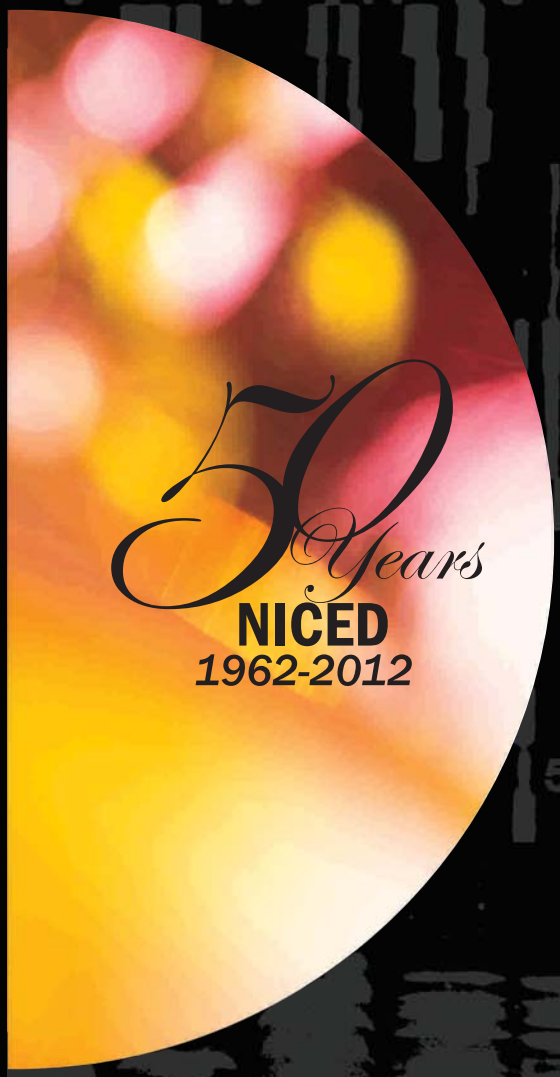
NICED ANNUAL REPORT 2010-2011

वार्षिक रीपोर्ट
Annual Report
2010-2011



राष्ट्रीय कॉलरा और
आंत्र रोग संस्थान
(भारतीय आयुर्विज्ञान
अनुसंधान परिषद्)

National Institute
of Cholera and
Enteric Diseases
(Indian Council of
Medical Research)

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(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

(INDIAN COUNCIL OF MEDICAL RESEARCH)

P33, C. I. T. Road, Scheme-XM, Beliaghata, Kolkata-700010

From the Director's Desk

G. Balakrish Nair
PhD, FNA, FNASc, FAAM
Director

Since its humble inception in 1962 in four rooms on a floor rented in central Kolkata, the National Institute of Cholera and Enteric Diseases (NICED) has pushed ahead with the daunting task of harmonizing basic and applied research as a single functional entity. Initially, the thrust was on operational research, for example implementation of oral rehydration therapy in hospitals and community and studying the transmission of cholera in community, both of which were widely acclaimed research and had tangible implications for the national and global policy on the control of diarrhoeal diseases. This singular thrust in the 1970s and 1980s resulted in a major reduction in diarrhoeal death especially among children. Alongside the Divisions of Epidemiology and Microbiology, which catered to the core interest of NICED, Divisions like those of Immunology, Biochemistry, Pathophysiology, Virology and Electron Microscopy were created to pursue more fundamental research on the molecular and genetic characterization of enteric pathogens and their virulence factors like toxins, colonization factors and on the innate and adaptive immune response in enteric infections. Currently, NICED with several high-end equipments like Scanning and Transmission Electron Microscopes, Atomic Force Microscope, Confocal Microscope, Fluroscence Activated Cell Sorter (FACS), Differential Scanning and Isothermal Titration Calorimeters has one of the best infrastructures in Eastern India for doing in-depth research in biomedical sciences. I am happy that the enrichment in infrastructure is reflected in the marked improvement in the quality of publications from the NICED in recent years. The ideal situation would be the utility of this basic research to address real-time problems on diarrhoea in this country, may it be creating a vaccine or may it be understanding virulence factors and their mechanisms of action at the fundamental level. The debate between basic and applied research has been a tenuous one but at NICED we have attempted to harmonize these two arms of research to derive maximum benefit and this is an effort which goes on.

A glimpse of some of the on-going projects that are of more visible relevance to public health include a

major initiative to strengthen diarrhoea surveillance that covers both inpatients and outpatients in hospitals, urban community and travelers and involves monitoring of a total of26 bacterial, viral and parasitic pathogens associated with diarrhoea. We are planning to expand the surveillance to include rural population as well and a proposal to develop a multi-center National Diarrhoea Surveillance is in the evaluation stages. A clinical trial of a heat- and formalin-killed combination of bivalent *Vibrio cholerae* 01 and 0139 strains, the two serotypes associated with epidemic outbreaks of cholera, has been successfully conducted and the vaccine is now licensed and is commercially available in India. A live oral recombinant vaccine, developed by three Institutes in India is in the process of Phase III clinical trial. It is worthwhile to remember that development of new vaccines is a continuous rather than a one-time venture. This is so, partly because new pathogens emerge and the old pathogens change themselves for survival in the environment and human host.

Diarrhoeal diseases have continued to be the main focus of the NICED. In 1984 when the first AIDS case was detected in Kolkata, the mandate of NICED was expanded to include basic and operational research on the Human Immunodeficiency Virus (HIV). As in previous years, NICED had been called upon to strengthen the efforts of the Central and State Governments by lending our manpower and expertise in emergency-like situations arising from outbreaks of diarrhoeal diseases and occasionally, of some non-enteric viral diseases like avian influenza H5N1 and pandemic H1N1. We are happy to be associated with such national exigencies.

What NICED is today would be impossible without the encouragement and support of the Director-General, ICMR and the Secretary, Department of Health Research, Ministry of Health and Family Welfare, Government of India. Finally, it is my pleasure to acknowledge the Faculty, students and Staff of NICED for their efforts and dedication.

निदेशक की मेज से

जी बालाकृष्णा नायर
पी.एच.डी., एफ.एन.ए., एफ.एन.ए.एस.सी., एफ.ए.ए.एम.
निदेशक

हमारे संस्थान की विनम्रशुरूआत सन् 1962 में हुई थी। सेंट्रल कोलकाता में चार कमरे किराए पर लेकर इसकी स्थापना हुई थी। कॉलरा और आंत्र रोग (एन आई सी ई डी) के नेशनल इंस्टीटयूट आगे एक संयुक्त कार्यात्मक एकाई के रूप में बुनियादी और अनुप्रयुक्त अनुसंधान के चुनौतीपूर्ण काम के साथ आगे बढा। प्रारंभ में, परिचालन अनुसंधान पर जोर दिया था। अस्पतालों और समुदाय में हैजा के संचारण के अध्ययन में मौखिक पुनर्जलीकरण चिकित्सा के उदाहरण के कार्यान्वयन के लिए जो दोनें के व्यापक रूप से प्रसंसित थे, अतिसारीय रोगों के अनुसंधान और नियंत्रण पर राष्ट्रीय और वैश्विक निति के लिए ठोस प्रभाव पड़ा। सन् 1970 के दसक में यह विलक्षण जोर दिया गया और सन् 1980 में बच्चों में विशेष रूप से एक अतिसारीय मौत में प्रमुख कमी के परिणामस्वरूप जानपदिक रोग विज्ञान और सूक्ष्म जीव विज्ञान, एन आई सी ई डी के मुख्य विभाग, इम्यूनोलॉजी, जैव रसायन, पैथोफिसियोलॉजी, विषाणु विज्ञान और इलेक्ट्रॉन माइक्रोस्कोपी के तरह के प्रबागों के लिए और उनके द्वेष की आणविक और आनुवांशिक लक्षण के बारे में अधिक मौलिक शोध को आगे बढ़ाने के लिए बनाये गये थे। टॉक्सिन जैसे कारकों, बसाना कारकों और सहज और अनुकूली आंत्र में संक्रमण प्रतिरक्षा प्रतिक्रिया पर वर्तमान में, स्कैन और ट्रांसमिशन इलेकट्रॉन माइक्रोस्कोप, परमाणु शक्ति सूक्ष्मदर्शी, कॉनफोकल सूक्ष्मदर्शी, फलूरोसेंस सक्रिय सेल सॉटेर (एक ए सी एस) विभेदकों स्कैन आइजोतर्मल टर्ट्रेशन के कैलोरीमिटर्स और उच्चस्तरीय उपकारणों के साथ एन आई सी ई डी सर्वोत्तम संस्थाओं में से एक है। विज्ञान में गहन अनुसंधान से मुझे खुशी है कि बुनियादी ढांचे में संवर्धन से प्रकाशनों की गुणवत्ता में उल्लेखनीय सुधार में परिलक्षित होता है। हाल के वर्षों में एन आई सी ई डी की आदर्श स्थिति इस बुनियादी इस देश में हैजा पर वास्तविक समय समस्थाओं से निपटने में अनुसंधान की उपयोगिता है, यह एक टीका बनाने या इसे बुनियादी स्तर पर बि-बैलापन कारकों और कार्रवाई के अपने तंत्र को समझाने में हो सकता है, बुनियादी और अनुप्रयुक्त अनुसंधान के बीज एक कमजोर बहस है। हम अनुसंधान के इन दो हथियारों को अनूरूप करने के लिए अधिकतम लाभ प्राप्त करने के लिए रत है।

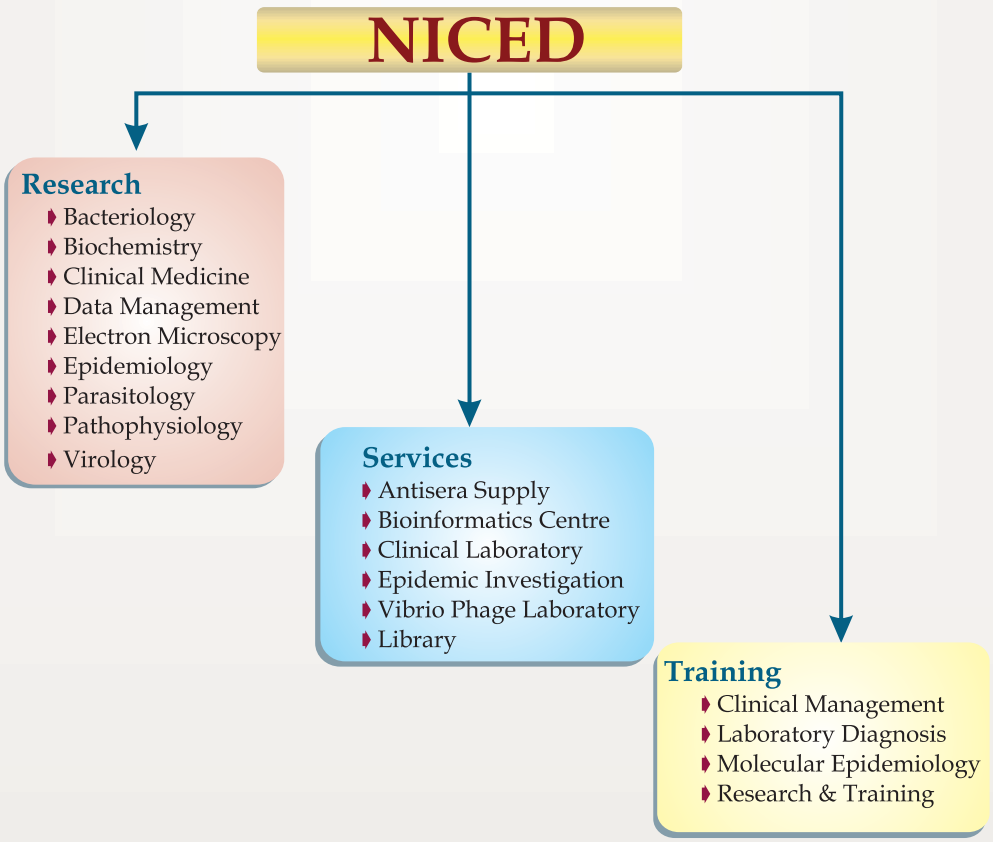
वर्तमान परियोजनाएं अधिक प्रासंगिकता के साथ सार्वजनिक स्वास्थ्य के लिए कार्यरत हैं। जिनकी एक झलक मूलरूप से दस्त निगरानी है जैसे कि दोनो प्रकार के दाखिल मरीज या रोगी और अस्पतालों में बहिरंग विभाग, शहरी समुदाय, यात्रीयों और कुल 26 जीवाणुओं की निगरानी में सामिल किया गया है। यह मजबूत पहल में शामिल हैं। हम वायरल और परजीवी दस्त रोग जनकों के निगरानी के विस्तार की योजना के साथ जुड़े हैं।

ग्रामीण जनसंख्या के रूप में अच्छी तरह से और एक के लिए एक बहु – राष्ट्रीय केन्द्र अतिसार निगरानी विकसित प्रस्ताव सामिल मूल्यांकन के चरण में है। विबरीयो कॉलरा 01 और 0139 उपभेदों, दो हैजा की महामारी फैलाने के साथ जुडे सीरसप्रकारों की गर्मी और फॉरमेलिन मारे संयोजन का एक नैदानिक परीक्षण, सफलतापूर्वक आयोजित किया गया है और टीका के लिये अब लाइसन्स प्राप्त है और भारत में व्यवसायिक रूप से उपलब्ध है। एक जीवित मौखिक टीका पुन संयोजक, भारत में तीन संस्थानों द्वारा विकसित चरण की प्रक्रिया में नैदानिक परीक्षण है यह महत्वपूर्ण है कि नये टीकों का विकास एक बार उधम के बजाय एक निरनतर प्रक्रि ?ा है। यह तो है, आशिक रूप क्योंकि नये रोग जनकों के उभरने और पुराने रोग जनकों स्वंय प्रर्यावरण और मानव अस्तित्व के लिए मेजबान में बदल जाते हैं।

अतिसारी रोगों पर एन आई सी ई डी ने मुख्य ध्यान केनिद्रत किया जाना जारी रखा है। सन 1984 में जब महली बार एड्स का मामला कोलकाता में पाया गया, एन आई सी ई डी को जनादेश के लिए ह्यूमन इम्यूनो डेफिसियेंसी वायरस (एच आई वी) पर बुनियादी और आपरेशनल रिसर्च में विस्तार के लिए शामिल किया गया था। जैसा कि पिछले वर्षों में एन आई सी ई डी को हमारे मानव शक्ति और आपतकालीन जैसे अतिसारीय रोगों और एवियन इन्फलूएंजा (एच 5 एन 1) जैसे कुछ गैर अंत्र वायरल रोंगों (एच 5 एन 1, एच 1 एन 1) के कभी–कभी, के प्रकोप से उत्पन्न होने वाली स्थितियों में विशेज्ञता उदाहरण देकर केंद्रीय और राज्य सरकारों के प्रयासों को मजबूत करने के लिए बुलाया गया था। हम इस तरह के राष्ट्रीय एवं अंत्रराष्ट्रीय आपात संकट के साथ जुडे होने से खुश हैं।

एन आई सी ई डी ने आज अनुसंधान के क्षेत्र में अंत्रराष्ट्रीय स्तर पर जो ख्याति प्राप्त किया है उसके लिए भारत सरकार के स्वास्थ्य अनुसंधान के सचिव तता भारतीय आयुर्विज्ञान अनुसंधान परिषद के महानिदेशक के समर्थन एवं प्रोत्साहन के बिना संभव नहीं था।

अंत में सभी वैज्ञानिकों, छात्रों एवं प्रशासनिक तथा तकनिकि कर्मचारियों के प्रयास एवं समर्पण की सराहना करते हुये मुझे अत्यंत खुशी है।



ADMINISTRATION

Redesignated as a"WHO Collaborating Centre for Research and Training on Diarrhoeal Diseases" by WHO from April 2010-April 2014

CONTENTS

OUR RESEARCH ACTIVITIES

BACTERIOLOGY

Research at the Division of Bacteriology involves characterization of enteric bacteria including *Vibrio cholerae*, *V. parahaemolyticus*, *Salmonella* spp., *Helicobacter pylori* and *Shigella* spp., isolated from hospital and community surveillance today by applying molecular genetic and classical microbiological techniques. The Division provides referral services for identification and characterization of different enteric bacteria and also laboratory support during investigation of outbreaks / epidemics of diarrhoeal diseases in West Bengal and other parts of the country. In the recent past, the Division has focused on in-depth analysis of novel serotypes and virulence genes relevant to changes in drug resistance pattern, transmission characteristics and clinical features of the recent isolates. Data on clonality of El Tor hybrid strains from Indian and other Asian countries will be shared with members of the PulseNet Asia-Pacific. Facilities for molecular methods will be established for the rapid identification of enteric pathogens from stool specimens that were negative by conventional assay systems. Phage typing and phage therapy study are two ongoing activities of this division. This Division is also actively engaged in exploring the role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal in relation to cholera incidence. The department is also involved in studies in relation to the pattern of colonization of the neonatal gut with Gram negative bacilli and its association with neonatal sepsis.

Scientist :
S. K. Niyogi, Scientist F
T. Ramamurthy, Scientist F
S. Dutta, Scientist F
A. Palit, Scientist E
B. L. Sarkar, Scientist E
R. K. Nandy, Scientist D
A. K. Mukhopadhyay, Scientist D
S. Basu, Scientist C
H. Koley, Scientist C

Staff :
J. Kharwar, Technical Officer A
S. K. Bhowmik, Technical Officer A
S. K. Dutta, Technical Officer A [retired on 31 Jan 2011]
A. K. Mondal, Technical Officer A
N. K. Sikdar, Technical Officer A
R. B. Bose, Technical Officer A
S. R. Ghosh, Technical Assistant
A. Ganai, Technical Assistant
S. De, Technical Assistant
S. C. Saha, Technician B
R. Balmiki, Technician B
M. L. Gupta, Technician B
K. Roy, Technician B
S. De,Technician B
A. K. Saha, Attendant Services
P. Samanta, Attendant Services

B. Roy, Attendant Services
K. Ghosal, Attendant Services
V. K. Singh, Attendant Services
S. Mondal, Attendant Services

Fellows : J. Alam (Pre-doctoral Fellow)
P. Mukherjee (Pre-doctoral Fellow)
A. Naha (Pre-doctoral Fellow)
P. Ghosh (Pre-doctoral llow)
K. Bhattacharya (SRF)
P. Batabyal (SRF)
G. Chowdhury (SRF)
H. Mukherjee (SRF)
P. Das (SRF)
S. Dutta (SRF)
S. Barman (SRF)
S. Ghosh (SRF)
S. Roy (SRF)
T. Patra (SRF)
J. Banerjee (Ghosh) (SRF)
A. Sinha (SRF)
R. De (SRF)
T. Nag (SRF)
S. Mitra (SRF)
D. Dutta (RA)
S. Nandy (RA)
A. Jaiswal (JRF)
F. Calcattawala (JRF)
P. Jain (JRF)
S. Mukherjee (JRF)
S. Dutta (JRF)
A. Sarkar (JRF)

Plasmid-mediated quinolone resistance in clinical isolates of *Vibrio fluvialis*.

