, Shizuoka, Japan	13.02.2001	Interferon therapy of chronic hepapatis C
Dr. Wanpen Chaicumpa, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand	15.02.2001	Diagnostic test kits for enteric bacteria

#### 14.5. Training of W.H.O. Fellow

During the period under report the institute imparted training on different aspects of diarrhoeal diseases to the following W.H.O. fellows :

Name	Country	Period
Dr. Kim Sung Do	DPR Korea	05.06.2000 to 28.07.2001
Dr. Hwang Mun Sop	- do -	- do -
Dr. Kim Son II	- do -	- do -

#### 14.6. Foreign scientist received training in the Institute

**Dr. Hanna Szpilewska**, Scientist of the Polish Academy of Sciences, Marine Biology Center, Poland visited Microbial Genetics Division of our institute for three weeks under Indian National Science Academy - Polish Academy of Sciences (INSA-PAS) Exchange Programme in the month of December - 2000

**15. Scientific Conference /seminars/meeting attended by the scientists/  
research fellows**

<b>Participants</b>	<b>Conference/seminars/Meetings</b>	<b>Title of the Papers/Talk</b>
Dr.S.K. Bhattacharya	Second World Conference on Digestology at Beijing, China from September 8-11,2000	Management of acute diarrhoea
	Regional Action Conference for Surveillance and Response to Infectious Diseases Outbreaks in South-East Asia at Bali, Indonesia from September 12-15, 2000	Over view on Cholera and Shigellosis
	5 <sup>th</sup> International Conference on Emerging Infectious Diseases in the Pacific Rim, Manila, Phillippines, January 13-15, 2001	Emerging diarrhoeal pathogens
	WHO Workshop on Epidemic Preparedness and Rapid Response held at NICD, Delhi during 10-19 April, 2000	
	Training Course – Johns Hopkins Summer Institute in HIV Epidemiology, USA during June-July, 2000	
	ICMR/WHO Symposium on Treatment and Control of Kala-azar, New Delhi, 18-19 December, 2000	
Dr. S. ChaKraborty	Quality Assurance on HIV Testing" organized by WHO/NACO at NICED, Calcutta during April 14-15, 2000.	
	"Ethical issues on HIV/AIDS" organized by WHO/UNAIDS at New Delhi during June 28-20, 2000.	
	International Symposium on Woman's Health at Science City, Calcutta on November 11th, 2000.	HIV and Developing Country
	National AIDS Society of India, Mumbai on November 18th, 2000.	"HIV Vaccines
	Workshop on "Application of Gag HMA for HIV-1 subtype determination" at Antwerp, Belgium during December 4-8, 2000	
Dr. P. Dutta	East Zone Conference of Indian Academy of Pediatrics held at Manipur during 2-4 April, 2000	Should gastrointestinal infection be treated with fluonoquinolone?
	Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000.	
	Seminar on the occasion of World Breast Feeding Week organised by IPHA sponsored by UNICEF at All India Institute of Hygiene &	

	Public Health on 1st August, 2000	
	Seminar on the occasion of National Nutrition Week organised by IPHA sponsored by UNICEF at Medical College and Hospital, Calcutta on 14th September, 2000	
	IMA & IAP workshop held at Howrah on 17th June, 2000	Status of Diarrhoeal Diseases in West Bengal
	NICED/JICA workshop on molecular epidemiology on diarrhoeal diseases held at NICED during 27th November to 6th December, 2000	Management of Diarrhoea
	East Zone Conference of IAP held at Santiniketan during 5-7 January, 2001	Role of lactobacillus in diarrhoeal diseases
	Attended Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000.	
Dr. T.N. Naik	34th joint working conference on viral diseases, Japan-US Cooperative Medical Science Programme at Inuyama City, Japan between July 20-22, 2000.	Sequence analysis of group B human rotavirus detected in Calcutta, India
	3rd Congress of Federation of Indian Physiological Societies (FIPS) at the Science City, Calcutta, November 24-26, 2000.	An evolutionary pathway for the rotaviruses
Dr. D. Dutta	Attended the seminar on "Road to Health" in July, 2000 held at New Delhi.	
	Training programme on controlled clinical trials held at National Institute of Epidemiology, Chetput, Chennai 30th October to 10th November, 2000	
Dr. R. Kumar	Visva Bharati, Santiniketan for refresher course for college teachers in Life Sciences during March 11 to 29, 2001	i)Molecular aspects of enteropathogenic vaccine development, special reference to <i>Shigella dysenteriae</i> 1" ii) Biodegradation of petroleum hydrocarbons in coastal waters of deltaic Sunderbans".
Dr. D.N. Gupta	Scientific Session on "Observation of World Breast Feeding Week" on 1st August, 2000 at AIHH&PH, Calcutta.	
	Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000	
	Seminar on "Observation of Nutrition Week" organised by IPHA HQ at Medical College, Calcutta on 14 September, 2000.	
	Symposium on "Prevention of Ischaemic Heart	

	Disease" on 15th October, 2000 organised by IMA, Calcutta	
	Participated in training on "Application of Behavioural Sciences in Health System Research" held on 23rd March, 2001 at NICED, Calcutta.	
Dr. A.N. Ghosh	"Professor N.N. Dasgupta Memorial Workshop on Microscopic Techniques" was held in the Division of Electron Microscopy, NICED, during September 19-22, 2000.	
Dr. P. Das	A.I.C.T.E. sponsored Quality Improvement Programme, 3rd short term course from 17-28 April, 2000, organized by Jadavpur University	Natural products and related aspects
	5th International Meeting on Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases (MEEGID-5) held at Hyderabad, 14th November, 2000	Characterization of genes encoding for 29 kDa ptypeptide of <i>E.histolytica</i> and its importance in amoebiasis
	XXIV All India Cell Biology Conference" held at New Delhi, 4th December, 2000.	Processing of ribosomal RNA of <i>G.lamblia</i> : involvement of fibrillar protein and small nuclear RNAs, snRNA
	ICMR-WHO sponsored symposium on "Treatment and control of Kala-azar" held at New Delhi on 18-29 December, 2000	
	7th Training Course on Immunodiagnosics for Infectious Diseases, sponsored by DBT, organized by JB Tropical Disease Research Centre, MGIMS, Sevagram on 5th February, 2001	Antigens of diagnostic and immunoprophylac importance in <i>E.histolytica</i>
	U.G.C. sponsored Academic Staff an invited talk on " College of Calcutta University, for teachers' refresher course, organized by Department of Physiology, Calcutta University on the 7th February, 2001	<i>Cryptosporidium</i> a newer enteric parasite of human
	U.G.C. sponsored Academic Staff College of Calcutta University, for teachers' orientation programme, organized by Department of Zoology, Calcutta University on the 16-18 February, 2001.	Immunological techniques and its application in medical and basic science
	U.G.C. sponsored Academic Staff College of Visva Bharati University for 5th refresher course, organized by Department of Zoology and Botany, Visva Bharati on the 22-23 March, 2001.	Application Hybridoma technology : its and PCR technology
Dr.M.K. Chakrabarti	UGC refresher centres for college teachers in Physiology, Academic Staff College, Calcutta	Recent advances in Diarrhoeal Disease Research with special

	University, 10th March, 2001.	reference to <i>Escherichia coli</i>
	3rd Congress of FIPS, 24-26 November, 2000.	Mechanism of action of <i>Escherichia coli</i> heat-stable enterotoxin in COLO 205 cell line : role of protein kinase C,
	UGC orientation programme for College Teachers, Academic Staff College, Calcutta University, 26th February, 2001.	Science in Health Care
	88th session of the Indian Science Congress held at Indian Agricultural Research Institute, New Delhi, January 3-7, 2001.	A 6.5 kb nonconjugative plasmic of the strain <i>Shigella dysenteriae</i> type 1 (dt66) encodes erythromycin, vanadium resistance characters and OMP biosynthesis.
Dr. S.K. Mondal	Seminar on "Breast Feeding is your right" on the occasion of World Breast Feeding Week organised by BPNI sponsored by UNICEF at Academy of Fine Arts on August 1, 2000	
	Seminar on the occasion of National Nutrition Week organised by IPHA sponsored by UNICEF at Medical College and Hospital, Calcutta on 14th September, 2000.	
	Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000	
	Participated in training on "Application of Behavioural Sciences in Health System Research" held on 23rd March, 2001 at NICED, Calcutta.	
Dr. K.K. Banerjee	27 Annual Congress of the Indian Immunology Society & Symposium on Immunology of Infectious Disease, Agra, 10-13 December 2000	Monoclonal antibodies as probes molecular interpretation of the mechanism of pore formation by <i>V. cholerae</i> hemolysin
Dr. A.K. Sinha	Workshop on HIV/AIDS Training Programme by NACP Phase-II from 14th to 16th March, 2001, organised with the Concurrence of Director of Medical Education, Govt. of West Bengal, held at Medical College,	
Dr. T. Ramamurthy	5th International meeting of Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases held at Hyderabad during November 12-16, 2000	Molecular analysis of enterotoxigenic <i>Escherichia coli</i> associated with an outbreak of diarrhoea in Ahmedabad by pulsed field gel electrophoresis

Dr. S. Dutta	5th International meeting of Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases held at Hyderabad during November 12-16, 2000	Recent trends in the epidemiology of shigellosis in children in Calcutta, India
	51st meeting of IAMM (Indian Association of Medical Microbiologists) WB Chapter held in Nilratan Sirkar Medical College on 22nd April, 2000.	
	52nd Meeting of IAMM WB Chapter held in Calcutta Medical College on 24th February, 2001.	
	69th Annual Meeting of Society of Biological Chemists held at Science City, Calcutta, December 7-9, 2000.	Evaluation of 4.5 kb plasmid of the epidemic strain of <i>S. dysenteriae</i> I in relation to drug resistance character & virulence potentials.
Dr. T. Biswas	30 <sup>th</sup> Meeting of Japanese society for Immunology, Sendai, Japan, 14-16 November, 2000.	
Dr. D. Sur	Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000	
	Annual State Conference of IAPSM at R.G. Kar Medical College & Hospital, Calcutta on 8th July, 2000.	
	Seminar on the occasion of World Breast Feeding Week organised by IPHA sponsored by UNICEF at All India Institute of Hygiene & Public Health on 1st August, 2000	
	Seminar on the occasion of National Nutrition Week organised by IPHA sponsored by UNICEF at Medical College and Hospital, Calcutta on 14th September, 2000	
	Training programme on "Application of Behavioural Sciences in Health Systems Research" organised jointly by NICED and UNICEF, Calcutta on 23 <sup>rd</sup> March, 2001.	
Dr. U. Mitra	Annual State Conference of IPHA at IPHA Bhavan, Salt Lake, Calcutta on 3rd June, 2000	
	Seminar on the occasion of World Breast Feeding Week organised by IPHA sponsored by UNICEF at All India Institute of Hygiene & Public Health on 1st August, 2000	
	Attended a seminar on the occasions of National Nutrition Week organised by IPHA sponsored by UNICEF at Medical College and Hospital, Calcutta on 14th September, 2000	

	Workshop on "Concepts in Human Diversity" organized at the Central Forensic Science Laboratory, Kolkata from March 12th to 16th, 2001.	
Dr.A. Pal	69th Annual Meeting of Society of Biological Chemists held at Science City, Calcutta, December 7-9, 2000	Enterotoxicity in <i>Vibrio cholerae</i> non O1 non O139 strains in an <i>in vivo</i> rat intestinal model
Dr. T. Krishnan	48th Annual Meeting of the Japanese Society for Virology held at Tsu City, Mie Prefecture, Japan, between 12th - 18th, October, 2000.	
	Department of Microbiology, Akita University School of Medicine, Hondo, Akita, Japan on 5th March, 2001	Rotaviruses - An overview of their puzzling diversity over the past 25 years.
	Department of Microbiology, Akita University School of Medicine, Hondo, Akita, Japan on 9th March, 2001.	Analysis of genome profiles and molecular characterization of the subgroup and serotype nature of rotaviruses from Calcutta and Manipur.
	Department of Developmental Medical Sciences, Graduate School of Medicine. The University of Tokyo, Bunkyo-ku, Tokyo, Japan on 13th March, 2001	Electron microscopic studies of some diarrhoeagenic viruses and newly emerging trends in rotaviral infections from Calcutta, India.
Dr. M.K. Saha	Japanese Virology Society 48th Annual Meeting held at Tsu (Mie Prefecture), Japan during 12th to 14th October, 2000	
	International Association for Study of Liver and Asia-Pacific Association for Study of Liver Joint Meeting 2000 held at Fukuoka, Japan during 2nd to 7th June, 2000	
Dr. A. Pandey	Attended a seminar on the occasion of World Breast Feeding Week organised by IPHA sponsored by UNICEF at All India Institute of Hygiene & Public Health on 1st August, 2000	
	Attended a seminar on the occasion of National Nutrition Week organised by IPHA sponsored by UNICEF at Medical College and Hospital, Calcutta on 14th September, 2000	
	Training programme on "Application of Behavioural Sciences in Health Systems Research" organised jointly by NICED and UNICEF, Calcutta on 23 <sup>rd</sup> March, 2001.	
Mr. S. Sengupta	XIVth Seminar on Amoebiasis, November 27-30, 2000, Centro de Investigacion y Estudios Avanzados del IPN, Mexico City, Mexico	Role of Excretory-Secretory Products of <i>E.histolytica</i> in Human Amoebiasis
	XIVth Seminar on Amoebiasis, November 27-	Characterization of plasma