Investigators : T. Ramamurthy, G. Chowdhury, G. P. Pazhani, G. B. Nair and A. Ghosh.

Vibrio fluvialis, which causes cholera-like diarrhoea in humans, is one of the aetiological agents of acute diarrhoea in Kolkata, India, and is resistant to many antimicrobial agents. Two *V. fluvialis* isolates resistant to fluoroquinolones and β-lactam antimicrobials were found to have mutations in the quinolone resistance-determining regions (QRDRs) of GyrA at position 83 and of ParC at position 85 as well as carrying a 150-kb plasmid harbouring the quinolone resistance gene qnrA1, the ciprofloxacin-modifying enzyme-encoding gene aac(6')-Ib-cr and genes encoding for extended-spectrum β-lactamases such as blaSHV and blaCTX-M-3. The qnrA1 gene was identified in a complex sul1-type integron in a plasmid of the transconjugants. Southern hybridisation and sequence analysis of qnrA1 and its flanking regions confirmed the presence of aac(6')-Ib-cr and blaCTX-M-3 but these were not associated with the sul1-type integrin (Fig.). An orf513 was found upstream of the qnrA1, and arr-

3 was identified further upstream. The downstream sequence of the second 3-CS region of class 1 integron was also analysed. These sul1-type integrons were found to have 100% homology with the In37 integron identified in an *E. coli* isolated from China, except for the gene cassettes aac(6)-Ib, blaOXA-30 and catB3 upstream of orf513 (Fig). Although the presence of many qnr alleles has been reported amongst enteric bacteria in Asian countries, this is the first report on the emergence of qnrA1 in India. qnrA1 along with aac(6')-Ib-cr and blaCTX-M-3 genes on a mobile plasmid may spread to other bacterial species that are under the selective pressure of fluoroquinolones and β-lactam antimicrobials in this region.

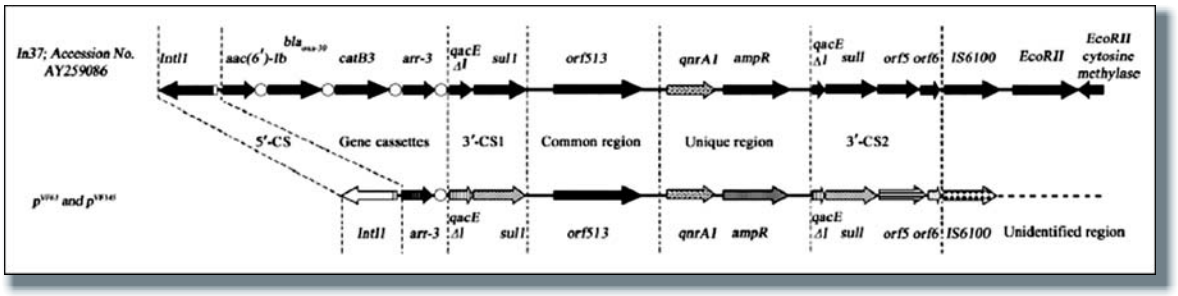


Fig. Comparison of the sequences of sul1-type integrons of *E. coli* and *V. fluvialis* strains harbouring qnrA1.

Genetic characteristics and changing antimicrobial resistance among *Shigella* spp. isolated from hospitalized diarrhoeal patients.

Investigators : T. Ramamurthy, S. Ghosh, G. P. Pazhani, G. Chowdhury, S. Guin, S. Dutta, K. Rajendran, M. K. Bhattacharya, Y. Takeda, S. K. Niyogi, and G. B. Nair

During 2007-2010, 212 (6.5%) Shigella strains were identified from 3262 diarrheal patients. The proportion of different Shigella serogroups were: 160 (75.5%) *S. flexneri*, 33 (15.6%) *S. sonnei*, 14 (6.6%) *S. boydii* and 5 (2.3%) *S. dysenteriae*. Throughout the study period, *S. flexneri* was the most common serogroup with predominance of serotype 2a during 2009. Among 14 *S. boydii* strains, type 12 was common.

All the Shigella strains isolated in this study are resistant to multidrugs. Overall, these strains exhibited high-level resistance to trimethoprim-sulfamethoxazole (95%), nalidixic acid (90%), tetracycline (90%), ciprofloxacin (89%), norfloxacin (83%), ofloxacin (83%), furazolidone (78%) and chloramphenicol (67%). The resistance to ampicillin (49%) and azithromycin (33%) was moderate while very low resistance to third generation cephalosporin and ceftriaxone (1%) was observed. Among *S. flexneri*, serotype 2a was totally resistant to nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin and streptomycin. Ninety six per cent of the strains were resistant to co-trimxazole, tetracycline and 90% of strains were resistant to ampicllin and chloramphenicol. Resistance to azithromycin was detected among 32% of the strains while 2.5% strains were susceptible to ceftriaxone. Majority of the *S. sonnei* strains were resistant to ciprofloxacin (94%), norfloxacin (94%), ofloxacin (94%), co-trimoxazole (94%), tetracycline (85%), and azithromycin (27%). Fluoroquinolone resistance was less in *S. boydii* with the exception of one strain (serotype 18) that was highly resistant to all the antimicrobial agents tested. Only one *S. dysenteriae* type 1 was isolated in this study during 2010, which was resistant to all the antimicrobials tested except ceftraixone and azithromycin. All the *S. dysenteriae* type 2 strains exhibited reduced susceptibility to fluoroquinolones.

Resistant Profile	Serotype
AM, AZM, C, CIP, CRO, FX, NA, NOR, OFX, SXT, TE	Sb 18 (1), Sf UT (1)
AM, AZM, CIP, CRO, FX, NA, NOR, OFX, SXT, TE	Sf UT (1)
AM, C, CIP, CRO, FX, NA, NOR, OFX, SXT, TE	Sf 2a (1)
AM, AZM, C, CIP, FX, NA, NOR, OFX, SXT, TE	Sf 2a (24), Sf 3a (2), Sf UT (1)
AM, C, CIP, FX, NA, NOR, OFX, SXT, TE	Sf 1b (1), Sf 2a (32), Sf 3a (2), Sf Y (1), Sf 2b (1), Sd 1 (1)
AZM, C, CIP, FX, NA, NOR, OFX, SXT, TE	Sf 2a (1), Sf 3a (9), Sf 3b (1), SS (1)
AM, AZM, CIP, FX, NA, NOR, OFX, SXT, TE	Sf UT (2)
AM, C, CIP, NA, NOR, OFX, SXT, TE	Sf 2a (11), Sf 3a (1), Sf 4 (1)
AM, CIP, FX, NA, NOR, OFX, SXT, TE	Sf 2a (1), Sb 9 (1), Sf UT (1), Sb 12 (2)
AM, AZM, C, CIP, NA, NOR, OFX, SXT	Sd 2 (1)
C, CIP, FX, NA, NOR, OFX, SXT, TE	Sf 3a (17), Sf 3b (1), SS (1)
AZM, CIP, FX, NA, NOR, OFX, SXT, TE	SS (5), Sf 6 (2), Sb 1 (1)
AZM, C, CIP, NA, NOR, OFX, SXT, TE	Sf 3a (1)
AM, C, CIP, FX, NA, NOR, OFX, TE	Sf 2a (2)
CIP, FX, NA, NOR, OFX, SXT, TE	Sf 2a (3), SS (18), Sf Y (1)
C, CIP, NA, NOR, OFX, SXT, TE	Sf 3a (10), Sf 3b (1)
AM, AZM, CIP, NA, NOR, OFX, SXT	Sd 2 (2)
AZM, CIP, FX, NA, NOR, OFX, SXT	SS (1), Sf 2a (1)
AM, C, CIP, NA, NOR, OFX, TE	Sf 3a (1), Sf 2a (1)
AM, AZM, CIP, FX, NA, NOR, OFX,	Sb 12 (1),
AZM, CIP, NA, NOR, OFX, SXT, TE	SS (1),
CIP, FX, NA, NOR, OFX, SXT	Sf 2a (1), SS (1)
CIP, NA, NOR, OFX, SXT, TE	Sf 2a (2), SS (1), Sf 6 (1)
C, CIP, FX, NA, NOR, OFX, TE	SS (1)
AM, AZM, FX, NA, SXT, TE	Sf UT(1)
AZM, FX, NA, SXT, TE	SS (1), Sf UT(1)
CIP, FX, NA, NOR, OFX	SS (1), Sf 3a (1)
AM, AZM, FX, SXT, TE	Sf UT (2)
AM, FX, NA, SXT, TE	Sf UT (3), Sb 1 (1)
C, FX, NA, SXT, TE	Sf 3a (1)

Table . Antimicrobial Resistance Patterns of *Shigella* species and their Serotypes

Serogroup abbreviation:

Sf, *S. flexneri*; Sf UT, *S. flexneri* Untypable, Sb, *S. boydii*, SS, *S. sonnei*, Sd, *S. dysenteriae*; Numbers in parenthesis indicate number of isolates for each serotype.

Molecular characterization of *Salmonella enterica* serovar Typhi isolated from blood of clinically suspected typhoid fever cases in children in Kolkata

Principal Investigator : S. Dutta

Classically typhoid fever is diagnosed by isolation of *Salm. enterica* serovar Typhi organism from blood samples using standard microbiological culture method, which has only 50% sensitivity and requires about one week. The available serology based tests like The Widal, Typhidot and Tubex kit tests are neither specific nor sensitive and could not fulfill the criteria of an ideal test. Therefore molecular methods have been used directly on clinical samples for better performance. PCR is the most common method and we used one PCR method amplifying flic-d gene for rapid diagnosis of typhoid fever even after antimicrobial therapy. Although culture positive cases are usually considered as gold standard, yet lack of growth of *S. typhi* after antimicrobial therapy limits its use.

So far 242 blood samples were collected from clinically suspected typhoid fever cases and processed by culture, Widal serology and PCR. Total 17 (7%) samples were positive by culture (15 *S. typhi* and 2 *S. paratyphi* A), 81 by Widal (TO >80; 33.5%) and 110 (45.5%) by typhoid specific PCR showing better performance by PCR method. More samples are being collected and tested. Antimicrobial resistance pattern showed increasing quinolone resistance (>90%) among the isolates.

We have included a total of 378 isolates of *Salmonella typhi* from Kolkata during 2003 to 2006, for molecular typing (SNP typing) and its relation with antimicrobial resistance pattern is shown in the Table. H58B was the major subtype.

Table 1. Nalidixic acid resistance phenotypes split by *Salmonella typhi* haplotype

A. S. Typhi haplotype	H14	H16	H37	H42	H50	H52	H55	H58	H8	H85	All
Nal^S Cip^S	6	4	1	35	2	1	1	120	1	0	171
Nal^R Cip^S	14	1	0	24	4	1	0	98	0	5	147
Nal^R Cip^I	5	0	0	6	0	0	0	40	0	0	51
Nal^R Cip^R	0	0	0	0	0	0	0	2	0	0	2

B.H58 (sub) haplotype	A	B	E2	G	G0	H64	H65	I1	I3	I4	K1	All H58
Nal^S Cip^S	5	112	0	3	0	0	0	0	0	0	0	120
Nal^R Cip^S	14	23	1	43	1	12	1	0	1	1	1	98
Nal^R Cip^I	2	13	0	20	0	4	0	0	0	0	0	40
Nal^R Cip^R	0	0	0	0	0	0	0	2	0	0	0	2

MICs of the antimicrobials were: NalS, nalidixic acid sensitive (MIC < 8 µg/mL); NalR, nalidixic acid resistant (MIC > 256 µg/mL); CipS, ciprofloxacin susceptible (MIC < 0.125µg/mL); CipI, ciprofloxacin reduced susceptible (MIC ⩾ 0.125 µg/mL); CipR, ciprofloxacin resistant (MIC >16 µg/mL).

Evaluation of Anti-Typhoid and Anti-Diarrhoeal Activity of three Ethnomedicinal Plants of Tribal use from different parts of India.

Principal Investigator : S. Dutta

Primarily anti-Typhoid (*Salmonella enterica serovar* typhi) activity of the decoction and crude alcoholic extract of *Shorea robusta* L. (Dipterocarpaceae) as practiced by the Kaatabhai tribes of Maharashtra, was determined. Anti-typhoid activity of crude aqueous and hydroalcoholic extracts of *Achyranthes aspera* Linn. (Amaranthaceae) and *Ephedra ciliate* Fisch. (Ephedraceae), other two medicinal plants, used by the tribes of Uttar Pradesh, Madhya Pradesh and Rajasthan were also determined. Secondarily the antidiarrhoeal and antidysenteric activity of decoction and hydroalcoholic extract of all three medicinal plants as practiced by some tribal population was determined.

The preliminary *in vitro* antibacterial study revealed that, both aqueous and methanolic extract of *Shorea robusta* has considerable antibacterial activity against *S. typhi*, and *Shigella flexneri* and *Shigella dysenteriae* with MIC_{90}<1000 µg/ml. Considerable synergistic activity was observed when tested in vitro by 1000-2500 µg and 5000 µg of the extract along with 30 µg of chloramphenicol. Both TLC and repeat study with HPLTC revealed that at least two major compounds were present in the aqueous extract of *S. robusta*. Baseline and peak display also revealed the same.

Role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal: relation with cholera incidence

Investigators : A. Palit, B. L. Sarkar and G. B. Nair

Objectives of the study :

The present project involves the investigation of the environmental *Vibrio* dynamics. The aims are:-

- Monitoring of physical-chemical features (e.g., pH, salinity, TDS, conductivity) of the estuarine water along the same transect
- Culture of *Vibrio*s and monitoring its abundance along the transect
- Record the direct viable counts of *V. cholerae* with direct fluorescent antibody method to reveal the VBNC state of the pathogenic bacteria
- Biochemical differentiation of isolated Vibrio strains to observe its diversity change in different parts of the estuary

- Identification of different O1, O139 and non-O1 non-O139 strains of *V. cholerae* by serological methods
- The assessment of the existence of different aquatic and benthic *Vibrio* communities with own characteristic composition and their seasonal dynamics
- The influence of river bank erosion and sediment resuspension - i.e. benthic inputs, by e.g. cyclones or peak rainfall on *Vibrio* diversity and abundance in the water column.
- The relationship of vibrio dynamics with seasonal change and cholera incidence patterns in the Kolkata region

Results : Three different zones have been identified as a result of this study.

- "high-salinity," (30–15 ppt) sector (upto 20 km inland),
- "transition" (15–3 ppt) (20–80 km inland) &
- "low-salinity" (3.0–0.1 ppt) (80 km away from river mouth towards inland).

Physico chemical analysis shows variation in salinity level (along with seasonal as well as tidal changes) at a single site has been observed in these sites except Kolkata (Hooghly). Salinity varied from 28.5 ppt at the estuary's mouth to 0.2 ppt at 130 km inland. The total dissolve solids (TDS) ranges between 14.5–1380 mg/L. High TDS has been observed in estuarine region (may be due to higher tidal influence) in comparison to inland river. However, at Diamond Harbour higher TDS has been observed only after heavy wind (which accelerate the tidal influence) and after heavy rain. At Kolkata, Hoogly site the lower range of conductivity (250-650µs/cm) is related to reduced salinity.

Bacteriological analysis shows that till now Total bacterial count (TBC) ranges between 8 X 101 to 5 X 109. Cultivable Vibrio count (CVC) seems to be very high at Basonti (Site-1) (0.6 x 101 to 6 X 103cfu/ml) in comparison to Namkhana (Site-2) (0.3 X 101 to 3 X 103 cfu/ml). An increasing prevalence of *Vibrio* sp. has been distinctly recorded in summer and pre-monsoon. Surprisingly, at Diamond Harbour and Howrah very less number of cultivable Vibrio organism has been identified in winter season. In all the study sites, coliform contamination has been observed (TCC) (2-293cfu/ 10ml). Though the higher prevalence of TEC along with TCC has been observed at

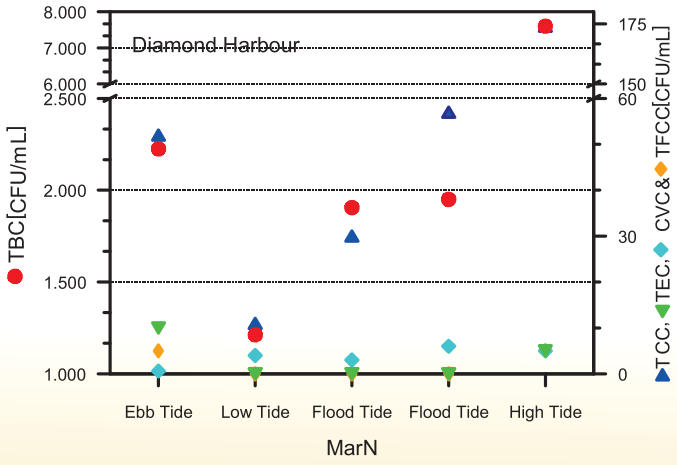


Fig. I. Comparison of TBC, CVC, TEC, TCC and TFCC value for the months of March,2011 at Diamond Harbour showing tidal variation

Kolkata and Basonti, prevalence of TFCC could only be noticed in Kolkata (Hooghly) site. In Diamond Harbour (Site-3), tidal influence has been distinctly observed on the prevalence of different bacterial community in terms of seasonality. During high tide, the intrusion of saline brackish water triggers up the salinity as well as the halophillic bacterial community and on the other hand low tide depicts the lowest prevalence of all type of bacterial community (Fig-I).