	30, 2000, Centro de Investigacion y Estudios Avanzados del IPN, Mexico City, Mexico	membrane associated antigens of diagnostic & prophylactic importance in <i>E. histolytica</i>
Mr.P. Mukhopadhyay	5th International Meeting on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID V) held at Ramoji Film City, Hyderabad, India, November 12-16, 2000.	Role of electron dense granules in pathogenicity of <i>Entamoeba histolytica</i>
	69th Society of Biological Chemists of India, held at Indian Institute of Chemical Biology, Calcutta, December 7-9, 2000	Biochemical and immunological studies on electron dense granules containing collagenase activity of <i>Entamoeba histolytica</i>
Mr. A. Debnath	69th Annual Meeting of the Society of Biological Chemists (India), held at Science City, Calcutta, India, from December 7-9, 2000.	Characterization of differentially expressed genes in <i>Entamoeba histolytica</i> during human collagen type 1 and Ca <sup>2+</sup> incubation
Md. A. Akbar	69th Annual Meeting of the Society of Biological Chemists (India), held at Science City, Calcutta, India, from December 7-9, 2000	Characterization of genes encoding for 29 kDa polypeptide of <i>Entamoeba histolytica</i> and its importance in amoebiasis
Miss. B. Sarkar	Indian Science Congress, 88th Session, New Delhi, 3-7 January, 2001	Incidence of heterogenous clones of <i>Vibrio parahaemolyticus</i> carrying <i>tdh</i> gene in untreated sewage water used for irrigation at Bantala, Koltala.
Miss. S. Chakraborty	Indian Science Congress, 88th Session, New Delhi, 3-7 January, 2001	Characterisation of <i>Escherichia coli</i> strains harbouring <i>elt</i> and <i>est</i> genes with reference to serotype and colonization factor antigens.
Mr. A. Khan	4th International Symposium and Workshop on "Shiga Toxin (Verocytotoxin) Producing <i>Escherichia coli</i> Infections" Kyoto, Japan	Prevalence and genetic profiling of virulence traits of Shiga toxin producing <i>Escherichia coli</i> isolated in Calcutta, India
	5th International Meeting on "Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases" Hyderabad, India	Prevalence and genetic profiling of virulence traits of Shiga toxin producing <i>Escherichia coli</i> isolated in Calcutta, India
Miss. N. Roy Chowdhury	5th International Meeting on "Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases" Hyderabad, India	<i>Vibrio parahaemolyticus</i> infections on a fast forward origin of a new pandemic

Miss. S. Dutta	5th International Meeting on "Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases" Hyderabad, India	Molecular genetics of <i>Helicobacter pylori</i> in India (i) distinctiveness of strains in Bengal, and (ii) sensitive ELISA-based analyses of vacuolating cytotoxin (VacA) levels.
Mr. A. Sen	5th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases at Hyderabad, India, November 12-16, 2000.	Evidence for a human group B CAL-like strain as the common ancestor of rotaviruses
	69th Annual Meeting of the Society of Biological Chemist (India) held at Science City, Calcutta, December 7-9, 2000.	Non-neutral evolution and jumps across the species barrier suggest group B ancestry for rotaviruses
Mr. S. Das	5th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases at Hyderabad, India, November 12-16, 2000.	Molecular characterization of a naturally occurring human group A rotavirus mutant
	69th Annual Meeting of the Society of Biological Chemist (India) held at Science City, Calcutta, December 7-9, 2000.	Molecular characterization of a naturally occurring human group A rotavirus mutant
Ms. G. Uma	5th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases at Hyderabad, India, November 12-16, 2000.	Molecular evidence for the association of human Caliciviruses with infantile diarrhoea in India
	69th Annual Meeting of the Society of Biological Chemist (India) held at Science City, Calcutta, December 7-9, 2000	Detection and partial sequencing of human Caliciviruses in Calcutta
Mr. V. Vici	5th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases at Hyderabad, India, November 12-16, 2000.	Evidence for the association of the rotavirus reassortants in an outbreak of epidemic gastroenteritis in Manipur, India
Mr. K.M. Hoque	69th Annual Meeting of Society of Biological Chemists held at Science City, Calcutta, December 7-9, 2000	Mechanism of action of heat-stable enterotoxin of <i>Vibrio cholerae</i> non-O1 : Role of cytosolic calcium in the stimulation of calcium influx in isolated rat enterocytes
	88th Session of the Indian Science Congress held at Indian Agricultural Research Institute, New Delhi, January 3-7, 2001.	Aggregative <i>Klebsiella pneumoniae</i> strains isolated from cases of diarrhoea causes rise in intracellular free calcium in HeLa cells
	69th Society of Biological Chemists of India held at Indian Institute of Chemical Biology,	Mechanism of action of heat-stable enterotoxin of <i>Vibrio</i>