Confirmation of biochemically identified Vibrio isolates: Altogether, 116 suspected *V. cholerae* have been identified through biochemical tests. 87% of the isolates are found to be *V. cholerae* non O1. A few 13% *V. cholerae* O1 isolates have also been identified so far (Mostly from Diamond Harbour i.e. Site-3 and Howrah i.e Site-4). Serology demonstrated the predominance of *V. cholerae* Ogawa serotype. Molecular analysis of *V. cholerae* O1 isolates has revealed that quite a few number of isolates posses both ctx and tcp gene and another few isolates only tcp gene. Antibiotic sensitivity profile revealed that most of the environmental *V. cholerae* O1 isolates are highly sensitive against most of the conventional antibiotics (viz. fluroquinolon, cephalosporin, tetracycline etc.). A few virulent *V. cholerae* O1 isolates from Kolkata Hooghly sites showed some resemblance with clinical isolates in terms of drug sensitivity pattern. Presence of *V. parahaemolyticus*, although few, have been noted in medium saline zone, the implications of which are under analysis. Prevalence of Sucrose fermenting vibrio was higher in low saline region, a fact not collating with results from high saline zones (higher prevalence of non-sucrose fermenting vibrio). Implications are under review.

Role of estuarine biogeochemistry on abundance and types of *Vibrio cholerae* in West Bengal: seasonality and relation with cholera incidence

Investigators : G. B. Nair, A. Palit & R. J. Lara

Objectives:

- To understand the influence of *seasonally-driven variations* of the biogeochemical setting of different coastal ecosystems on changes of *Vibrio* diversity and abundance along with different aquatic and benthic *Vibrio* communities.
- To evaluate the influence of river bank erosion and sediment re-suspension, i.e. benthic inputs, by cyclones or peak rainfall on *Vibrio* diversity and abundance in the water column
- To evaluate the relationship of *seasonally-driven variations* and existence of different aquatic and benthic *Vibrio* communities to cholera incidence patterns in the Kolkata region.

Salient observations:

A. Summary of Hooghly river observations (Dec'2010- Mar,2011)

Diamond Harbour

- TBC : 1000 to 30,000 cfu/ml
- Total coliform : 10 to 1000 cfu/ml
- Total *E. coli* count : <1 to 15 cfu/ml

- Total Fecal Coliform : <1 to 75 cfu/ml
- CVC count :1 to 300 cfu/ml

Howrah Bridge

- TBC ranges between: 1,000 to 15,000 cfu/ml
- Total coliform count: 100 to 1,000 cfu/ml
- Total *E. coli* count: 50 to 800 cfu/ml
- Total Fecal Coliform: 50 to 500 cfu/ml
- CVC count: 0.1 to 20 cfu/ml

Highlights:

- Higher CVC at Diamond Harbour Site may be because of higher turbidity & Salinity.
- Tidal influence on bacterial counts is evident but not consistent in every sampling.
- Increase in temperature enhances bacterial counts.

B. Summary of Hooghly transect and Sunderban Matla transect (Dec'2010- Mar,2011)

Diamond Harbour

- Salinity: 2 to 6 PSU
- Turbidity: 50 to 900 NTU
- TBC : 10^3 to 10^4 cfu/ml
- Total coliform: 10 to 1,000 cfu/ml
- CVC: 1 to 50 cfu/ml
- 10 to 300 cfu/ml

Matla

- Salinity: 20 to 30 PSU
- Turbidity: 5 to 30 NTU (Dec, Jan,Feb)
  50 to 350 NTU (March)
- TBC ranges between: 10^4 to 10^8 cfu/ml
- Total coliform count: 0.01 to 0.4 cfu/ml
- CVC : <1 to 150 (Dec, Jan,Feb)
  50 to 1,000 cfu/ml (March)

Highlights:

1. Increase in temperature lowers CVC in >20 µ fraction
   but enhances CVC in <20 µ fraction
2. Turbidity: In Dec, Jan, higher in high saline zone near Bay of Bengal
   In Mar, higher in comparatively low saline zone
3. Low salinity gradient in each cruise
   (e.g., 21-24, 23-26, 26-30 in Jan, Apr & May, respectively)

Nationwide screening of phage types of *V. cholerae* O1 and O139.

Investigator : B. L. Sarkar.

During the period under study, a total of 878 strains of *V. cholerae* were received from different parts of the country for serotyping, biotyping and phage typing. Of these, 629 (71.6%) representative strains confirmed as *V. cholerae* O1 biotype ElTor were included in phage typing study. This year, highest number of strains was received from Maharashtra state. Majority of the strains belonged to Ogawa (97.9%) followed by Inaba 9 (2.0%). A total of 22 (3.4%) strains were found to be untypeable with the conventional scheme of Basu and Mukerjee. These strains were grouped under type 2 with Basu and Mukerjee scheme. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (81.0%) followed by type 26 (3.8%), 23 (1.1%), type 13 (5.2%), 12 (1.5%) respectively. It has been observed that type 27 was the predominant phage type circulating in this country. During the current year, not a single strain of *V. cholerae* O139 was received for phage typing study from any parts of the country.

Biotype, serotype and phagetype of *V.Cholerae* strains received during 2010-2011

State	No of Strains	Biotype		Serotype		Basu & Mukherjee			New Phage													
		Eltor	Classical	Ogawa	Inaba	T-2	T-4	UT	4	7	12	13	14	17	19	20	21	23	24	25	26	27
Andhra Pradesh	86	86	-	81	5	80	-	6	-	2	3	6	-	-	-	-	-	-	-	-	4	71
Gujrat	60	60	-	60	-	60	-	-	-	-	-	2	-	-	-	1	2	1	-	-	1	53
Karnataka	8	8	-	8	-	7	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	7
Maharashtra	266	266	-	266	-	254	-	12	-	4	7	16	4	-	-	2	3	4	5	2	12	207
Punjab	22	22	-	22	-	22	-	-	-	1	-	-	-	-	1	-	-	-	-	-	1	19
Tamil Nadu	152	-	-	144	8	149	-	3	3	2	-	9	-	2	2	-	3	2	1	1	3	124
West Bengal	35	35	-	35	-	35	-	-	-	-	-	-	2	-	-	1	1	-	-	-	3	28
Grand Total	629	629	-	616	13	607	-	22	3	9	10	33	7	2	3	4	9	7	6	3	24	509

Studies on effect of *V. cholerae* phages in RITARD model

Investigators : B. L. Sarkar, H. Koley and D. R. Saha

During the period under study, *Vibrio cholerae* O1 strain MAK 757 and the cocktail phage of *V. cholera* O1 (five phages) were challenged in Rabbit Ileal Loop (RIL) model. Two sets of rabbits were used in this purpose, one was infected with only *V. cholerae* MAK 757 strain and in another one MAK 757 and a cocktail of respective vibriophages were used. In both the rabbits, diarrhoeagenic inflammation was observed but it was found lesser in the rabbit where vibriophages were challenged.

After that, similar experiment was carried out in RITARD model. In one set (control), the rabbit was infected with only *V. cholerae* MAK 757 strain. In another set, rabbit was challenged with MAK 757 with cocktail phage of *V. cholerae* O1. This study concerns the feasibility of possible exploitation of bacteriophages as a biocontrol agent to eliminate the pathogen *V. cholerae* in the

gut. Phage-treated rabbit euthenized 24h post infection had 100 fold less infectious cells (1.3×109 CFU/ml) compares to the untreated control. It also showed the presence of 100 fold increase in phage titre (0.9×1010 PFU/ml) compared to the initially administered dose. On the other hand, histological results revealed that in control rabbit (MAK 757 treated) vili lost its normal shape and that it displayed more inflammatory cellular infiltration in lamina propia. In experimental rabbit (phage-treated) on the other hand vili of the intestinal mucosal almost appeared normal.

In the second phase of animal experiment, cocktail phage was used against *V. cholerae* in mice model. There is well established previous experiments were done to evaluate reproducibility in oral mice cholera model. In present study three groups (5 mice each) of mice were taken. Control group, Post cholera treatment group, and Pre cholera treatment group. To observe the phage activity we took intestinal colonization model in CFU as a measure at different time periods (12hours, 24 hours, 36 hours, 48 hours, and 60 hours). In control group only bacteria(1x109CFU/ml) was fed, in post treatment group bacteria was given in 0 hours and the cocktail phage(1x 108 PFU/ml) is given at +6 hours and +12 hours. In pretreatment group bacteria in 0 hours and the cocktail phage is given in - 6 hours and - 12 hours. It has been found that both pre and post treatment are effective but post treatment against cholera is more effective than the pre treatment. So it can be said that the cocktail phage are also able to decrease the *V. cholerae* in vivo as well as in vitro. The studies are under way to confirm that cholera phage could be the alternate to antibiotic.

Functional Entner Doudoroff (ED) is essential for *Vibrio cholerae* pathogenesis.

Investigator : R. K. Nandy

The organism *Vibrio cholerae* is autochthonous to estuaries. However, pathogenic counterparts have adapted enormously to survive in very disparate environment of human intestinal milieu to cause cholera. *V. cholerae* can utilize different types of carbohydrates during in-vivo survival in the human intestine. In microbial community breakdown of sugars through Entner Doudoroff (ED) pathway has recently been shown to play important roles in the physiology of many pathogenic bacteria. However, no study has yet been carried out for *V. cholerae*. In silico genomic analysis of *V. cholerae* N16961 revealed ED pathway genes (edd and eda) linked to GNT kinase (gntK) and permease (gntP) and their regulatory element (gntR), essential for gluconate (GNT) catabolism. Such organization is very unique as compared to other enterobacteriaceae (Fig. 1). Gene manipulation studies established sole involvement of ED pathway in GNT catabolism by *V. cholerae* (Fig. 2) which in turn induced expression of virulence genes in vitro (Fig. 3). Deactivation of ED pathway caused severe attenuation in virulence that were established through in vitro as well as in vivo studies using animal models. Comparative analysis on the transcriptome profiles between cells grown in media supplemented with glucose and gluconate revealed 1285 genes were up regulated in GNT grown cells and these include most of the genes that are known to be induced during in vivo growth. *V. cholerae* being a facultative anaerobe that survives in the intestinal milieu may receive some signals for triggering functional ED pathway to maintain its pathogenesis which can be mimicked in vitro by functional activation of ED pathway through gluconate supplementation. This study may help to initiate a situation for drug designing or probiotic therapy to treatment of diarrheal patients in future.

Intact cag Pathogenicity Island of *Helicobacter pylori*: impact on disease manifestations in Kolkata

Investigator : A. K. Mukhopadhyay

Helicobacter pylori is genetically more diverse than most bacterial species. Strain specific genetic diversity has been postulated to be involved in the organism's ability to cause different diseases. The severity of *H. pylori*-related disease correlates with the presence of a cag pathogenicity island (cag PAI) in western countries. Genetic diversity within the cag PAI may have profound effect on the pathogenic potential of the infecting strain. So, variation in the genetic structure of the cag PAI might be responsible for different clinical outcomes. The cag PAI is a 40-kb locus containing around 31 genes, 6 of which are thought to be encoded by a putative type IV secretion system and several other genes are responsible for cytokines induction triggering severe host inflammatory responses. In this project we characterized the cag PAI of *H. pylori* strains from duodenal ulcer (DU) patients and asymptomatic or non-ulcer dyspepsia (NUD/AV) subjects from Kolkata, India. Unlike *H. pylori* strains isolated from other part of India, 82.6% of the strains used in this study had intact cag PAI, 9.6 % had partially deleted cag PAI and 7.7% of the strains lacked the entire cag PAI. Furthermore, the expression of cagA mRNA was confirmed by RT-PCR for the representative strains from both DU and NUD/AV subjects indicating the active cagA promoter regions of these strains. 66.7% of Kolkata strains produced ~390-bp shorter amplicon than standard strain 26695 for HP0527 gene, homologue of virB10 (Figure 1). However, sequence analyses confirmed that the deletion did not alter the reading frame of the gene and mRNA transcripts were detected by RT-PCR analysis. The strains isolated from DU and NUD/AV express CagA protein and possess functional Type IV secretion system, as revealed by Western blot analyses (Figure 2). Interestingly, no significant differences in cag PAI genetic structure were found between DU and NUD/AS individuals suggesting other bacterial virulence factors, host susceptibility and environmental determinants also influence the disease outcome at least in certain geographical locations. Finally, although the cagPAI is an important virulence component for infection, the determination of the direction of disease development (gastritis, ulcers, or cancer) is likely to involve a highly complex interplay of many bacterial and/or host factors.

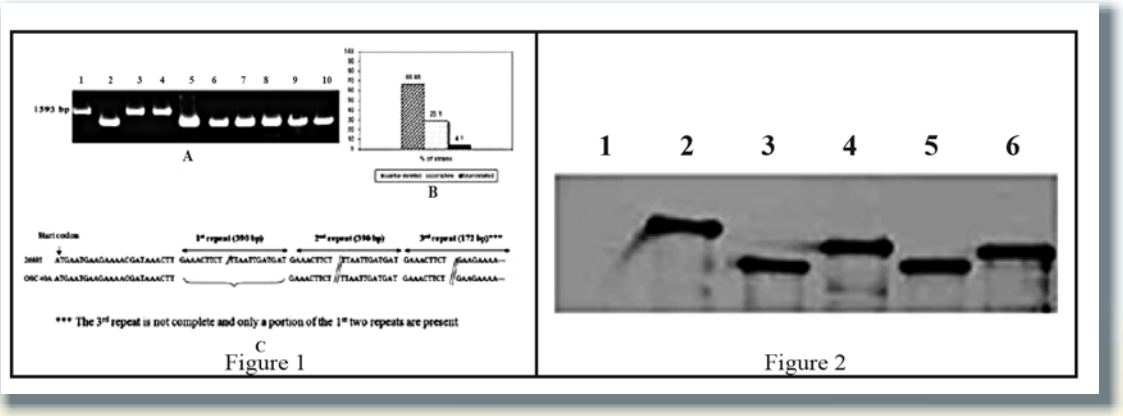


Figure 1

Figure 2

Figure 1 A) PCR assay of the representative strains of *H. pylori* from Kolkata with primers PAI-14S and 15AS amplifying part of HP0527(VirB10) showed shorter amplicon in most of the strains as compared to 26695. Lane 1 represents 26695 while lanes 2-10 represent different strains from Kolkata. Percentage of *H. pylori* strains having partially deleted, complete, and totally deleted HP0527 gene of *cag* PAI among Kolkata strain. 66.66% of the strains gave a shorter amplicon, 29.1% of the strains gave same amplicon as that of 26695 while only 4.1% of the strains showed complete deletion of the gene.

C) Nucleotide sequence alignment of 26695 and one representative strain having shorter amplicon of HP0527 gene of *cag* PAI from Kolkata. The first repeat of 390bp is absent in those strains that have a shorter gene length in HP0527 gene.

Figure 2 AGS cells were cocultured with *H. pylori* strains from DU (Lanes 3 and 4) and NUD/AV (Lanes 2, 5 and 6) subjects for 5 hours at 37°C before the cells were lysed and the samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting with polyclonal anti-CagA antibody. Lane 1 indicates the result of AM1 (one *cag* PAI negative strain) infected AGS cell.

Role of gut microflora in neonatal sepsis with special reference to gram-negative bacteria

Investigator : S. Basu, A. K. Singh and T. Ramamurthy

The gut of a neonate is colonized by bacteria immediately after birth. While not all colonization leads to infection, the pathogenicity of the aerobic Gram Negative Bacilli (GNB) may predispose the babies towards infection. With this in view, the study examines the pattern of colonization of the neonatal gut by aerobic Gram negative bacilli (GNB) and evaluates the association between gut colonization and sepsis. This deserves attention because of the high incidence of sepsis and the differences in hygienic environment in developing countries compared to the developed world.

A prospective study of neonates with and without clinical sepsis, was carried out for a year in a tertiary care hospital in India. The gut (gastric aspirate & stool) and the blood samples of the babies were analyzed. Antibiogram and Pulsed Field Gel Electrophoresis were carried out to evaluate the relatedness of the gut and blood isolates. Further, characterization of isolates of *K. pneumoniae* and *E. coli* from the gut and blood of newborns was carried out to understand the role of these organisms in sepsis

GNB was the cause of septicemia in majority of the cases, *K. pneumoniae* being the most frequently isolated GNB from the blood. Babies with GNB in the gut have higher incidence of clinical sepsis than those without. In 50% cases the genotypes of the organisms found in the blood were indistinguishable from their gut counterpart. An association of gut colonization with neonatal sepsis was observed. Characterization of the *E. coli* isolates from the blood highlighted the fact that *E. coli* considered to be commensals according to their phylogroups do cause bloodstream infection in neonates. The status of the host and the features that allow the organism to persist under adverse conditions could act as important contributors to bloodstream infection in neonates.

Studies on colonization ability of tcp^{-ve} *Vibrio cholerae* strains in animal model.

Investigator : H. Koley

Pathogenic *Vibrio cholerae* strains are the etiologic agents of cholera. Pathogenic O1 and O139 isolates typically encode two critical virulence factors, cholera toxin and toxin corregulated pilus. CT is primarily responsible for diarrheal purge where as TCP is an essential intestinal colonization factor of *V. cholerae*. TCP has been shown to be critical for colonization both in animal models and in humans. Most pathogenic non-O1, non-O139 strains are CTX-ve TCP -ve but can still cause diarrhea.

Comparative studies on the colonization ability of *V. cholerae* of diverse serogroups with a variety of combinations of different virulence factors were made. In the present study, we investigated the role of individual virulence factors and its influence on the colonization ability as well as diarrhoeagenicity and colonization ability in RITARD model.

All the strains examined could colonize in both the ileum and the jejunum. However, inter-strain differences in colonization ability were explicit and some strains showed better colonization capacity in the ileum than in the jejunum. Kelly et al (2006) and Tam et al (2008) two groups worked on EPEC and EHEC organism and showed that structural components of a type III secretion system has important role on colonization.

Screening of *V. cholerae* showed that K11857, *V. cholerae* O139 strain having t3ss gene positive. Then target specific vcsC2 gene knockout was done. T3SS deleted mutant showed relatively lower colonization ability in Mouse model. RITARD model support our data that T3SS deleted mutant showed relatively lower colonization and no diarrhea.

Awards & Honours

S. Dutta

- Invited reviewer of Journal of Health, Population and Nutrition (JHPN), official journal of International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka.
- Invited reviewer of Diagnostic Microbiology and Infectious Disease (DMID), official journal of Elsevier Publication.
- Invited reviewer of BMC infectious Diseases, an open access journal, official journal of Elsevier Publication.
- Reviewer of two e-based journals e.g. Scientific Research & Essays (SRE), Journal of Infection in developing countries (JIDC).
- Invited member of the Council of Healthcare Advisors for consultancy, Gerson Lehrman Group, New York, USA for 2010-2011 and consulted few projects on invitation.

H. Koley

- Delivered Dr. (Mrs) Chitralekha Mukherjee Memorial Oration on the 22nd Annual Conference of The Physiological Society of India (PSI) and 2nd Biennial Conference of South Asian Association of Physiologists (SAAP), on 15th – 17th December 2010 at St. John's Medical College, Bangalore, Karnataka, India.

A Palit

- Member, Drinking Water sectional Committee, FAD 25, Bureau of Indian Standards, Ministry of Consumer affairs, Food and Public distribution, GOI, 2010-11.
- Member, Water purification system sectional committee, MHD 22, Bureau of Indian Standards, Ministry of Consumer affairs, Food and Public distribution, GOI, 2010-11.
- Reviewer, STS (ICMR),2010-11 programme

Conferences/ Seminars/Workshops/Trainings Attended/Organised

S. K. Niyogi

- Acted as expert of the selection committee for the selection of Junior Bacteriologist at Union Public Service Commission, Dholpur House, New Delhi-110069 on 18.03.2010.
- Participated in IBSC General Meeting at Institute of Molecular Medicine (IIM), Kolkata on 11 th October, 2010.
- .Participated in the XXXIV National Conference of Indian Association of Medical Microbiologists held at Swabhumi, Kolkata from 24th-28th November 2010.
- Conducted PhD viva examination at PGI. Chandigarh on 29.01.2011
- Participated in the brain storming workshop of experts regarding "Containment of Antimicrobial Resistance" at "Magnolia" India Habitat Center, New Delhi on 20th October 2010
- Participated in the one day zonal workshop by M/s. Elsevier Group at NICED, Kolkata on 2nd December 2010 on "Information and Analytical Tools for the Medical Science Researchers for eastern region of ICMR Institutes".
- Participated in WHO Global Foodborne Infection Network training course during 14-18 March 2011 held at NICED, Kolkata.
- Delivered a talk entitled " Isolation and identification of Vibrio cholerae serogroups O1 and O139".
- Participated in WHO Global Foodborne Infection Network training course during 21-25 March 2011 held at NICED, Kolkata.
- Delivered a talk entitled "Isolation and identification of *Vibrio cholerae* serogroups O1 and O139".

T. Ramamurthy

- Delivered a Guest lecture on "Changing molecular characteristics of *Vibrio cholerae*". International Conference on Aquatic Microbiology" Center of Advanced Studies in Marine Biology, Annamalai University, September 3, 2010.
- Delivered a on talk on "Current status of diarrheal diseases in Kolkata, India" in the Okayama University Forum for Emerging Enteric Diseases, Okayama, Japan. December 3, 2010.
- 45th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections at Kyoto, Japan, December 6-8, 2010 and presented a poster on "Clinical spectrum and etiology of enteric pathogens among diarrheal children: Comparative account on hospitalized cases and outpatients in an endemic area"

- The 7th PulseNet Asia Pacific Strategic Planning Meeting held at the Public Health Laboratory Centre in Hong Kong from December 19-23, 2010 and gave on talks on "Update on *Global Vibrio cholerae* database (GVD)" and "Genomic characteristics of *Campylobacter* spp from Kolkata".
- Delivered a Guest lecture on "Molecular epidemiology of human pathogenic vibrios". National Training on Molecular Diagnostics and fingerprinting of Salmonella and pathogenic Vibrios associated with seafood and aquatic environments, Central Institute of Fisheries Technology, Cochin. February 25, 2011.
- Division of Bacteriology, NICED organised Global Food-borne Infections Network (GFN) Level-1 training and two consecutive training courses were held from March 14-25, 2011. About 30 participates from all over the country took part in this training which was supported by the World Health Organization, Geneva.

S. Dutta

- Expert group meeting to review the candidate vaccine (conjugate Vi vaccine) for typhoid available at Bharat Biotech International Limited, Hyderabad at New Delhi on 16 Sept 2010.
- 14th International Conference on Emerging Infectious Diseases in the Pacific Rim: Focus on Next generation diagnostics for infectious Diseases held at Penang, Malaysia 4-6 October 2010 and presented a poster on "Validation of simple, rapid and affordable point of care new generation test for the diagnosis of typhoid fever."
- Invited to participate in a two day meeting in London on "Diagnostics for typhoid and paratyphoid fever" on 27-28 Oct 2010 organised by London School of Hygiene and Tropical Medicine and the Oxford University of Clinical Research Unit, Vietnam.
- Invited to participate in the 22nd National Congress of Parasitology held at Dept of Zoology, University of Kalyani, West Bengal, India from 30 Oct to 1 Nov 2010 and delivered an invited talk tilted "Water and Diarrhoeal Diseases" in the mini symposium on "Water and Health" held on 1 Nov 2010 at the same place.
- Participated in the 34th National Conference of Indian Association of Microbiologists "MICROCON 2010" held at Kolkata from 24-28 Nov 2010 and presented a paper titled "Changing serovars and antimicrobial resistance of Salmonella enterica blood culture isolates from a community based study in Kolkata".
- Participated in the National conference on "Emerging Trends in Natural Product Research" organized by School of Natural Product Studies held at Jadavpur University campus on 12-13 Feb 2011, Kolkata, India

A. Palit

- Organized & participated as Local Principal Coordinator for ICMR assisted "Public Consultation" (Eastern region) of "ICMR-DBT Guidelines for Stem Cell Research and Therapy" at CGCRI, Kolkata, India, 17 April, 2010.
- Organised & participated in ICMR, New Delhi assisted Public Opinion on "Knowledge Management Policy for Health – Service, Education and Research, 18 November, 2010, at NICED, Kolkata
- Organized & participated in ICMR, New Delhi assisted meeting "National Health Research Policy", 18 November, 2010, at NICED, Kolkata

- Organised and participated in "Informal Consultation for Development of Research Proposal on Communicable Diseases", at NICED, Kolkata, 23-24 December, 2010 (WHO-SEARO-APW).
- Participated and presented paper in 22nd National Congress of Parasitology, at Department of Zoology, Kalyani University, Kalyani, India, 30 Oct- 1 Nov, 2010.
- Organised & participated as joint Principal Investigator in "Kick off" meeting and subsequent programme specific field and local visits for conduction of project work in the DST-DFG (Indo-German collaboration) project "Role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal: relation with cholera incidence" along with our international counterparts (German and Japan), 10-27 January, 2011.
- Organised as joint–organiser and participated in Indo-Swedish sponsored "Internationl Symposium in Molecular & Pathological Research on Enteropthogens" at NICED, Kolkata 27-29 Janury, 2011.
- Organized as joint–organizer and participated in "International CME-2 on Tropical & Infectious Diseases", jointly orgnised by School of Tropical Medicine, Kolkata and NICED at NICED Kolkata, 5- 6 March, 2011.
- Organised as joint-organiser WHO assisted Global Food Nework (GFN) meeting for National and International participants in two phases at NICED,Kolkata,14-18 March & 21-25 March, 2011
- Participated in "Rajbhasa meeting" at Puri, Orissa, India, April, 2010.

B. L. Sarkar

- Invited as keynote speaker, "Importance of bacteriophages in cholera disease" at the Golden jubilee symposium on contemporary trends on Microbial Sciences held at Department of Microbiology, University of Burdwan on 18 May 2010.

R. K. Nandy

- Attended International Symposium on Molecular and Pathophysiological Research on Enteric Pathogens; held at Kolkata during 27-29 January 2011, organized jointly by NICED, Kolkata and Umea University, Sweden
- Oral presentation "Entner Doudoroff pathway is functional in *Vibrio cholerae* and plays an important role in the pathogenesis of cholera" in 45th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections at Kyoto, Japan during 6-8 December 2010.
- Attended 14th International Conference on Emerging Infectious Diseases held in Penang, Malaysia during 4-6 October 2010, organized by National Institute of Allergy and Infectious Disease (NIAID), USA.

S. Basu

- Presented a poster '*Acinetobacter baumannii* in gut of hospitalized neonates with special reference to cabapenem resistance'. International Symposium on Molecular and

pathophysiological research on enteric pathogens. 27-29 January 2011 Kolkata , India.

- Presented a poster 'Multidrug-resistant Gram negative bacilli in the neonatal gut and their role in sepsis.' Hot topics in Neonatology ,2010 ,American Academy of Pediatrics, Northwest Point Blvd, Elk Grove Village, IL ,USA, 5-7 December 2010
- Presented a poster '*Acinetobacter baumanni* and other nonfermenting Gram negative bacilli- an emerging problem in neonatal intensive care units'. Microcon, 2010. Kolkata, India, 25-28 December 2010
- Attended Indo-US workshop on Maternal and Neonatal Sepsis , Department of Health Research and National Institute of Health, New Delhi, 2010

H. Koley

- S. Barman, R. Kumar, N. Roy, D. R. Saha, H. Koley. 'The efficacy and immunogenicity of a live transconjugant hybrid strain of *Shigella dysenteriae* type 1 in guinea pig model'. 45th Annual Joint Panel Meeting on Cholera & Other Bacterial Enteric Infections, an United States-Japan Cooperative Medical Science Program from 6-8 December 2010 in Kyoto, Japan.
- H. Koley, S. Barman, D. R. Saha, R. Kumar. 'Protective efficacy and Immunogenicity of a live transconjugant hybrid strain of *Shigella dysenteriae* type 1 in Animal models'. 22nd Annual Conference of The Physiological Society of India (PSI) and 2nd Biennial Conference of South Asian Association of Physiologists (SAAP), scheduled to be held during 15 – 17 December 2010 in the St. John's Medical College, Bangalore, Karnataka, India.
- S. Mitra, S. Barman, D. R. Saha, A. Pal H. Koley. 'Haemagglutinating activity shigella and their role on colonization in colonization'. International Symposium on Molecular and Pathophysiological Research Enteric Pathogens, 27-29 January 2011, Kolkata, India.

BIOCHEMISTRY

The focus of the Division of Biochemistry lies in elucidating the molecular mechanism of host-pathogen interactions in diarrheal diseases. Therefore first we attempt to identify and isolate surface-associated microbial proteins that are thought to play a critical role in pathogenesis of disease by mediating adhesion and colonization of host intestine by alteration of host cell physiology or cell death. In the next step, we characterize the proteins in terms of their solution structure, receptor-specificity and thermodynamics of association with host ligands and finally take up elucidation of their biochemical functions. This involves extensive use of the techniques of molecular genetics and biophysical chemistry, like cloning and site-directed mutagenesis, amino acid and nucleotide sequencing, spectrofluorimetry, spectropolarimetry, microcalorimetry and analytical ultracentrifugation. This being a frontier area of biomedical research we ventured into this area after developing our infrastructure. Our research interests are structure-function relationship and mode of action of *V. cholerae* cytolysin, characterization of *V. cholerae* chitin-binding protein and its role in colonization in gut and the structure-function relationship of the colonization factor of enterotoxigenic *E. coli* (ETEC).

Scientist	:	K. K. Banerjee, Scientist F N. S. Chatterjee, Scientist D
Staff	:	K. C. Paramanik, Technical Officer A T. Roy, Technical Assistant
Research Fellow	:	S. Acharya (PDF) A. Ghosh (SRF) S. Sabui (SRF) S. Ganguly (SRF) M. Mondal (SRF) A. Debnath (JRF) A. Mukherjee (JRF)

Vibrio choleracytolysin/hemolysin (VCC): Structure-function relationship of a pore-forming toxin (PFT) with multiple biological functions

Principal Investigator	:	K. K. Banerjee
Co-Investigator	:	N. S. Chatterjee

A unique feature of VCC, a pore-forming toxin (PFT) from *V. cholerae* El Tor O1and non-O, is the presence of two contiguous carbohydrate-binding domains at the C-terminus that are absent in other PFTs with a similar domain architecture and mode of action. Deletion of the 15 kDa β-prism lectin domain bearing homology to the sugar-binding domain of the plant lectinjacalin caused a decrease in specific haemolytic activity by more than three orders of magnitude. Recent 3D-cryoelectron microscopic studies of the 65 and 50 kDa

oligomers revealed that a structural realignment of the lectin domain prior to insertion is critical for efficiency of pore formation. Moreover, deletion of the lectin domain profoundly affected its immunomodulatory activity, suggesting that the domain is critical for understanding of the structure-function relationship of the toxin. Earlier, we showed by ELISA and spectrofluorimetry that the lectin domain was not a prerequisite for initial events like membrane-binding and oligomerization. Because we observed that tryptophan fluorescence emission maximum of VCC shows a red shift in lipid-water interface and also because tryptophan prefers the polar-nonpolar interface and therefore seems to be implicated in membrane-insertion, we selected the two tryptophan residues in the lectin domain for site-directed mutagenesis. Substitution of tryptophan by alanine caused a drastic reduction in hemolytic activity. Interestingly, substitution of tryptophan rendered the β-prism lectin domain extremely susceptible to proteolysis by trypsin even at a low enzyme:substrate concentration of 1:1000 in comparison to the wild-type toxin, suggesting that apart from modulating its pore-forming activity, tryptophan played a critical role in correct folding of the β-prism lectin domain. Presently, we are in the process of substituting tryptophan by the aromatic amino acids tyrosine and phenylalanine and also by the polar amino acid serine to pinpoint the role of specific structural feature of tryptophan involved in activity.

Molecular characterization of enterotoxigenic *Escherichia coli* colonization factors

Principal Investigator : N. S. Chatterjee

Co-Investigator : T. Ramamurthy

Enterotoxigenic *Escherichia coli* (ETEC) infection is the leading cause of infantile diarrhea in developing countries and an important etiologic agent for traveler's diarrhea. Previous studies including ours have indicated that CS6 is a prevalent colonization factor of ETEC. Thus CS6 has become an important vaccine candidate. Using a multiplex PCR method, we found that the structural genes of CS6 cssA had three alleles (AI, AII and AIII) and cssB had two (BI and BII). BI along with AI/AIII showed stronger binding during ETEC colonization. The unique combinations of CS6 subgroups were AIBI and AIIBII, where alterations in both subunits were observed. AIIBII displayed different oligomerization pattern and had changes in polyacrylamide gel pattern. CS6 variants bound to different epithelial cells in a CFU-dependent saturable manner. CS6 with AIBI allelic subtypes showed on an average 3.6-fold more adherence than AIIBII in intestinal cells. Qualitatively similar results were obtained by Giemsa staining and florescence microscopy. Inhibition assays suggested that specific involvement of CS6 in cellular adhesion. We also show that CS6 could bind to rabbit mucin along with cellular fibronectin. Purified CS6 subgroups (AIBI and AIIBII) bound to immobilize rabbit mucin in concentration-dependent, saturable manner but the dissociation constant (Kd) for AIBI and AIIBII were determined as 309.09 ± 52.45 nM and 1.2 ± 0.05 μM, respectively. This indicated AIIBII showed a weaker binding with rabbit mucin compared to AIBI. Significant difference was not observed during fibronectin (Fn) binding. Screening results showed that AIIBII expressing ETEC strains were found in control strains mainly. From the result obtained, it may be predicated that AIBI variant might be better target in developing effective ETEC vaccine. Further work is in progress.

Studies on *Vibrio cholerae* adherence and survival in gut and environment

Principal Investigator : N. S. Chatterjee

Co-Investigator : K. K. Banerjee

Vibrio cholerae O1, a cause of epidemic diarrheal disease, normally resides in marine ecosystems and remains associated with the chitinous exoskeletons of zooplankton. The principal objective of our study is to understand the mechanism how these bacteria adhere to the gut and survive in the environment using some common factors. Amongst these ChiA2 (2.55 kb, locus VCA0027, 91 kDa) is a chitinase that help it to survive in the environment as well as in the human intestine. *V.cholerae* chiA2 promoter was amplified fused with reporter gene alkaline phosphatase (phoA). The fused construct was then transformed into *V. cholerae* N16961 and assayed in the native environment for its maximum activity. The aklkaline phosphatase assay showed that the promoter activity was 2-fold more at alkaline pH, maximum at 30C and maximum when the salt concentration was 500 mM. This result suggested that sea water may be the best environment for maximum chiA2 promoter activity. The chiA2 promoter showed 90-fold up-regulation in presence of chitin in the minimal media and 20-fold upregulation in presence of mucin relative to the control. For better understanding of the promoter, we have constructed deletion mutants of the chiA2 promoter from its 5-end in 100 base pair increments. 100-bp deletion from the upstream of the chiA2 promoter shows 75% reduction in the chiA2 activity, whereas 200 and 300-bp deletion from the upstream of the chiA2 promoter shows 95% reduction of the chiA2 promoter activity. Deletion of the -10 and -35 region of the chiA2 promoter shows total reduction of the promoter activity. Further promoter assays under different environmental conditions are in progress.

Conferences/ Seminars/Workshops/Trainings Attended/Organised

N. S. Chatterjee

- 14th International Conference on Emerging Infectious Diseases in the Pacific Rim held at Penang, Malaysia during 4-6 October 2010. Poster Title: Detection of common colonization factor antigens of enterotoxigenic *Escherichia coli* by multiplex PCR-based method.
- Okayama University Forum for Emerging Enteric Diseases held in Okayama University, Okayama, Japan on 3 December 2010. Title of the talk: Enterotoxigenic *Escherichia coli*
- 45th US-Japan Conference on Cholera and Other Bacterial Enteric Infections held in Kyoto University, Kyoto, Japan during 6-8 December 2010. Title of the talk: Intestinal Adherence with Colonization factor CS6 variants isolated from enterotoxigenic *Escherichia coli*.
- DAE-BRNS Life Sciences Symposium 2010 (LSS-2010) on Current Trends in Biology and Medicine held in BARC, Mumbai, India during 22-24 December 2010. Title of the talk: Biochemical characterization of a colonization factor expressed by enterotoxigenic *Escherichia coli*.
- International Symposium on Molecular and Pathophysiological Research on Enteric Pathogens held in Kolkata, India during 27-29 January 2011. Title of the talk: Involvement of a *Vibrio cholerae* chitin-binding protein GbpA in intestinal adherence.