	Calcutta, December 7-9, 2000.	<i>cholerae</i> non-O1 : Role of cytosolic calcium in the stimulation of calcium influx in isolated rat enterocytes
Mr. S. Samanta	69th Annual Meeting of Society of Biological Chemists held at Science City, Calcutta, December 7-9, 2000.	Species-specific differences in the density of <i>Escherichia coli</i> heat-stable enterotoxin receptors and guanylyl cyclase activity in the intestinal brush-border membrane of guinea pigs and hamsters
	88th Session of the Indian Science Congress held at Indian Agricultural Research Institute, New Delhi, January 3-7, 2001.	Evaluation of the density of <i>Escherichia coli</i> heat-stable enterotoxin receptors and guanylyl cyclase activity in hamster and guinea pig enterocytes
Mr.A.Mukhopadhyay	88th Session of the Indian Science Congress held at Indian Agricultural Research Institute, New Delhi, January 3-7, 2001.	Protection against shigellosis by killed oral Shigella vaccine made from <i>Shigella flexneri</i> 2a using rabbit model for shigellosis

## 16. TRAINING RECEIVED BY THE SCIENTISTS IN ABROAD

### **Dr. T. ramamurthy**

Visited Department of Appropriate Technology Development and Transfer, International Medical Center of Japan, Tokyo, Japan from March 4-18, 2001 to work on Drug Resistance of *Vibrio cholerae*, *Vibrio parahaemolyticus* and ETEC strains isolated in Calcutta.

### **Dr. S.K. Niyogi**

Awarded WHO fellowship for 6 weeks in the field of Pathology and Microbiology from 2<sup>nd</sup> June to 7th July, 2000. In the fellowship programme, I visited following laboratories in Canada:

Health Protection Branch, Food Directorate, Bureau of Microbial Hazard, Ottawa, Ontario, Canada (5th to 9th June, 2000).

National Laboratory for Enteric Pathogens, Winnipeg, Manitoba, Canada (12th to 23rd June, 2000).

Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada (26th June to 7th July, 2000).

### **Dr. T. Biswas**

Received JICA sponsored counterpart individual training for six months (August 22, 2000 to February 19, 2001) in the field of Immunology (Mucosal Immunity) in association with Dr. Taeko Dohi, Director, Department of Gastroenterology, Research Institute, International Medical Center of Japan, Shinjuku-ku, Tokyo, Japan.

Dr. Biswas received certificate(s) from i) International Medical Center of Japan, Ministry of Health, Labor and Welfare for training in the field of Immunology and ii) JICA under the International Cooperation Programme, Govt. of Japan.

### **Dr. T. Krishnan**

Visited Japan under the Counterpart Exchange Programme of JICA for research work in the area of astroviruses and human caliciviruses (Norwalk virus and Sapporo virus) from August 2000 to June 2001 in the laboratory of Prof. Shunzo Chiba, Chairman, Department of Pediatrics, School of Medicine, Sapporo Medical University under the able guidance of Dr. Shuji Nakata, Assistant Professor. She also visited the laboratory of Prof. Osamu Nakagomi to be acquainted with latest advancements in the genogrouping of rotaviruses for two weeks. During the course of her programme she also visited to the laboratories of Prof. Hiroshi Ushijima, Dr. N. Okabe, Prof. Yoshifumi Takeda, Dr. Shinji Yamasaki and Prof. H. Kida in Japan.

### **Dr. M.K. Saha**

Acquired training on Molecular Virology at Virology Division - 2, National Institute of Infectious Diseases, Tokyo, Japan, organized by JICA (Govt. of Japan) under the counterpart training programme in connection with the project for prevention of Emerging Diarrhoeal Diseases for the period of ten months (28th March, 2000 to 27th January, 2001).

### **Pallavi Garg**

- i) visited the laboratory of Prof. J. Glenn Morris, Jr., Director, Department of Epidemiology & Preventive Medicine University of Maryland, School of Medicine, Baltimore, USA under UNESCO-ASM Travel Award, 2000 for 4 months (September to December, 2000).
- ii) visited the laboratory of Prof. Douglas E. Berg, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA for 4 months

(March to July, 2001).

## **17. AWARD/HONOUR RECEIVED BY THE SCIENTISTS**

### **Dr. S.K. Bhattacharya**

Awarded Fellow of the National Science Academy (FNA), New Delhi

PDT Member, PDT Meeting on Miltefosine, WHO/SEARO, New Delhi, 20 December, 2000.

Delivered an oration "Molecular Epidemiology of Diarrhoeal Pathogen with Special Reference to *Vibrio cholerae*" in "Professor(Dr.) S.C. Seal Memorial Inaugural Oration of the Indian Association of Public Health on 3<sup>rd</sup> June, 2000.

### **Dr. M.K. Chakraborty**

Elected as the President, Section of Physiology, 89th Session of Science Congress to be held at Lucknow University during 3-7 January, 2001.

### **Dr. R. Kumar**

Awarded WHO fellowship for 6 weeks in the field of Pathology and Microbiology from 2nd June to 7th July, 2000. In the fellowship programme, he visited following laboratories in Canada: Health Protection Branch, Foiod Directorate, Bureau of Microbial Hazard, Ottawa, Ontario, Canada (5th to 9th June, 2000).

National Laboratory for Enteric Pathogens, Winnipeg, Manitoba, Canada (12th to 23rd June, 2000).

Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada (26th June to 7th July, 2000)

### **Dr. S.K. Mondal**

Conferred Post Graduate Diploma of the London School of Hygiene & Tropical Medicine in Community Health in Developing Countries.

### **Dr. S. Dutta**

Selected as a RONPAKU Fellow of Japanese Society of Promotion of Science (JSPS) under the JSPS RONPAKU (Dissertation Ph.D) Program for JFY 2001.

Biological profile has been selected for publication in the forthcoming 6th Edition of Who'swho in Science and Engineering due to be published in 2002-2003 by Marquis Who's Who.

### **Dr. A. Pandey**

During the year 1999, I was nominated for the Master degree course on Primary Health Care Management (MPHM) at ASEAN Institute of Health Development (AIHD), Mahidol University, Thailand.

**Mr. A. Sen**

Received prestigious Ely Lilly Foundation Post Doctoral Fellowship on Emerging and Reemerging Infectious Diseases to do Post Doctoral Research at Centers for Diseases Control and Prevention (CDC) at Atlanta, USA (Senior Research Fellow of Division of Virology)

Poster Non-neutral evolution and jumps across the species barrier suggest group B ancestry for rotaviruses presented at the 69th Annual Meeting of the Society of Biological Chemist (India) held at Science City, Calcutta, December 7-9, 2000 was adjourned as one of the Best Poster of the Conference.