CLINICAL MEDICINE

The Division of Clinical Medicine is conducting two studies on hospital based surveillance of diarrhoeal disease. One surveillance project is conducted at Infectious Diseases Hospital where every 5th hospitalized patient of all age groups is surveyed on randomly selected two consecutive days in a week. Another surveillance project is in progress at Dr. BC Roy Memorial Hospital for Children, Kolkata where children up to the age of 12 years suffering from diarrhoea or dysentery and attending Out Patient Department are enrolled. One of the scientists is involved in basic research to explore the mechanisms behind the regulation of antimicrobial peptide expression over the mucosal surfaces and to identify novel virulence factors of Salmonella Typhi and study host-pathogen interactions in human Salmonellosis.

Scientists have also conducted various research projects funded by external funding agencies. A study to determine the immune response to novel conserved Shigella protein antigens in patients with recent onset shigellosis and another study to find out the immunogenicity of two doses of modified killed whole cell oral cholera vaccine (WC-OCV) under two alternative vaccination schedules.

Recent studies showed that most of the drugs usually use in cholera now-a day is more or less resistant to causative agent of the disease. Recently, few studies showed that NORFLOXACIN and AZITHROMYCIN both are very sensitive to the said organism. A very recent outbreak investigation of cholera conducted by a clinical team from National Institute of Cholera and Enteric Diseases in Purba Midnapur showed 100% sensitivity towards Norfloxacin and Azithromycin. A hospital based clinical study on efficacy of single dose Azithromycin and standard dose of Norfloxacin in the treatment of cholera in adult is going on and we are getting much encouraging result.

Scientists are trying to develop better formulation of Oral Rehydration Therapy with high amylase resistant maize starch in addition to reduced-osmolar ORS for treatment of dehydrating acute diarrhoea in children. Scientists are also evaluating the role of probiotics for the better management of rotavirus associated diarrhoea in children. An extramural grant has been received to study the regulation of antimicrobial peptide expression in the intestinal epithelial cells.

One of the scientists has recently identified a novel virulence protein of Salmonella Typhi, which is a potential candidate for vaccine development because of strong immunogenicity in humans. The scientist reported this discovery in the PNAS, USA (February 22, 2011. vol. 108, no. 8, pp. 3348–3353), which was also highlighted by 'Nature' (17 February, 2011. Vol 470, pp. 308).

Scientists are involved in investigation of epidemics of diarrhoeal diseases and unknown fever. They are also involved in human resource development by providing training to the service providers like doctors and para-medical staff.

Scientist : U. Mitra, Scientist E
M. K. Bhattacharya, Scientist E
S. S. Das, Scientist D

Staff : A. Pal, Technical Officer A
M. Dey, Sr. Laboratory Assistant
[Retired on 30 April 2010]
K. G. Saha, Technician B
S. Turi, Attendant Services
S. Dey, Attendant Services

Outpatient based surveillance of diarrhoeal diseases at Dr. B. C. Ray Memorial Hospital for Children, Kolkata

Investigator : U. Mitra

Objectives of this study are to establish a systematic surveillance of diarrhoeal diseases and to identify the enteropathogens among the surveyed children who are attending Out Patients Department (Diarrhoea Treatment and Training Unit, run by NICED) at Dr. B.C. Ray Memorial Hospital for Children, Kolkata. This OPD based surveillance on diahhroeal diseases in children has been initiated in January 2010. This project has been achieved to determine the etiological identity of these diarrhoeal episodes which may help for better management of these patients and planning to develop strategies for prevention also.

The systematic surveillance has been initiated of every 5th patient of first 5 days of the week who are attending OPD with the history of diarrhea. The clinical set up has been standardized with special reference to evaluation of Clinical Research Form (CRF), process of having written informed consent, sample collection and in time transportation of sample to the laboratory.

During the period of report a total of 7708 diarrhoea patients attended to the OPD of which 1355 (17.6%) children were enrolled in the systematic surveillance system during January,2010 to April, 2011. Of these children 60% were male and 40% were female. Amongst them 99% children were below 5 years of age. Sixty percent of these children were Hindu and 31% were Muslim. Average monthly family income of majority of these families (82.2%) was Rs.2500 to 5000. Fifty nine percent children were from surrounding urban areas, 41% from rural areas. Fig.1 shows the types of diarrhoea of enrolled children. Eighty four percent children had the history of loose stools (unformed soft stools), 13% had watery diarrhea, 3% children had had frank bloody diarrhea (dysentery). Fig 2. Shows the presenting clinical features of the children.. Fig.3 shows the dehydration status of the enrolled children. Majority (96.7%) of children had "No Dehydration", 3.1% children had "Some" dehydration and 0.2%children had severe dehydration at the time of enrollment as per classified by WHO guidelines. The Project is continuing

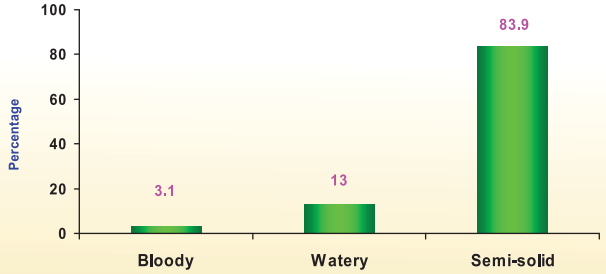


Fig.1 Type of diarrhoea of under-five children

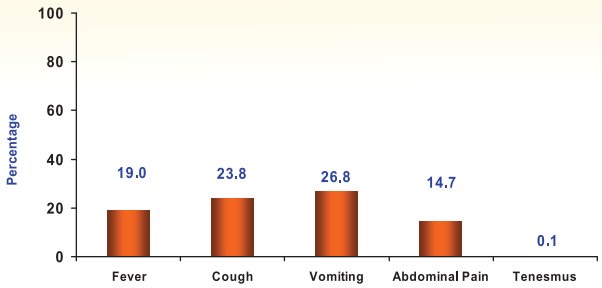


Fig 2 Clinical Symptoms of diarrhoea of under-five children

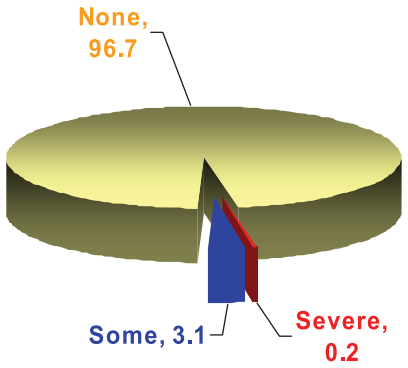


Fig.3 Dehydration status of under-five children before OPD

Hospital based clinical study on efficacy of single dose Azithromycin and standard dose of Norfloxacin in the treatment of cholera in adult.

Principal Investigator	:	M. K. Bhattacharya
Co Investigators	:	S. Kanungo, T. Ramamurthy, M. Ghosh

This study is conducted to evaluate the efficacy of single dose Azithromycin in treatment of cholera in adult. During this period from 05-10-2010 to 01-06-2011 a total of 67 patients have been included in the study who fulfilled inclusion and exclusion criteria. Of these 67 patient twenty six (26) were positive for *V. cholerae*, of these twenty six (26) positive cases, eleven (11) patients received Group – A drug and Fifteen (15) patients received Group – B drug. Forty One (41) cases were negative for *V. cholerae*. Both the groups received either Azithromycin single dose or Norfloxacin twice daily for 3 days according to random number table. Twelve patients received the Group A drug and thirteen patients received the Group B drug. The total intake of IV Fluid (in ml), intake of ORS (in ml), Stool output (in ml) and Duration of diarrhoea (in hours) were (4269.7±866.2 vs 4173.5±825.5), (2154.5±795.3 vs 2568.2±1159.0), (1071.2±528.1 vs 1322.1±566.4), (25.6±5.9 vs 26.8±8.1) respectively in both groups. All patients were successfully recovered. The study is in progress.

Hospital based surveillance system for diarrhoeal diseases

Principal Investigator	:	G. B. Nair
Co Investigators	:	M. K. Bhattacharya, T. Ramamurthy, K. Rajendran.

Objectives of the study are:

(1) To monitor changes in disease pattern,
(2) To create a database on diarrhoeal diseases,
(3) To provide regular reports to the Govt. and other agencies and to improvement in better patients care and preventive measures.

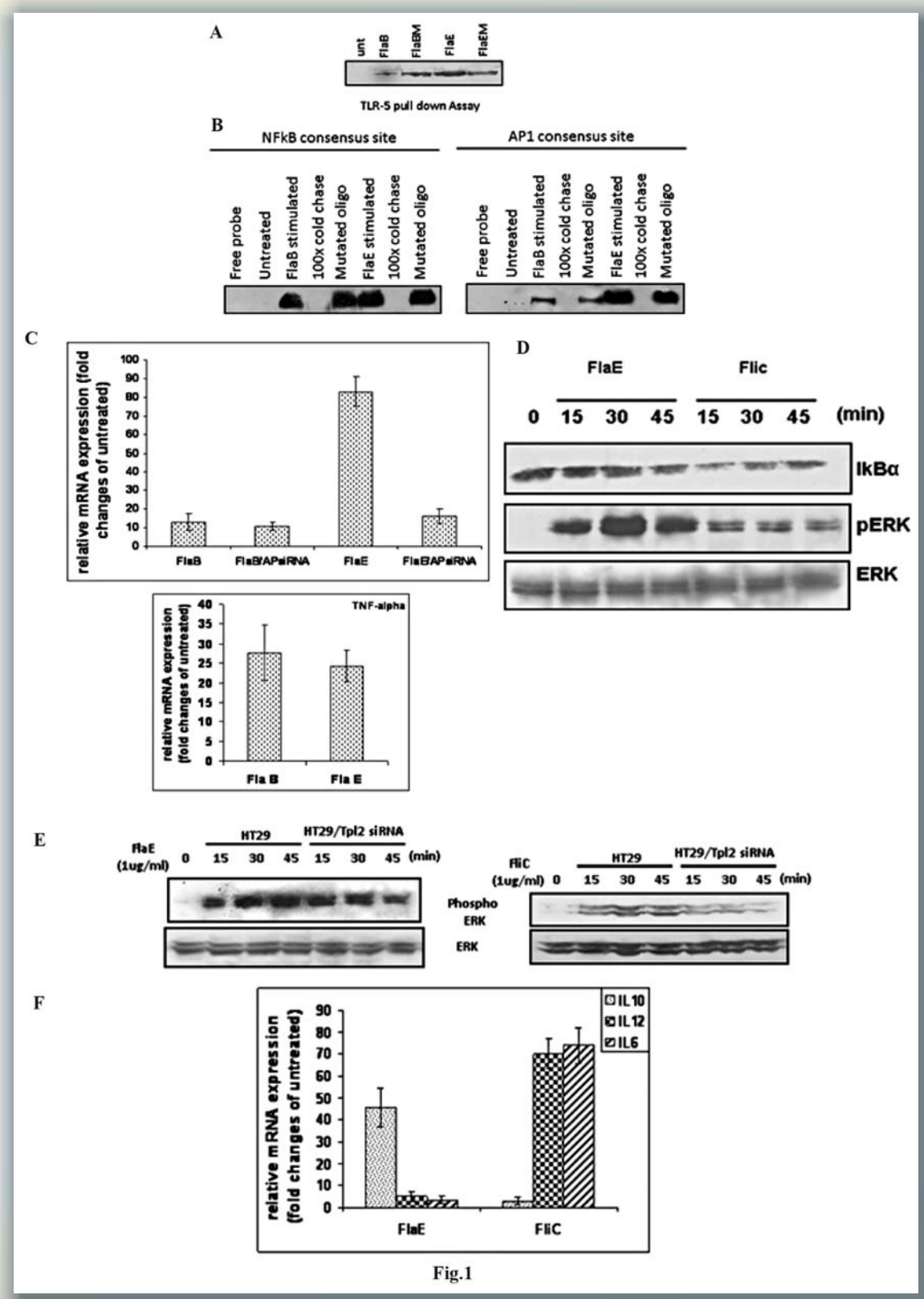
During 01st Apr, 2010 – 31st March, 2011, a total of 21323 diarrhoea cases admitted at ID &BG Hospital. Out of these 707 diarrhoea cases were enrolled in the surveillance. Majority cases were presented with dehydration (94.3%). Of which 72.1% acute watery diarrhea. The death rate was 1%. In children below 5 years of age rotavirus and *V.Cholerae* were main athogens. *V.cholerae* strains were resistant to tetracycline and susceptible to Azithromycin and Norfloxacin. Shigella isolation was only 6.9%. Weekly reports sent to Govt. and other agencies for control and improvement for better patients care.

Study of the pro-inflammatory functions of *V. cholerae* flagellins and their role in reactogenicity and immune response

Principal Investigator	:	S. S. Das
Co-investigator	:	H. Koley

We investigated the pro-inflammatory potential of individual subunit proteins of *V. cholerae* flagella. Monomeric flagellar subunits (flagellins) bind cell surface TLR5 and induce pro-inflammatory signals. We have shown that flagellins of *Vibrio cholerae* have different affinity for TLR5 (Fig 1A), determined by several critical amino acid residues over the conserved domains of the flagellins, most notably an alanine residue at the 100th position and differentially activate intracellular signaling pathways. While a weak ligand FlaB and a much stronger ligand FlaE equally activate NF-B, only FlaE significantly activates AP-1 as determined by EMSA (Fig 1B). It results in much higher induction of IL-8 by FlaE, while predominantly NF-B-regulated genes, such as TNF-alpha and IL-1beta were equally induced as studied by qPCR (Fig 1C). The above results suggest that a stronger receptor-ligand interaction may recruit an additional intracellular signaling pathway downstream of TLR5.

In another study, we found that *Salmonella typhimurium* flagellin FliC is a significantly stronger activator of NF-B compared with FlaB and FlaE. In contrast, FlaE-induced activation of ERK that in turn activates AP-1 is much stronger than both FliC and FlaB (Fig 1D). Although ERK activation downstream of TLR is largely TPL2-dependent, we found that FlaE induces ERK independent of TPL2, while FliC-induced ERK activation was Tpl2-dependent (Fig 1E). However, the former was dependent on TLR5-binding by FlaE and recruitment of MyD88 and TRAF6. Moreover, ERK was found to be activated downstream of cAMP-PKA-Src-Rap1-B-raf. Differential NF-kappaB and AP-1 activation by FliC and FlaE, respectively results in the induction of Th1 cytokines by the former, while the latter predominantly induces Th2/T-regulatory cytokines (Fig 1F). We failed to observe in our system any role of intracellular Ca2+ release that was earlier reported to mediate ERK activation by TLR5.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TLR-5 pull down Assay
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AP1 consensus site
Free probe
Untreated
FlaB stimulated
100x cold chase
Mutated oligo
FlaE stimulated
100x cold chase
Mutated oligo
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relative mRNA expression (fold changes of untreated)
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FlaE
FlaB/APsiRNA
TNF-alpha
relative mRNA expression (fold changes of untreated)
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IL12
IL6
FlaE
FliC

Fig.1

A study on the identification of novel virulence factors of *Salmonella typhi* and their role in pathogenesis and immune responses

Principal investigator : S. S. Das
Co-investigator : H. Koley

We have recently reported identification and characterization of a virulence-associated protein of *Salmonella* Typhi, which was also found to induce strong protective immunity (Ghosh S *et al*, ***Proc Natl Acad Sci U S A*. 108**:3348-3353). With continued search to identify novel virulence factor(s) of *S. typhi*, we characterized a putative serine-threonine kinase (STK) that is part of an operon, which includes another kinase and a phoaphatase. The STK was found to be secreted outside the bacteria (Fig 2A) and the kinase property was confirmed by an *in vitro* kinase assay using myelin basic protein (MBP) as a substrate (Fig 2B). Deletion mutation of the STK did not affect growth of bacteria *in vitro* (Fig 2C). However, the mutant had reduced survival within human macrophage cell line Thp1 (Fig 2D), but not within epithelial cells (Fig 2E). Macrophage survival was not related to suppression of the generation of ROS or RNS. Further studies revealed that the STK transcriptionally upregulates cathepsin D, which is known to contribute to the maintenance of Salmonella-containing vacuoles (SCVs). Ectopic expression of the STK resulted in degradation of IkB-α, leading to NF-kB-mediated transcription of pro-inflammatory cytokines and chemokines, which may help spread of bacteria to newly recruited inflammatory cells. Finally, LD_{50} of the STK mutant was 1-log higher compared with the wild type bacteria.

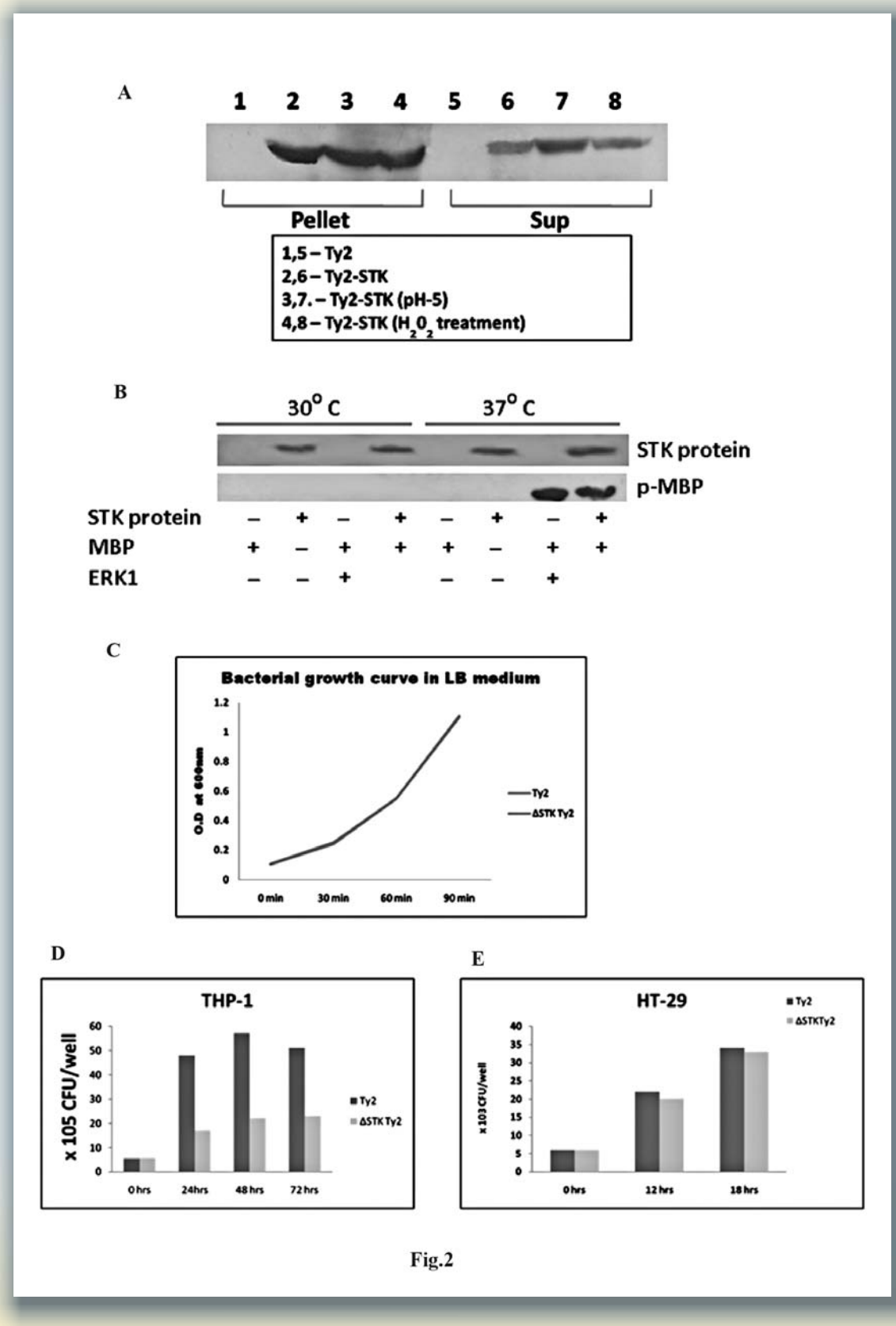

A
1 2 3 4 5 6 7 8
Pellet
Sup
1,5 – Ty2
2,6 – Ty2-STK
3,7. – Ty2-STK (pH-5)
4,8 – Ty2-STK (H_2O_2 treatment)
B
30° C
37° C
STK protein
p-MBP
STK protein – + – + – + – +
MBP + – + + + – + +
ERK1 – – + – – +
C
Bacterial growth curve in LB medium
O.D at 600nm
1.2
1
0.8
0.6
0.4
0.2
0
0 min
30 min
60 min
90 min
Ty2
ΔSTK Ty2
D
THP-1
x 105 CFU/well
60
50
40
30
20
10
0
0 hrs
24hrs
48 hrs
72 hrs
Ty2
ΔSTK Ty2
E
HT-29
Ty2
ΔSTKTy2
x 103 CFU/well
40
35
30
25
20
15
10
5
0
0 hrs
12 hrs
18 hrs

Fig.2

Award and Honours

S. S. Das

- Travel Award from Okayama University, Japan to attend the 45th Joint Panel Meeting of the US-Japan Cooperative Medical Science Program on Cholera and Other Bacterial Enteric Infections, held at Kyoto, Japan during 6-8 December 2010.
- Travel Award from Okayama University, Japan to attend the 7th International Conference on Innate Immunity sponsored by Aegean Conferences held at the Imperial Rhodes Hotel, Ixia, Rhodes, Greece from 4-9 July 2010.
- Selected as Guest Lecturer, Paper Setter and External Examiner of M.Sc. (Microbiology-part II), University of Calcutta.
- Invited lectures on 'Laboratory Biosafety: Principles and Practices' – Indian Institute of Chemical Biology, Kolkata on 30 September 2010
- Invited lecture on 'Antimicrobial peptides' – Department of Pharmacology, Okayama University, Japan on 3 December 2010.
- Invited lecture on 'War and Peace in the Human Gut' – S.N. Bose National Center for Basic Sciences, Kolkata on 18 February 2011.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

U. Mitra

- Nestle Nutrition Institute, South Asia Region on "Emerging trends in pediatric nutrition" at Hotel Oberoi Grand, Kolkata on 28 April, 2010
- Environment Day Celebration organized by Indian Science congress Association, Kolkata chapter at NICED, Kolkata on 7 June, 2010
- Nestle Nutrition Institute, South Asia Region on "Start Healthy Stay Healthy" at Hotel Park, Kolkata on 30 July, 2010
- Nestle Nutrition Institute, South Asia Region on "Pediatrics and clinical nutrition" at ITC Sonar Bangla, Kolkata on 22 September, 2010
- Nestle Nutrition Institute, South Asia Region on "Challenges in preterm care and nutrition" at The Park, Kolkata on 15 March, 2011
- Nestle Nutrition Institute, South Asia Region on "Role of probiotics in maternal and child health: What is the current evidence?" at ITC Sonar Bangla, Kolkata on 14 April, 2011

M. K. Bhattacharya

- Guest lecturer, delivered a talk on Health & Hygiene Management in Schools on 17th March 2011 at Administrative Training Institute, Govt of West Bengal.
- Guest lecturer, delivered a talk on management of acute Diarrheal Disease among the MO, BMOH and ACMOH of Health Service Doctor's Minister of Health, Govt. of West Bengal on August & September,2010 at ID & BG Hospital

S. S. Das

- Oral Presentation at the 45th Joint Panel Meeting of the US-Japan Cooperative Medical Science Program on Cholera and Other Bacterial Enteric Infections, held at Kyoto, Japan during 6-8 December 2010.
- Oral presentation at the International symposium on "Molecular and Pathophysiological Research on Enteric Pathogens", held in Kolkata on 27-29 January 2011.
- Presented a poster at the 7th International Conference on Innate Immunity sponsored by Aegean Conferences held at the Imperial Rhodes Hotel, Ixia, Rhodes, Greece from July 4-9, 2010.
- Participated at the training on Continued Medical Education, 2011, Society of Tropical Medicine and Infectious Diseases in India, held on March 5-6, 2011 at NICED, Kolkata.

DATA MANAGEMENT

The Division of Data Management primarily focuses on good data management practices to produce reliable, complete and accurate data from the various health research projects of this Institute. Hospital based diarrhoeal diseases surveillance study at Infectious Disease Hospital (IDH), Kolkata is an ongoing project to identify various diarroeagenic enteric pathogens. The information on causative organism and antimicrobial resistance pattern is being communicated on weekly basis to IDH and different departments of State Government to help physicians for proper treatment and management of diarrhoeal diseases.

The division has direct access to the data from all divisions of NICED and hence, it is in a position to provide data management support including data entry/verification to various studies undertaken by this institute in collaboration with the project on HIV sentinel surveillance of National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India, Integrated Diseases Surveillance Project (IDSP) and International Collaborators like International Vaccine Institute, Korea, and Centre for Vaccine Development, University of Maryland, Baltimore. This division is capable of advanced electronic data transfer from country to country and also GIS implementation. The division rendered statistical help for epidemiological, clinical and microbiological research. It has future plans to conduct local and country level training on research methodology, basic Bio-Statistics, Epi-info and SPSS for health researchers. This would eventually provide a comprehensible vision of basic and operational research in diarrheal diseases.

Scientist : B. Manna, Scientist E
K. Rajendran, Scientist B

Generation of a database on cholera outbreaks in India

Principal Investigator : B. Manna

A huge number of diarrhoeal outbreaks have been reported and investigated in different parts of India during last 30 years. All the investigation reports are usually submitted to the respective State Government as well as Ministry of Health, Govt. of India. But unfortunately, some of the outbreak reports are published in the indexed journal depending on the research interest of the investigators. So, there is a limited scope for any researcher or health policy maker to get the access the information about all outbreaks electronically. Therefore continued monitoring & surveillance of all cholera outbreaks become necessary and there is a need to create database on all cholera outbreaks in India which will facilitate the health planners to make policy for combating future outbreak and to make control strategy based on the evidences gathered from this study. So, keeping this in mind, study has been undertaken with the following objectives.

I. To establish a cholera outbreak database including all relevant information

II. To analyze the pattern of causative organism for cholera outbreak.

III. To look on antimicrobial resistance pattern of *Vibrio cholerae.*

IV. To plot spot/GIS mapping of outbreaks over time

V. To analyze the general clinical signs & symptoms for epidemic and treatment management

VI. To determine the possible risk factors for outbreaks

VII. To see the effect of source of transmission for outbreak

VIII. To make control strategy for future outbreaks based on the emergence of such antibiotics resistance

IX. To find out the relationship between climate factors and cholera outbreak

The published articles on diarrhoea outbreak /epidemic are searched through Free medical journals, Medexplorer, Medscape, Medhunt and PubMed. All relevant articles Other than full free-text articles will be collected on payment basis. Attempt is going on for collection of unpublished data from different sources viz, NCDC (National Centre for Disease Control, Delhi)-annual report, NICED- annual reports, Integrated Disease Surveillance Projects (IDSP) from different States of India and National Institute of Epidemiology – FETP(Field Epidemiology Training Programme) report. The metrological data corresponding to time and place of outbreak is being collected from the respective metrological department, Govt. of India or can be abstracted from the website of Indiastat.com /

Creation of Database**:** A customized Data Entry Programme on Visaual Basic at the front end will be developed to feed the necessary data from epidemics and Data will be saved in MS access in the back end. The input variables are: Time, Place, State, total person affected, attack rate, causative organism, isolation rate, antimicrobial sensitivity of causative organism, treatment management, clinical sign & symptoms, mode of transmission, identification of risk factors for outbreaks

Climate data (Rainfall, minimum, maximum temperature humidity) during epidemic period will be collected from "Indiastat.com" website on yearly subscription or from free website weatherunderground.com or from specific epidemic place metrological department.

Progress so far : Data collection has been started from different published articles and unpublished documents. Data structure has been created and data entry is ongoing. Study is in progress.

Time series model study for prediction of cholera and diarrhoea using atmospheric Temperature, Relative Humidity and Rainfall in Kolkata, India.

Investigator : K. Rajendran

The objectives of the study is to compare the climatic characteristics such as Temperature, Relative Humidity (RH) and Rainfall with observed infection of diarrhoea and Cholera in the Infectious Diseases Hospital, Kolkata and to assess long term changes to develop Time series model and Mathematical Statistical Models. In climatic factors, the difference of RH and temperature [i.e., morning (max)-evening (min)] were used in the analysis. This procedure was relevant to identify the actual causative factors instead of mean

factors. The mean factors purposefully have been averted to avoid the influence of high variation in the series.

Periodic comparison for diarrhoeal pathogens: Active surveillance data generated from the Infectious Diseases Hospital, was used by general liner model (GLM). Two sets of data were considered during 1996-97 and 2008-09 as they met all the criteria in the study design. Data on relative humidity (RH), temperature, rainfall and sunshine duration are the climatic factors collected during the study period and assessed on the prevalence of cholera caused by *Vibrio cholerae* O1, rotavirus and parasite infection using GLM. These climate factors were also considered for Time series analysis of Seasonal Auto-Regressive Integrated Moving Average (SARIMA) model to investigate relative impact of climatic on cholera.

Seasonal Auto integrative Moving Average (SARIMA): Time series model: Seasonal Auto integrative Moving Average (SARIMA) has created candidate model of month wise *V. cholerae* infection with predictor variables of RH, temperature, sunshine duration and rainfall were in SARIMA (1, 0, 0) (0, 1, 1) along with seasonal difference to stabilize model. The periodicity, seasonality and pattern of Diarrhoeal pathogens infection were investigated for future prevention. Heavy rain fall indirectly stimulated the *V. cholerae* infection. High RH favours *V. cholerae* infection, that was linearly related where as high temperature (mean) does not favour the *V. cholerae* infection(Fig-1-2).

Generalized Linear Model (GLM): The GLM revealed increase of average temperature (1C) and RH (15%) and 50% decrease in average sunshine duration (3 hrs) in December during the considered study period. Climate significantly fluctuated and the consistency was not similar during the study periods. In 1996-97, V. cholerae O1 favoured by day time RH ($p<0.001$) with minimum sun shine duration (3.65hrs ±.82) and highest rainfall (11.47cm, $p<0.001$). However, the trend during 2007-08 was not significant, as there was a shifted to 10-30% range of variation perhaps due to La Niña effect. Rotaviral infection was less influenced by 10-20% RH variation (70.52±8.36) with maximum sunshine duration (6.38±.80), no rainfall and low temperature (21.698±2.47) during 1996-97. During 2008-09, the influence was very high and significant with 30-40% RH (65.74±6.4, $p=0.027$) and highest sun shine duration (7.22±1.56) (fig-2). Since, the parasitic infection was found along with V. cholerae O1 and rotavirus (co-infection), influence of climate on these pathogens remained difficult to determine

Pathogens progresses rapid climate adaptation?! GLM has identified the changing temporal patterns of enteric pathogens in relation to climatic conditions in Kolkata. When the sunshine duration was less, prevalence of cholera was less but the sunshine duration increases while rotavirus infection was high. Prevalence of cholera was high during monsoon when the RH was high. In contrast, the rotavirus infection was in peak during winter season when the temperature and RH were at minimum. The El Niño and La Niña has definite role in determining the prevalence of diarrhea in Kolkata. The study is addressing that the pathogens had quick climate adaptation even though high variability which may have chance for mutation of organism. Climatic factors are the real challenges for diarrhoeal diseases which have to be diagnosed in time for welfare of next generation

Relation between diarrhoea and climatic factors in Kolkata: Twenty years retrospective study

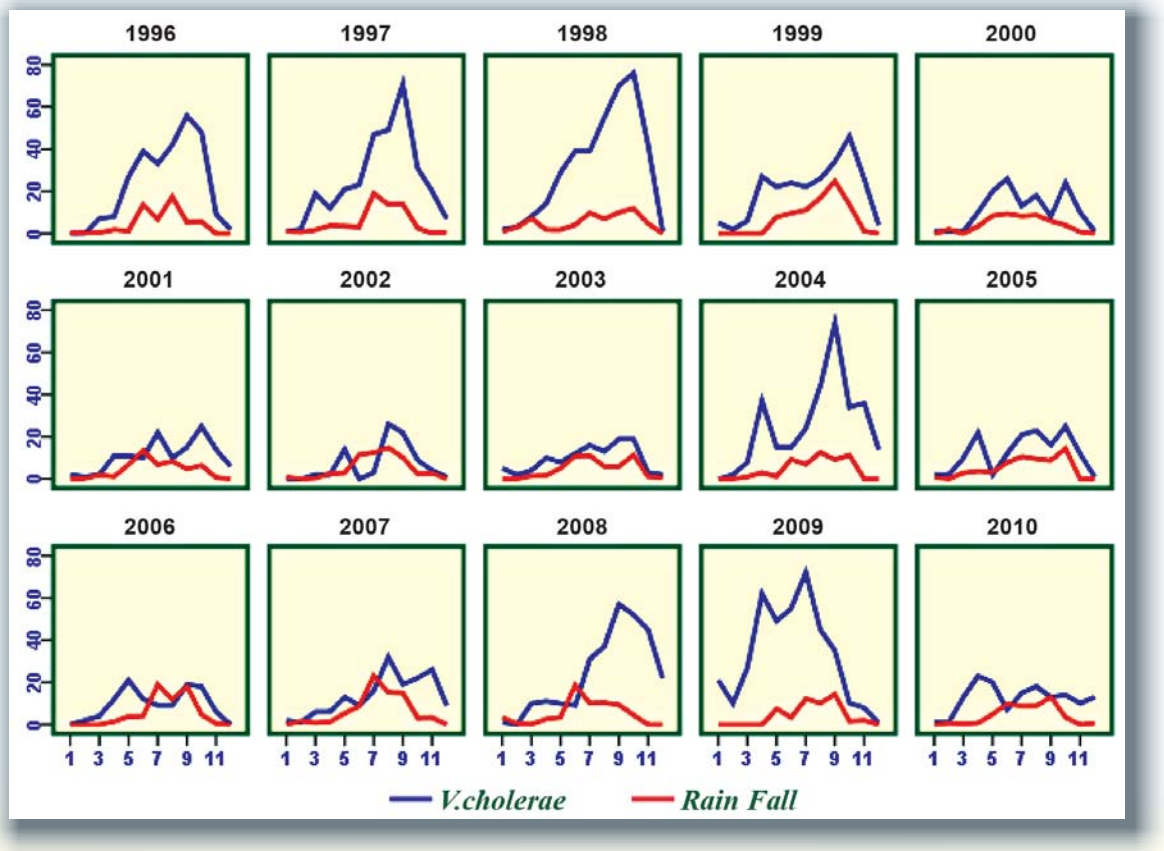
Investigator : K. Rajendran

Objective:

- Comparison of diarrhoea and climatic factors using collected data from 1980-96 in Kolkata
- Application of time series model to determine disease pattern and its long term variations
- Relate the influence of climatic factors on diarrhoea and estimated Cholera using Generalized Linear model

Data collection is being progressed at Infectious Diseases Hospital.

Figure-1: Relational impact Rainfall associated *V. cholerae* infection at Kolkata



Identified ARIMA(1,0,0)(0,1,1) Model depicts climatic impact for Rain fall,Temperature,sunshine and Relative Humidity of the *V.cholerae* infection at IDH, Kokata.