**Kazi Mirajul Hoque**

Received the Young Scientist, 2001 of Indian Science Congress during 88th Session of Indian Science Congress held at New Delhi during 3-7 January, 2001 (Research Fellow, Division of Pathophysiology).

**Saurajyoti Samanta**

Received the Best Poster Award, 2001 of Indian Science Congress during 88th Session of Indian Science Congress held at New Delhi during 3-7 January, 2001 (Research Fellow, Division of Pathophysiology).

**Arunika Mukhopadhaya**

Received the Best Poster Presentation Award, 2001 of Indian Science Congress during 88th Session of Indian Science Congress held at New Delhi during 3-7 January, 2001 (Research Fellow, Division of Pathophysiology).

**Mr. P. Mukhopadhyay**

Society of Biological Chemists (India) presented the Certificate of Commendation for the presentation of B.S. Narasingha Rao Best Poster at the Scientific Sessions of Enzymology, 69th Annual General Body Meeting held at Science City, Calcutta from 7-9 December, 2000 (Research Fellow, Division of Parasitology).

**18. OTHER ACTIVITIES BY THE SCIENTISTS****Dr. P. Dutta**

Editor, Indian Journal of Public Health  
Divisional Editor (Gastroenterology), Child and New Born

**Dr. M.R. Saha**

Dr. M.R. Saha was invited as M.D. (Medical Microbiology) examiner of Calcutta University in the month of May, 2000.

**Dr. S. Dutta**

Invited to become expert of H<sub>2</sub>S strip committee and become one of the nodal persons to

complete phase-I and II part of the multicentric study entitled "H<sub>2</sub>S strip test for safe drinking water supply in rural areas" jointly sponsored by Rajiv Gandhi National Drinking Water Mission and UNICEF.

Conducted a school survey to test the H<sub>2</sub>S strip test kit for drinking water analysis in collaboration with the teachers of Kendriya Vidyalaya, Joka.

Became co-guide of students for MVPH (Master in Veterinary Public Health) course under Calcutta University.

Became guide of a M.Sc. student of Rani Durgawati University, Jabbalpur to complete her dissertation work under her supervision.

### **Dr. M.K. Chakrabarti**

Organized a seminar on "Low Cost Balanced Diet" at Raja N.L. Khan Women's College, Midnapore, WB on 27th July, 2000.

Conducted Viva-Voce Examination of Ph.D. thesis of Calcutta University.

Honorary Lecturer of M.Tech (Bio-Tech) and M.Sc. Part II course (Physiology and Botany) of Jadavpur and Vidyasagar University respectively.

Member of Editorial Board of Indian Journal of Physiology and Allied Sciences and Reviewer of Comparative Medicine.

Member, Under-graduate Board of Studies in Physiology of Calcutta University.

Member of Board of Examiners of Post-graduate Departments of Jadavpur, Calcutta, Burdwan and Tripura University.

Honorary Assistant General Secretary of the Physiological Society of India.

Honorary General Secretary, Prof. N.M. Basu Memorial Committee in Physiological Sciences.

Organized the 3rd Congress of Federation of Indian Physiological Societies held at Science City, Calcutta on 24-26th November, 2000 and 12th Annual Conference of the Physiological Society of India held at ROHC, Salt Lake on 22-23 December, 2000 as Joint Organizing Secretary.

Conducted Scientific Sessions in the Section of Physiology during 88th Session of Indian Science Congress held in New Delhi from January 3-7, 2001 as Recorder of the Section.

Resource Person for UGC sponsored Academic Staff College of Calcutta University for College Teachers Orientation and Refresher Courses.

### **Dr. D. Sur**

Nominated as member of the Editorial Board of Indian Journal of Public Health.

Elected Joint Secretary General and Member of Central Council of All India Body of Indian

Public Health Association.

Selected as District Level Committee Member for Protection of Women's Rights by Deputy Commissioner of Police, Eastern Subdivision of Calcutta police.

Advisor to International Consultation on "Action Research on Health Care Utilisation" being conducted in India by Catholique University of Louvain, Brussels.

Invited by All India Radio to speak on "Anaemia" - broadcast on 22nd October, 2000.

Lecture to school children on "Prevention and Control of Diarrhoeal Diseases" on the occasion of National Science Day at NICED, Calcutta on 8th March, 2001.

Advisor to International Consultation on "Action Research on Health Care Utilisation" being conducted in India by Catholique University of Louvain, Brussels.

### **FUND RECEIVED**

**Dr. S. Dutta**

#### **Title of the multicentric study**

"Operational Research on H<sub>2</sub>S Strip Test for Drinking Water Analysis" to be completed in two phases I and II for accomplishing the objectives of the R & D project.

Funded by : Rajiv Gandhi National Drinking Water Mission (RGNDWM), Ministry of Rural Areas and Employment, Govt. of India and UNICEF.

Duration of the study : 2000-2001

Total sanctioned fund Rs.1,57,000.00

Total fund received Rs.1,57,000.00

## 19. COMMITTEES OF THE INSTITUTE:

### Scientific Advisory Committee (SAC)

Prof. N.K. Ganguly : Chairperson  
 Director General  
 Indian Council of Medical Research  
 Ansari Nagar  
 New Delhi - 110 029

Dr. V.I. Mathan : Member  
 Flat 2A, Mayflower  
 10 Haddows Road, First Street  
 Nungambakkam  
 Chennai 600 006

Dr. M.K. Bhan : Member  
 Professor  
 Department of Paediatrics  
 All India Institute of Medical Sciences  
 Ansari Nagar  
 New Delhi 110 029

Dr. Sandip K. Basu : Member  
 Director  
 National Institute of Immunology  
 Aruna Asaf Ali Marg  
 New Delhi 110 067

Dr. D.C.S. Reddy : Member  
 Prof. of Preventive & Social Medicine  
 Institute of Medical Sciences  
 Banaras Hindu University  
 Varanasi - 221 005

Dr. D. Mahalanabis : Member  
 Director  
 Society for Applied Studies  
 108, Manicktala Main Road  
 Flat No. 3/21  
 Calcutta - 700 054

Dr. K.P. Das : Member  
 Superintendent  
 I.D. & B.G. Hospital  
 Beliaghata  
 Calcutta 700 010