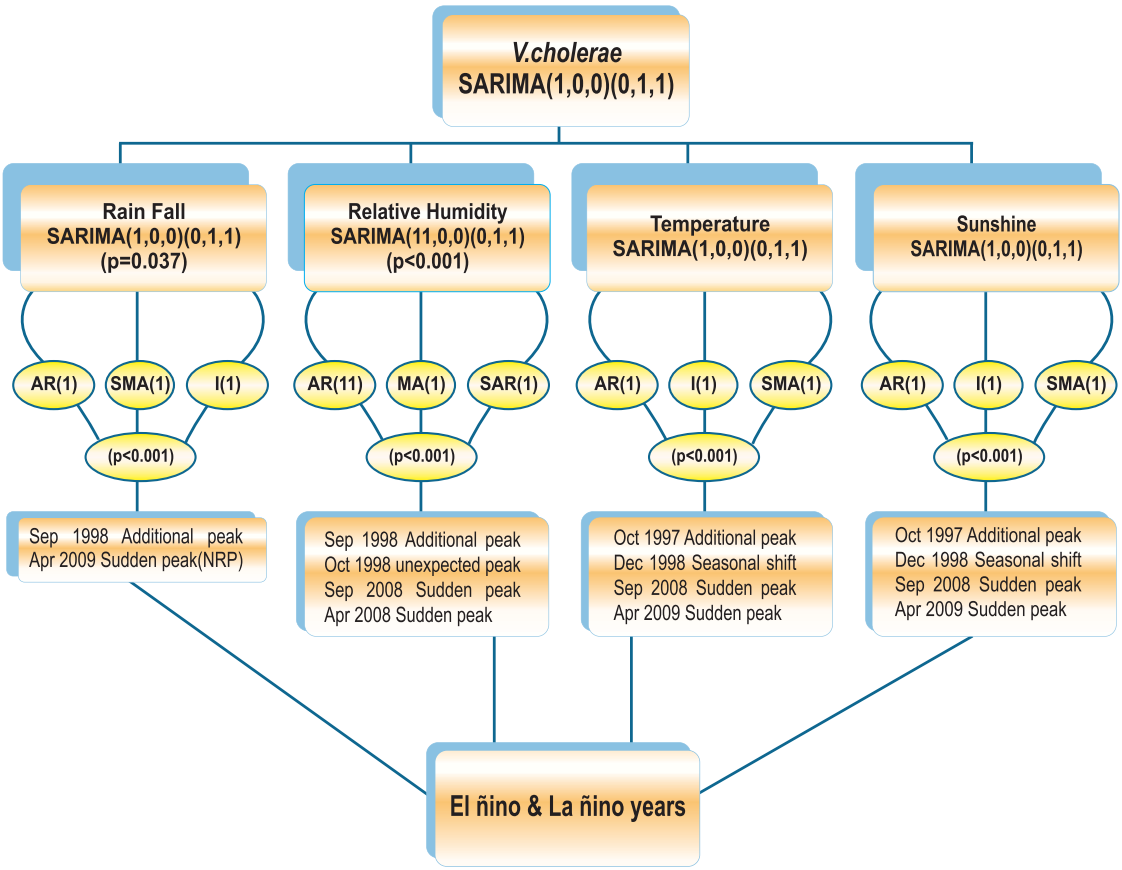


Table-1: Generalized linear model (GLM) Explored the *V. cholerae* infection with no Rain fall events (n=637) associated with interval rang of Relative Humidity and Temperature.

R.H. Difference (Morning-Evening)	Mean±SD	*V. cholerae* (n=637)	Parameter Value	SE 95% likelihood CI	Wald Chi-square	P-Value
Evening high RH (n=132)	79.17±8.98 29.28±2.78 T	132	1.182	0.26(-.43-2.79)	20.33	<0.001**
Day's equal RH (n=22)	76.18±6.94 29.29±2.83 T	22	0.750	0.40(-.97-2.47)	3.55	<0.001**
<=10% (n=270)	73.63±6.90 28.41±3.69 T	270	0.976	0.24(-.62-2.57)	16.23	<0.001**
>10-20% (n=132)	71.87±6.89 27.84±3.98 T	132	0.856	0.254(-.75-2.47)	11.39	0.001**
>20-30% (n=60)	65.82±8.78 27.33±4.62 T	60	0.233	0.25(-1.40-1.87)	0.88	0.349
>30-40% (n=17	65.21±4.27 29.96±2.07 T	17	0.338	0.27(-1.42-2.10)	1.53	0.217
>40% (n=4)	60.62±2.95 29.35±1.46 T	4	Reference category			
Temperature difference						
<=5° C (n=17)	82.62±4.19 29.28±2.14	17	0.118	0.90(-1.65-1.88)	0.017	0.897
>5-10°C (n=419)	75.69±7.47 29.69±2.59	419	0.325	0.87((-1.27-1.92)	0.139	0.709
>10-15°C (n=197)	68.10±7.91 25.84±4.22	197	0.239	0.87(-1.84-1.37)	0.075	0.784
>15°C (n=4)	65.00±12.36 23.20±3.68	4	Reference category			
Season						
Pre monsoon (n=200)	68.90±8.23 30.75±2.09	200	0.352	0.13(-.07-.77)	7.62	0.006**
Monsoon (n=151)	79.10±6.31 30.86±1.34	151	0.769	0.19(.31-1.23)	16.47	<0.001**
Post monsoon (n=211)	74.72±7.31 26.95±2.30	211	1.09	0.17(.67-1.51)	39.54	<0.001**
Winter (n=75)	70.72±8.30 21.67±2.38	75	Reference category			

** highly significant

Award and Honours

K. Rajendran

- Deliver an invited talk at AMIST University, Malaysia on 6 October 2010: Talk Title:" Essential Statistics for Bio-Medical Research"
- Reviewer of two International Journals:

Conferences/ Seminars/Workshops /Trainings Attended/Organised

B. Manna

- Delivered lectures to Ph.D. students on "statistical methods in laboratory science"
- Attended the Investigators' Meeting for the project titled " Diarrhoeal Disease in Infants and Young Children in developing Countries (The Global Enteric Multi Centric Studies) in Maputo, Mozambique from 28-30 September, 2010

K. Rajendran

- The 14th International Conference on Emerging Infectious Diseases of the Pacific Rim: Next Generation Diagnostics for Infectious Diseases during 4-6 October 2010 Penang, Malaysia.

  Title: Influence of Climatic Factors on Prevalence of Enteric Pathogens in Kolkata, India: General linear model .

ELECTRON MICROSCOPY

The Division of Electron Microscopy is engaged in research and diagnosis in the field of diarrheal diseases. There are several projects going on in the laboratory that can be categorized as follows.

Cryoelectron microscopy and 3-D image reconstruction

Three-dimensional structure of protein molecules are studied using cryoelectron microscopy and single-particle analysis. The 3-D structure of hemolysin oligomer, a pore-forming toxin of *Vibrio cholerae*, has already been studied. Also the 3-D structure of several vibriophages and packaging pattern of DNA inside the phage head have been determined using cryoelectron microscopy. Three-dimensional structure of pili that play a vital role in the attachment of bacteria to the intestinal cell wall is being worked out using cryoelectron microscopy.

Vibriophage research

Morphology of different vibriophages isolated from different sources as well as those used in different phage typing schemes has been determined. Conformation of the genomes of these phages, genetic relatedness amongst them and studies on the biological processes like replication of these vibriophages, packaging of the genome inside the phage head have been carried out. This laboratory, for the first time, showed the filamentous nature of RS1-KmΦ phage of *V. cholerae*.

Nanobiotechnology

The bacterial flagellum consists of a flagellar motor,a hook and a long filament. The flagellar motor, not more than 40 nm wide, can rotate at a tremendous speed of about 1,00,000 rpm which propels the cell. How torque is generated for such high speed and also how the cell changes its direction of swimming are very important factors. Knowledge of these factors is essential for the design of an artificial nanomachine like a propeller-driven one that can dispense drug. Elastic properties of the flagella of several *Vibrio* spp. have been studied.

Histopathological studies

Histopathological changes caused by different enteric pathogens have been studied by light microscopy. Surface structural changes and in-depth ultrastructural changes are being studied using scanning and transmission electron microscopy. Few of the important enteropathogens studied so far are: *Vibrio cholera*e, *Helicabacter pylori*, *Shigella* spp. and *Aeromonas hydrophila*.

Scientist	:	A. N. Ghosh, Scientist F
		D. R. Saha, Scientist E
Staff	:	A. Sarbajna, Technical Officer A
		S. Kumar, Technican B
		B. R. Mallick, Attendant Services
Senior Research Fellow	:	Somnath Dutta

Three-dimensional structure of *Vibrio cholerae* hemolysin oligomers by cryo-negative staining.

Principal Investigator : A. N. Ghosh

Co-Investigator : K. K. Banerjee

Vibrio cholerae produces several potent enterotoxins other than cholera-toxin (CT). Prominent among these non-CT enterotoxins is a water-soluble 65-kDa monomeric membrane-damaging protein, designated as *V. cholerae* hemolysin (HlyA) or cytolysin/hemolysin (VCC). HlyA is a pore-forming toxin (PFT) that causes lysis and death of a broad-spectrum of eukaryotic cells by forming oligomeric transmembrane heptameric diffusion channels in the plasma membrane lipid bilayer. HlyA is exported to the culture medium as 79-kDa prohemolysin (proHlyA). Proteolytic removal of 132-residues from the N-terminal region generates the mature 65-kDa hemolysin (HlyA). HlyA undergoes an additional proteolytic cleavage close to the C terminus to yield a second active species of about 50-kDa (HlyA50). Hemolytic activity of HlyA50 is about 1000 times less than that of HlyA. The aim of the project is to reconstruct the three-dimensional structure of HlyA and HlyA50 oligomers using cryo-negative staining and single particle analysis methods.

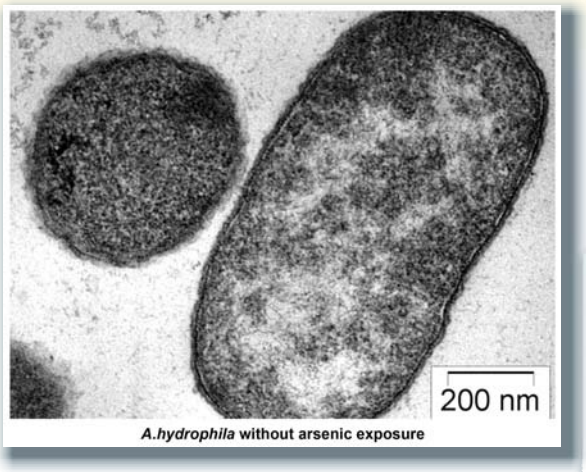
Symmetry of these oligomers was determined which shows that HlyA has 7-fold symmetry while HlyA50 is an asymmetric molecule. Further work is in progress.

Studies on the effect of arsenic on the pathophysiology of bacteria.

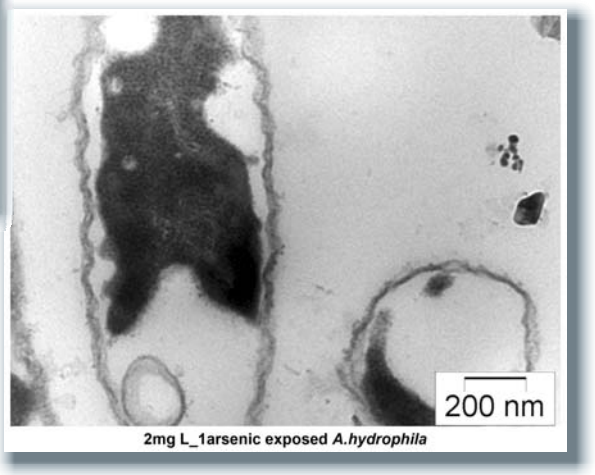
Principal Investigator : S. Mazumder

Co- Principal Investigator : D. R. Saha, R. Goswami

Arsenic is a well known top priority global pollutant',found in soil, air and water. *Aeromonas hydrophila*, being a ubiquitous organism is prone to arsenic exposure. The effect of environmental stress on *Aeromonas* spp.is not well documented though few reports suggested the influence of environmental factors like temperature, salinity and pH on the pathogenicity of the bacterium. Among the different metals ubiquitously distributed in nature, arsenic appears to be the most common. The present study was designed to determine the role of arsenic on growth and virulence of *A. hydrophila*. Exposure to arsenic (1mgL-l and 2 mgL-l) had no effect on growth but significantly inhibited the hemolytic and cytotoxic potential of exposed bacteria. Transmission electron microscopy revealed loss of membrane integrity and presence of condensed cytoplasm suggestive of acute stress in bacteria exposed to arsenic. Arsenic adadpted bacteria were developed by repeated sub-culturing in presence of arsenic. Arsenic-adaptation led to significant recovery in hemolytic and cytotoxic potential of exposed bacteria.The arsenic –adapted bacteria exhibited normal membrane integrity, decreased cytoplasmic condensation and possessed scattered polysome like structures in the cytoplasm. A positive correlation was observed between arsenic tolerance and resistance to several antimicrobials. Arsenic –adaptation failed to confer cross- protection to mercury and cadmium stress. SDS-PAGE analysis revealed the expression of two new proteins of approximately 85kDa and 79 kDa respectively in arsenic- adapted *A. hydrophila*. Further study is in progress.



A.hydrophila without arsenic exposure



2mg L_1arsenic exposed *A.hydrophila*

Award and Honours

A. N. Ghosh

- Enlisted in the reviewers' panel of Journal of Molecular Biology and Biochimie.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

A. N. Ghosh

- Resource person in the workshop entitled "Basic Principles, Preparatory Methods and Biological Application in Transmission Electron Microscopy" organised by the North Eastern Hill University, Shillong during 22-25 March 2011. Delivered two lectures entitled "Cryoelectron Microscopy" and "Electron Microscopy of DNA".

D. R. Saha

- 1 day seminar on the 100th birth anniversary of Prof. N. N Dasgupta at the Meghnad Saha Auditorium,University,College of Science,Kolkata on 30 April 2010 organised by Electron Microscope Society of India (Kolkata zonal chapter).
- The 14th International conference on 'Emerging Infectious Dsieases in the pacific Rim' sponsored by the United States- Japan Cooperative Medical science Program at Penang, Malaysia from 4-6 October 2010 and presented a poster on 'Fecal leucocyte-a rapid and simple way to assess diarrhoeal aetiology'.

EPIDEMIOLOGY

The span and horizon of the work of Division of Epidemiology extends from surveillance of diarrhea and HIV to intervention studies like vaccine trials. Highlights of the division for the recent years are as follows:

On diarrhoeal disease surveillance and intervention trials:

Phase III study on the efficacy of the bivalent whole cell killed oral cholera vaccine. It is a randomized double blind placebo controlled trial among 110,000 urban slum populations in Kolkata in 2006, being conducted by NICED in collaboration with International Vaccine Institute, Korea. In 2008, the protective efficacy (PE) of all age group was 67% and at the end of three years post vaccination (2009) it was 65%. These results have been instrumental for the new cholera vaccine being introduced in India in February 2010. Now we have a vaccine which is effective, cheap, safe, produced according to WHO and international norms, which can effectively implemented either preemptively in cholera prone areas or as reactionary measures for combating epidemics.

A multi centric study of the burden of diarrhoeal diseases among children under 5 years of age was started in 2007 in collaboration with University of Maryland. It is a large community based case control study among 2,00,000 populations who are urban slum dwellers in Kolkata. Although the study is ongoing, the initial results show higher rate of detection of rotavirus and Shigella in cases than in controls.

Diarrhoeal disease surveillance with emphasis on cholera, has been set up in urban slums of Kolkata in preparation for a Phase III trial of a live oral cholera vaccine (VA1.4) developed by Indian scientists and funded by Dept. of Biotechnology, Govt. of India

A community based epidemiologic study on Rotavirus infection among children below 2 yr of age has covered a little over 600 children and was carried out over a period of 5 and a half month in the district of south 24 Parganas in West Bengal. Participation of the local community members in decision making during different stages of execution of the study has been a major strength of this work.

Community based surveillance for diarrhoeal diseases in the rural community is also being carried out by the division

Surveillance for dengue fever in eastern Kolkata, West Bengal, India is being conducted to determine the incidence and burden attributable to dengue fever. DEN-1 was the most prevailing strain. Results suggested that dengue is a major public health problem in Kolkata. being as high as 2.3% which is almost similar to other endemic areas of the world. High incidence in younger age group makes it important for decision making for future trials of dengue vaccines for targeting this particular age group.

Projects on HIV

On Stigma

A major finding emerging from this piece of work is identification of factors associated with various domains of HIV stigma, which could help develop intervention. An on-going research from the division focuses on transmission of HIV in heterosexual married relationship in West Bengal. This is being conducted by NICED through collaboration with the civil society organization named Society for Positive Atmosphere and Related Support to HIV/AIDS (SPARSHA) and RG Kar Medical College & Hospital, Kolkata. This 3 yearlong study is currently in its final phase of recruitment of participants. Technical assistance support is also being provided by the scientist of the epidemiology division of NICED to National AIDS Control Organization (NACO), India in relation to baseline situation assessment of HIV among Injecting drug users (IDUs) of Punjab.

A prevalence study on oncogenic HPV among female population with and without HIV infection to understand the epidemiology and circulating genotypes of oncogenic HPV among HIV positive and negative female population in West Bengal, India showed that the prevalence of HPV 16, 18 among HIV positive females was higher than HIV negative females. Interestingly, oncogenic HPV was not found to be associated with age and duration of sexual exposure. But the presence of HIV was found to a statistically significant predictor oncogenic HPV.

Scientist	:	S. Ghosh, Scientist F [Retired on 28 February 2011] D. Sur, Scientist F S. Panda, Scientist E K. Sarkar, Scientist E A. K. Deb, Scientist D S. Kanungo, Scientist B
Staff	:	D. C. Das, Technical Officer A S. Manna, Technical Officer A R. L. Saha, Technical Officer A S. Shil, Technical Officer A C. Mondal, Technician B A. Chakraborty, Technician B

A randomized controlled trial (Phase-III) of the bivalent killed whole cell oral cholera vaccine in eastern Kolkata

Principal Investigator	:	D. Sur
Co Investigators	:	S. Kanungo, B. Manna, S. K. Neogi, B. L. Sarkar, In collaboration with International Vaccine Institute, Seoul, Korea

Objectives:

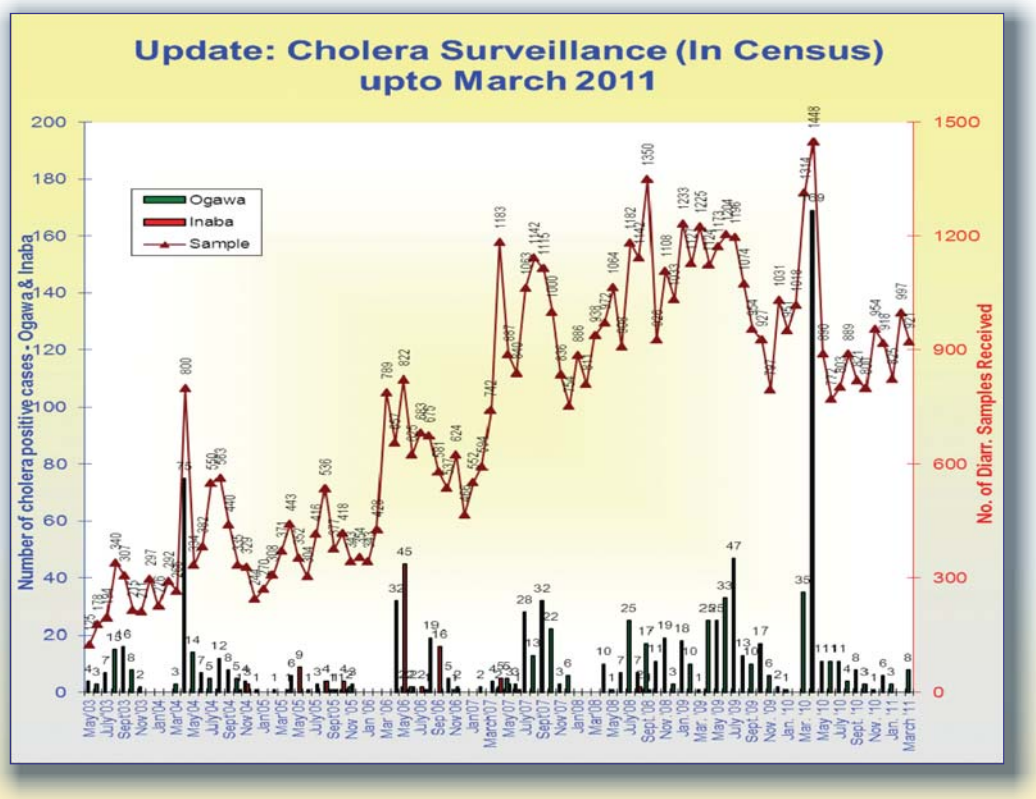
To estimate the efficacy of a two-dose regimen of the oral whole cell killed bivalent cholera vaccine in preventing culture-proven *V. cholerae* O1 diarrhoea episodes severe enough to require treatment in a health care facility in persons over one year of age.