- Dr. Amit Ghose : Member  
 Director  
 Institute of Microbial Technology  
 Sector 39-A  
 Chandigarh - 160 014
- Dr. Sujoy Das : Member  
 Director, Health Services  
 Department of Health and Family Welfare  
 Govt. of West Bengal  
 Writers Building  
 Calcutta - 700 001
- Dr. Shyamal Kanti Bandopadhyay : Member  
 Director of Medical Education  
 Govt. of West Bengal  
 Writers Building  
 Calcutta - 700 001
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 Jawaharlal Nehru University  
 Aruna Asaf Ali Marg  
 New Delhi - 110 067
- Dr.(Mrs.) Ira Ray : Member  
 Addl. Director General of Health Services  
 Directorate General of Health Services  
 Govt. of India  
 Nirman Bhawan, Room No. 252A  
 New Delhi - 110 011
- Dr. S.C. Sehgal : Member  
 Director  
 Regional Medical Research Centre  
 (Indian Council of Medical Research)  
 Post Bag No. 13  
 Port Blair - 744 101  
 Andaman and Nicobar Islands
- Dr. K.K. Datta : Member  
 Director  
 National Institute of Communicable Diseases  
 22, Shamnath Marg, Post Box No. 1492  
 Delhi - 110 054

- Dr.(Mrs.) Archana Ayyagari : Member  
 Professor & Head  
 Department of Microbiology  
 Sanjay Gandhi Post Graduate Institute of Medical Sciences  
 Raebareli Road  
 Lucknow - 226 014
- Dr. D.A. Gadkari : Member  
 Ex-Director  
 National Institute of Virology  
 2/13, Shilpayatan, 1/3 Erandwa  
 Erandujanc Road  
 Pune - 411 004
- Dr. Pradeep Seth : Member  
 Professor and Head  
 Department of Microbiology  
 All India Institute of Medical Sciences  
 Ansari Nagar  
 New Delhi 110 029
- Prof. R.C. Mahajan : Member  
 Emeritus Scientist  
 Department of Parasitology  
 Post Graduate Institute of Medical  
 Education and Research  
 Chandigarh - 160 012
- Dr. Lalit Kant : Member  
 Senior Deputy Director General  
 Indian Council of Medical Research  
 Ansari Nagar  
 New Delhi - 110 029
- Dr. S.K. Bhattacharya : Member Secretary  
 Director, N.I.C.E.D., Calcutta

### **Library Committee**

- Dr.(Smt.) A. Dutta - Chair-Person  
 Dr. S. Chakrabarti - Member  
 Dr. P. Dutta - Member  
 Dr. T.N. Naik - Member  
 Dr. G.B. Nair WPWPC - Member  
 Dr. M.K. Chakrabarti - Member  
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 Sri C.S. Dutta - Member

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Dr. S Chakraborty	-	Member
Dr. M.R. Saha	-	Member
Dr. R. Kumar	-	Member
Dr. P. Dutta	-	Member
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Dr. T.N. Naik	-	Member
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Dr. M.K. Chakrabarti	-	Member
Dr. P. Das	-	Member
Sri C.S. Dutta	-	Member
Sri P.K. Ghoshal	-	Member

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Dr. Tapas Biswas	-	Chairman
Dr.(Mrs.) Aparna Pandey	-	Member
Mr. C.S. Dutta	-	Member
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Mr. S. Ghosh	-	Member
Mr. C.R. Bose	-	Member
Mr. P. Sen	-	Member
Mr. Shyamal Banerjee	-	Member
Mr. Viswanath Besra	-	Member

**Animal Ethics Committee**

Dr. T.N. Naik	-	Chairman
Dr. R. Kumar	-	Member
Dr. M.K. Chakrabarti	-	Member
Dr. P. Das	-	Member
Dr. Ananga Mohan Chandra	-	Member
Dr. Ranatosh Chakrabarty	-	Member
Ms. Purnima Tulsidas	-	Representative Member, (Nominated by the CPCSEA)
Dr. R.K. Sarkar	-	Member Secretary

**Building Committee**

Dr. Sandip Basu	...	Chairman
Dr. Amit Ghosh	...	Member
Dr. A.C. Ghosh	...	Member
Dr. Santu Banerjee	...	Member
Dr. Shyamal Bandopadhyay	...	Member
Dr. K.P. Das	...	Member
Dr. S. Chakrabarti	...	Member

Dr. T.N. Naik	...	Member
Dr. G.B. Nair	...	Member
Dr. M.K. Chakrabarti	...	Member
Dr. P. Datta	...	Member
Dr. M.K. Bhattacharya	...	Member
Representative from ICMR Headquarter		
Mr. P.K. Ghoshal, Maintenance Engineer		
Dr. A. Datta	...	Member Secretary
Dr. S.K. Bhattacharya	...	Project Manager and Coordinator

## 20. LIST OF PUBLICATIONS:

### Publications in international journals

1. Basu A, Garg P, Dutta S, Chakraborty S, Bhattacharya A, Khan T, Bhattacharya SK, Yamasaki S, Takeda Y and Nair GB. *Vibrio cholerae* O139 in Calcutta, 1992-1998: Incidence, antibiograms, and genotypes. *Emerg Infect Dis* 2000; 6 : 139-147.
2. Basu A, Garg P, Datta S, Chakraborty S, Bhattacharya T, Khan A, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y and Nair GB. A seven-year analysis of the incidence, antibiogram and genotypes of *Vibrio cholerae* O139 in Calcutta, India and comparison of the Calcutta O139 genotypes with those isolated from other parts of India. *Emerg Infect Dis* 2000; 6: 20-28.
3. Bhattacharya M, Roy SS, Biswas D and Kumar R. Effect of Mg<sup>2+</sup> ion in protein secretion by magnesium-resistant strains of *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* isolated from the coastal water of Haldia port. *FEMS Microbiology Letters* 2000; 185: 151-156.
4. Bhattacharya M, Choudhury P and Kumar R. Antibiotic and metal resistance strains of *Vibrio parahaemolyticus* isolated from shrimps. *Microbial Drug Resistance* 2000; 6: 171-172.
5. Biswas T. Role of porin of *Shigella dysenteriae* type 1 in modulation of lipopolysaccharide mediated nitric oxide and interleukin-1 release by murine peritoneal macrophages. *FEMS Immunol Med Microbiol* 2000; 29: 129-136.
6. Chattopadhyay UK, Dutta S, Deb A and Pal D. Verotoxin producing *Escherichia coli*- an environment induced emerging zoonosis in and around Calcutta. *Internat J Environ Hlth Res* 2001; 11: 107-112.
7. Chowdhury NR, Chakraborty S, Eampokalap B, Chaicumpa W, Chongsa-Nguan M, Moolasart P, Mitra R, Ramamurthy T, Bhattacharya SK, Nishibuchi M, Takeda Y and Nair GB. Clonal dissemination of *Vibrio parahaemolyticus* displaying similar DNA fingerprint but belonging to two different serovars (O3:K6 and O4:K68) in Thailand and India. *Epidemiol Infect* 2000; 125: 17-25.
8. Chowdhury NR, Chakraborty S, Ramamurthy T, Nishibuchi M, Yamasaki S, Takeda Y and Nair GB. Molecular evidence of clonal *Vibrio parahaemolyticus* pandemic strains. *Emerg Infect Dis* 2000; 6: 631-636.
9. Chakrabarti AK, Ghosh AN, Nair GB, Niyogi SK, Bhattacharya SK and Sarkar BL. Development and evaluation of a phage typing scheme for *Vibrio cholerae* O139 Bengal. *J Clin Microbiol* 2000; 38: 44-49.
10. Chakraborty S, Mukhopadhyay AK, Bhadra RK, Ghosh AN, Mitra R, Shimada T, Yamasaki S, Faruque SM, Takeda Y, Colwell RR and Nair GB. Virulence genes in environmental strains of *Vibrio cholerae*. *Appl Environ Microbiol* 2000; 66: 4022-4028.

11. Dutta P, Mitra U, Dutta S, Manna B, Chatterjee MK, De A and Bhattacharya SK. Hypo-osmolar oral rehydration salts solution in dehydrating persistent diarrhoea in children :double-blind, randomized, controlled clinical trial. *Acta Paediatr* 2000; 89: 411-416.
12. Dutta P, Mitra U, Datta A, Niyogi SK, Dutta S, Manna B, Basak M, Mahapatra TS, Bhattacharya SK. Impact of zinc supplementation in malnourished children with acute watery diarrhoea. *J Trop Paediatr* 2000; 46: 259-263.
13. Dutta P, Mitra U, Manna B, Niyogi SK, Roy K, Mondal C, Bhattacharya SK. Double blind, randomised controlled clinical trial of hypo-osmolar oral rehydration salts solution in dehydrating acute diarrhoea in severely malnourished (marasmic) children. *Arch Dis Child* 2001; 84: 237-240.
14. Dutta S, Deb A, Chattopadhyay UK, Tsukamoto T. Isolation of Shigatoxin producing *Escherichia coli* including O157: H7 strains from dairy cattle and beef samples marketed in Calcutta, India. *J Med Microbiol* 2000; 49: 765-767.
15. Dutta S, Chatterjee A, Dutta P, Rajendran K, Roy S, Pramanik KC, Bhattacharya SK. Sensitivity and performance characteristics of a direct PCR with stool samples in comparison to conventional techniques for detection of *Shigella* and enteroinvasive *Escherichia coli* infection in children with acute diarrhoea in Calcutta, India. *Journal of Medical Microbiology* 2001; 50: 1-8.
16. Dutta D, Bhattacharya MK, Deb AK, Sarkar D, Chatterjee A, Biswas AB, Chatterjee K, Nair GB and Bhattacharya SK. Evaluation of oral hypoosmolar glucose-based and rice-based oral rehydration solutions in the treatment of cholera in children. *Acta Paediatrica* 2000; 89: 1-4.
17. Faruque SM, Saha MN, Asadulghani, Sack DA, Sack RB, Takeda Y and Nair GB. The O139 serogroup of *Vibrio cholerae* comprises diverse clones of epidemic and nonepidemic strains derived from multiple *V.cholerae* O1 or non-O1 progenitors. *J Infect Dis* 2000; 182: 1161-1168.
18. Faruque SM, Saha MN, Asadulghani, Bag PK, Bhadra RK, Bhattacharya SK, Sack RB, Takeda Y and Nair GB. Genomic diversity among *Vibrio cholerae* O139 strains isolated in Bangladesh and India between 1992 and 1998. *FEMS Microbiol Lett* 2000; 15: 279-284.
19. Garg P, Chakraborty S, Basu I, Datta S, Rajendran K, Bhattacharya T, Yamasami S, Bhattacharya SK, Takeda Y, Nair GB and Ramamurthy T. Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992-1997 in Calcutta, India. *Epidemiol Infect* 2000; 124: 393-399.
20. Garg P, Nandy RK, Chaudhury P, Roy Chowdhury N, De K, Ramamurthy T and Yamasaki S. Emergence of *Vibrio cholerae* O1 biotype ElTor serotype Inaba from the prevailing O1 Ogawa serotype strains in India. *J Clin Microbiol* 2000; 38: 4249-4253.
21. Garg P, Sinha S, Chakraborty R, Bhattacharya SK, Nair GB, Ramamurthy T and Takeda Y.

- Emergence of fluoroquinolone-resistance strains of *Vibrio cholerae* O1 biotype ElTor among hospitalized patients with cholera in Calcutta, India. *Antimicrobiol Agents Chemother* 2001; 45: 1605-1606.
22. Ghosh S, Debnath A, Sil A, De S, Chattopadhyay DJ and Das P. PCR detection of *Giardia lamblia* in stool: targeting intergenic spacer region of multicopy rRNA gene. *Mol Cell Probes* 2000; 14: 181.
  23. Hoque KM, Pal A, Nair GB, Chattopadhyay S and Chakrabarti MK. Evidence of calcium influx across the plasma membrane depends upon the initial rise of cytosolic calcium with activation of IP(3) in rat enterocytes by heat-stable enterotoxin of *Vibrio cholerae* non-O1. *FEMS Microbiol Lett* 2001; 196: 45-50.
  24. Jeong JY, Mukhopadhyay AK, Dailidiene D, Wang Y, Velapatino B, Gilman RH, Parkinson AJ, Nair GB, Wong BC, Lam SK, Mistry R, Segal I, Yuan Y, Gao H, Alarcon T, Brea ML, Ito Y, Kersulyte D, Lee HK, Gong Y, Goodwin A, Hoffman PS and Berg DE. Sequential inactivation of rdxA (HP0954) and frxA (HP0642) nitroreductase genes causes moderate and high-level metronidazole resistance in *Helicobacter pylori*. *J Bacteriol* 2000; 182: 5082-5090.
  25. Kurazono H, Yamamoto S, Nakano M, Nair GB, Terai A, Chaicumpa W and Hayashi H. Characterization of a putative virulence island in the chromosome of uropathogenic *Escherichia coli* possessing a gene encoding a uropathogenic-specific protein. *Microb Pathog* 2000; 28: 183-189.
  26. Mukhopadhyay AK, Kersulyte D, Jeong JY, Datta S, Ito V, Chowdhury A, Chowdhury S, Santra A, Bhattacharya SK, Asuma T, Nair GB and Berg DE. Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, Indian *J Bacteriol* 2000; 182: 3219-3227.
  27. Mukhopadhyay P, Sengupta S, Akbar A, Sengupta K, Bhattacharya S, Debnath A, Ganguly S and Das P. Characterization of plasma membrane associated antigens of diagnostic and prophylactic importance in *Entamoeba histolytica*. *Arch Med Res* 2000; 31: 21.
  28. Mitra SN, Mukhopadhyay R, Ghosh AN and Ghosh RK. Conversion of *Vibrio eltor* MAK757 to classical biotype: role of phage PS166. *Virology* 2000; 273: 36-43.
  29. Mitra R, Figueroa P, Mukhopadhyay AK, Shimada T, Takeda Y, Berg DE and Nair GB. Cell vacuolation, a manifestation of the ElTor hemolysin of *Vibrio cholerae*. *Infect Immun* 2000; 68: 1928-1933.
  30. Mitra RK, Nandy RK, Ramamurthy T, Bhattacharya SK, Yamasaki S, Shimada T, Takeda Y and Nair GB. Molecular characterization of rough variants of *Vibrio cholerae* isolated from hospitalized patients with diarrhoea. *J Med Microbiol* 2001; 50: 268-276.
  31. Nandi B, Nandy RK, Mukhopadhyay S, Nair GB, Shimada T, Ghose AC. Rapid method for species-specific identification of *Vibrio cholerae* using primers targeted to the gene of outer membrane protein OmpW. *J Clin Microbiol* 2000; 38: 4145-4151.

32. Panda S, Chatterjee A, Bhattacharya SK, Manna B, Singh PN, Sarkar S, Naik TN, Chakrabarti S and Detels R. Transmission of HIV from injecting drug users to their wives in India. *Int J STD & AIDS* 2000; 11: 468-473.
33. Roy Chowdhury N, Chakraborty S, Eampokalap B, Chaicumpa W, Chongsa-Nguan M, Moolasart P, Mitra R, Ramamurthy T, Bhattacharya SK, Nishibuchi M, Takeda Y and Nair GB. Clonal dissemination of *Vibrio parahaemolyticus* displaying similar DNA fingerprint but belonging to two different serovars (O3:K6 and O4:K68) in Thailand and India. *Epidemiol Infect* 2000; 125: 17-25.
34. Sen A, Kobayashi N, Das S, Krishnan T, Bhattacharya SK, Urasawa S, Naik TN. Amplification of various genes of human group B rotavirus from stool specimens by RT-PCR. *J Clin Virol* 2000; 17: 177-181.
35. Sen A, Kobayashi N, Das S, Krishnan T, Bhattacharya SK and Naik TN. The evolution of human group B rotaviruses. *The Lancet* 2001; 357: 198-199.
36. Sengupta S, Akbar A, Mukhopadhyay P, Ganguly S, Sen P and Das P. Role of excretory secretory products of *E.histolytica* in human amoebiasis. *Arch Med Res* 2000; 31: S226-S228.
37. Yamamoto S, Nakano M, Terai A, Yuri K, Nakata K, Nair GB, Kurazono H and Ogawa O. The presence of virulence island containing the USP gene in uropathogenic *Escherichia coli* is associated with urinary tract infection in an experimental mouse model. *J Urol* 2001; 165: 1347-1351.

#### **Publications in National Journals**

1. Bhattacharya MK, Ghosh S, Mukhopadhyay AK, Deb A and Bhattacharya SK. Outbreak of cholera caused by *Vibrio cholerae* O1 intermediately resistant to norfloxacin at Malda, West Bengal. *J Indian Med Assoc* 2000; 98: 389-390.
2. Bhattacharya J, Samanta S, Hoque KM, Mukherjee A and Chakrabarti MK. *Escherichia coli* heat stable enterotoxin receptors and guanylyl cyclase activity in the intestinal brush border membrane of hamsters and guinea pigs. *Indian J Med Res* 2001; 113: 5-10.
3. Bhowmik S, Ghosh S, Haldar KK, Mitra PK and Manna B. Risk of early onset neonatal septicemia in babies born to mothers with pre-eclampsia. *Indian Pediatr* 2000; 37: 775-779.
4. Chakrabarti S, Panda S, Chatterjee A, Sarkar S, Manna B, Singh NG. B, Naik TN, Detels R and Bhattacharya SK. HIV-1 subtypes in injecting drug users and their non-injecting wives in Manipur, India. *Indian J Med Res* 2000; 111: 189-194.
5. Dutta P. Lactobacillus (Lactic Acid Bacteria) in Diarrheal Diseases. *The Child and Newborn* 2000; 4: 124-129.
6. Ghosh S, Gupta DN, Mondal SK, Saha DR and Sen Gupta PG. Epidemiological profile of acute watery diarrhoea: Observation from a longitudinal study. *Bull RGKMC* 2000; 5: 5-10.

7. Mandal D, Jana S, Panda S, Bhattacharya S, Ghosh T, Bhattacharya SK and Chakrabarti S. Distribution of HIV-1 subtypes among female sex workers in Calcutta, India. *Indian J Med Res* 2000; 112: 165-172.
8. Niyogi SK, Dutta P, Mitra U. Trends in antimicrobial resistance of *Shigella* species isolated from children with acute diarrhoea. *Indian Pediatr* 2000; 37: 339-341.
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