Activities:

A bivalent whole cell killed oral cholera vaccine underwent a Phase III randomized double blind placebo controlled trial among 110,000 urban slum populations in Kolkata in 2006 being conducted by NICED in collaboration with International Vaccine Institute, Korea. In 2008, t**he protective efficacy (PE) of all age group was 67% and at the end of three years post vaccination (2009) it was 65%.** These results have been instrumental for the new cholera vaccine being introduced in India in February 2010. Post vaccination surveillance is going on.

Results:

The 4th Years surveillance (April 2010 to March 2011) shows that total number of diarrhea cases enrolled 11,449. Among these 241(2.1%) one cholera positive all are *V. cholerae* O1, the most common effected less than 15 yrs is mostly affected (1.3%).



Surveillance for Dengue Fever in Eastern Kolkata

Principal Investigator	:	S. Chakrabarti
Co-Principal Investigator	:	D. Sur
Co investigators	:	S. Kanungo, B. Manna, S. Chatterjee, P. Sadhukhan, S. Dutta

Objectives :

- To determine the incidence and burden attributable to dengue along with the epidemiologic, clinical and virologic characteristics.
- To assess the characteristics of severe dengue through health care facility based enhanced sentinel surveillance for febrile illness.

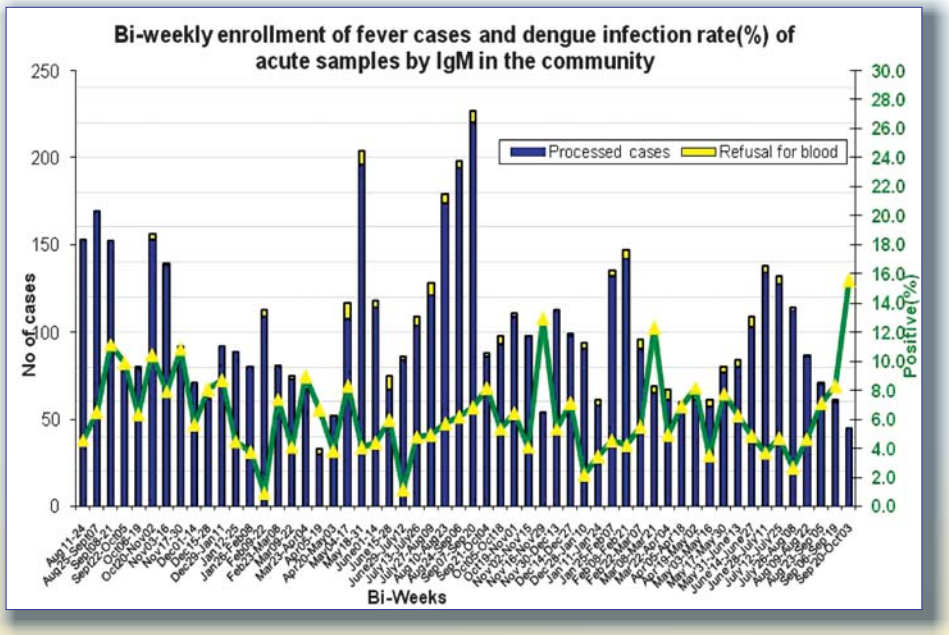
Results :

During 20 months of surveillance, a total of 5765 patients presented at the field outposts with 0-7 days of febrile illness and among them 5611 (97.3%) samples were collected. Overall, dengue detection rate by IgM ELISA among acute fever cases was 6.1%. It was higher in 0-15 years of age . The overall crude dengue 2.6/100/year but the highest incidence (7/100/year) was observed in 0-5 years age group of children. So far, DEN-1 is the most prevailing strain.

The percentages of RTPCR are DEN 1-43.3, DEN 2-24.7, DEN 3-0.6, DEN 4-31.3

Conclusion:

This data suggests that dengue is a major public health problem in Kolkata. High incidence in younger age group makes it important for decision making for future trials of dengue vaccines for targeting this particular age group. This is one of the pioneer studies in determining the population based incidence of dengue in our country.



A randomized controlled trial (Phase-II/III) of the live recombinant oral cholera vaccine (VA1.4) in eastern Kolkata

Principal investigator	:	D. Sur
Co-Investigator	:	S. Kanungo, B. Manna and T. Ramamurthy

Main target is to see the vaccine protective efficacy through a large scale phase III field trial in cholera endemic areas. For that population enumeration is tartgeted as well setting up of a community based surveillance is planned in preparation for Phase III cholera vaccination

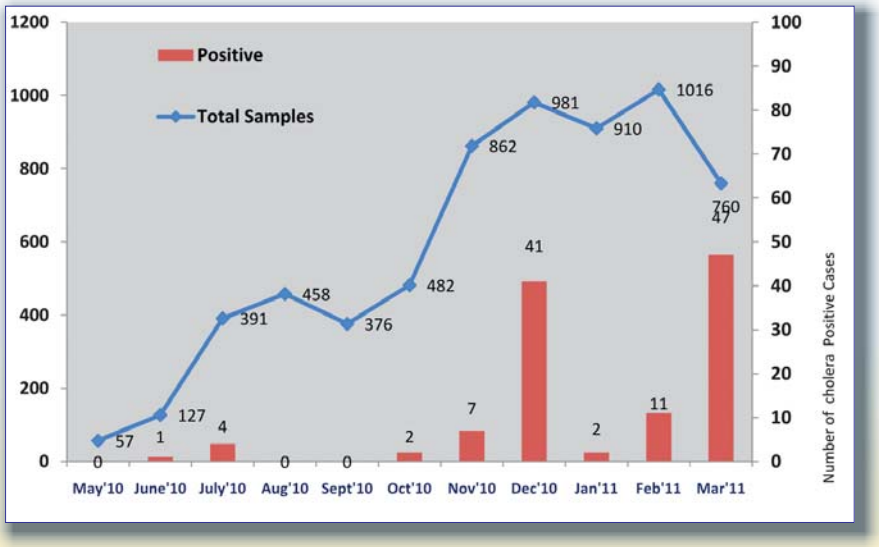
Rapid assessment of 1,30,000 population completed. Census is completed. After census, mainly two activities are going in the field.

Demographic Surveillance :

The community health worker (CHW) visits each household once in a month with pre-printed member's information in the surveillance log book for demographic surveillance to update the population from the collected information on birth, migration-in and migration-out and death. Usually each CHW visits 40-50 households per day. They also collect the information on diarrheal diseases occurrence of all individuals with a recall period of one month and promote them to report the diarrhea cases at the project based community health outposts.

Diarrhoeal Disease Surveillance :

This is an augmented passive surveillance where each community health outpost (HOP) caters about 10,000 – 12000 population. Each HOP is manned by one physician, one field supervisor, one male and one female sample collector. Besides HOPs, 2 hospitals have been identified where the patients usually come for seeking care and treatment for diarrhoea. Those 2 hospitals are Infectious Diseases Hospital, Beliaghata and B.C. Roy Children hospital, Phoolbagan. The specimens with case report forms (CRFs) are collected 3 times a day and those are processed in the NICED laboratory and the stool report is sent to the patient at the HOP or at household level through Community Health Worker.



Ward wise population distribution:

Ward	Period	In Census Population	Total No of Migration In	Total No of New Born Baby	Total No of Migration Out	Total No of Expired Cases	Active Population
35	15th May'2010-15th May'2011	25687	676	165	1245	317	24966
57	15th June'2010-15th May'2011	43753	924	423	4782	313	40005
66	1st Nov'2010-15th May'2011	68234	847	787	8311	223	61334

Age wise Diarrhoea and Cholera positive Cases:

Age	Diarrhoea(%)	Cholera(%)
0-5	2070(28.0)	51(2.5)
5-15	1731(23.4)	24(1.4)
>15	3592(48.6)	52(1.4)
Total	**7393(100.0)**	**127(1.7)**

Diarrhoeal Disease in Infants and Young Children in Developing Countries

Principal Investigator : D. Sur

Co- Investigators : T. Ramamurthy, B. Manna & S. Kanungo
In collaboration with University of Maryland,

Objective:

To estimate the population-based burden, etiology and consequences of severe diarrhea among children 0-59 months of age

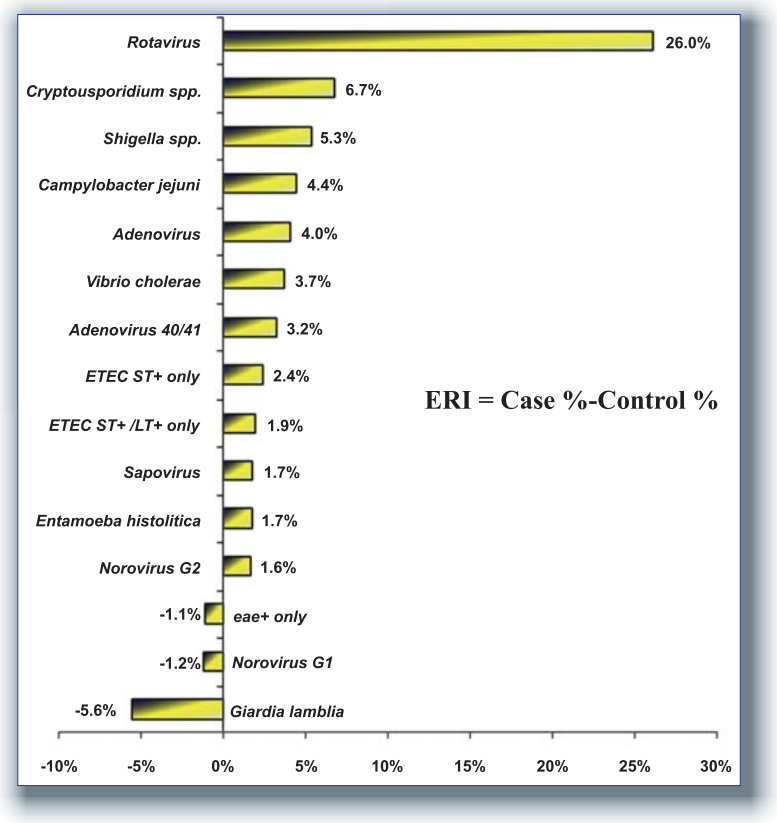
Activities:

The study site was selected in the slum areas within Wards 14, 31, 34, 58 and 59 of Kolkata Municipal Corporation (KMC) in eastern Kolkata. Currently, a total of 1,95,994 populations including approximately 13,517children are under Demographic Surveillance System (DSS) at 4 months interval for collection of demographic events. A Case-Control study was conducted with cases (from the study sites only) being enrolled from Two Sentinel Health Centres (Infectious Diseases Hospital and B.C. Roy Children Hospital) and also two SHC's at community level, controls matched for age and sex from the neighborhood of the case.

Results

Since the beginning of the study, (Dec'07 – Mar'11), a total of 5,618 births were detected and 253 deaths were reported from Demographic Surveillance System. A total of 1572 cases and 2019 controls of under-five children were enrolled with 674 cases in 0-11 month, 590 cases from 12-23 month and 308 from 24-59 months of age. The study is in progress.

ERI of causative organism for diarrhea



ERI: Excess Risk of Infection

A randomized controlled trial to evaluate the immunogenicity of two doses of the modified killed whole cell oral cholera vaccine (WC-OCV) under two alternative vaccination schedules

Principal Investigator	: D. Sur
Co Investigators	: S. Kanungo, B. Manna, M. K. Bhattacharya, R. K. Nandy, In collaboration with International Vaccine Institute, Seoul, Korea

Main study:

Primary:

Proportion of subjects exhibiting 4-fold or greater rises in titers of serum vibriocidal antibodies, relative to baseline, 14 days after last dose of study agent in each dose-interval group.

Secondary:

Proportion of subjects exhibiting 4-fold or greater rises in titers of serum vibriocidal antibodies, relative to baseline, 14 days after first dose of study agent

Geometric mean serum vibriocidal titers at baseline, 14 days after each dose of the study agent, in each dose-interval group.

Proportion of subjects given 2 doses of study agent given 14 and 28 days apart with any of the following adverse events:

Immediate reactions within 30 minutes after each dose: Serious Adverse Events occurring throughout the trial. Reactogenicity during three consecutive days following each dose: Headache, vomiting, nausea, abdominal pain/cramps, gas, diarrhea, fever, loss of appetite, general ill feeling. Diarrhea is defined as having 3 or more loose/watery stools within a 24 h period. Fever is defined as having an oral or tympanic temperature of $\geqslant 38^{\circ}$ C

Additional Exploratory Study:

Proportion of subjects given 2 doses of vaccine given 14 and 28 days apart with significant responses to: Fecal antibody to LPS, OMP micro ELISPOT, LPS-specific micro ELISPOT

IgA to LPS (by ELISA)

356 subjects in the main study population (Group A, healthy, non-pregnant adults of aged 18 years and above / Group B, children 1 to 17 years of age):

Current Status:

Age Group(Year)	Bld1(Day 0)	Bld2(Day 14)	Bld3(Day 28)	Bld4 (Day 42)
1-5	13	10	9	7
6-10	60	58	54	49
11-17	60	60	59	52
>=18	178	151	123	113
Total	**311**	**279**	**245**	**221**

No. of subjects with Non solicited adverse events: 2

No. of events: 2, NAUSEA, ABDOMINAL PAIN
No serious adverse events occur during the study period.
Among the 243 recruited 2 subjects were found to be screen failure at Day 0 and 7 subjects were considered as screen failure on Day 14 and 1 subjects were considered as screen failure on Day 28 and 3 subjects withdrew from the study. Data entry is on-going through RDC sys.

Identifying factors influencing HIV transmission in married couples- a step towards intervention development

Principal Investigator : S. Panda
Co-Investigators : S. K. Niyogi, S. Chakrabarti, S. S Das, M. K. Saha and D. Banerjee

The study is being carried out in collaboration with the civil society organization named 'Society for Positive Atmosphere and Related Support to HIV/AIDS (SPARSHA) and RG Kar Medical College & Hospital, Kolkata. The purpose of this research is to identify factors associated with discordant and concordant HIV sero-status of heterosexually married couples. Final stage of recruitment of study participants is currently on-going. This explorative research where people living with HIV take part an active role not only as

participants but also as research team members is expected to inform HIV prevention intervention in Indian setting.

A community based epidemiological study of Rotavirus in children below 2 yrs of age

Principal Investigator : S. Panda
Co-Investigators : A. Deb, T. Ramamurthy, .M. Chawla-Sarkar and S. Ganguly

Five field offices, one in each of the five different villages under Sonarpur Block of the South 24 parganas district of West Bengal were set up to conduct this epidemiologic study on Rotaviral diarrhea. A little over 600 children were covered under this study. Before initiating daily home visit to identify incidents of diarrhea in children the project staffs were trained extensively on various aspects of the study including 'good interviewing technique' and 'gaining confidence of community'. Information on socio-demographic factors, hand washing practice, health seeking behavior, weaning practice and accessing care were collected from different levels as appropriate such as family, unit and individual. Stool samples were collected and transported in room temperature on the same day to NICED laboratory for investigation. Data entry and cleaning has been over the analysis phase has presently started.

Technical Assistance Support to NACO – Baseline/Impact assessment study on HIV in IDUs in Punjab

Principal Investigator : S. Panda

Amritsar, Batala, Jalandhar, Ludhiana and Taran Taaran are the five areas in Punjab, which are being covered under this study. The concerned scientist of NICED has been responsible for designing the Baseline Situation assessment study, which might help in future impact assessment initiative. The project is being implemented by NICED through recruitment of short term project staff as well as consultants as appropriate for training, development of baseline assessment questionnaire, quality check, analysis and report writing. The study is currently being implemented in close partnership with the Punjab State AIDS Control Society, National AIDS Control Organization and Futures Group. Existing health care services in the State of Punjab have been leveraged during execution of this study. Collection of behavioral data through interviews and testing of blood samples collected from consenting injecting drug users (IDUs) and their wives have been completed. Presently analysis and laboratory quality control exercise is being undertaken and report writing plan based on data analysis is being finalized.

A study on the prescribing practices for diarrhoeal diseases in under-five children in primary, secondary and tertiary health care levels in West Bengal

Principal Investigator : S. Panda
Co-Principal Investigator : A. Mukherjee (RG Kar Medical College & Hospital)
Co-Investigators : T. Ramamurthy, S. K. Lahiri (RG Kar Medical College & Hospital)

Prevention and appropriate management of diarrhea in children is of primary importance as one in every five children – about 1.5 million globally – die each year due to diarrhea.. Although guidelines recommend the use of diagnostic stool cultures when prescribing antimicrobials, empirical antimicrobial therapy for diarrhea continues to be a part of the management in clinical practice in India. Investigation carried out by NICED in the district of Purbo-Medinipur following the Tropical Cyclone AILA has further recorded inappropriate prescription practices including antibiotic use. Against this backdrop, the current cross-sectional study intends to examine adherence to the recommended national guideline in the management of diarrhea by treating physicians and identify areas for improvement. The main objectives of the project are

a) to study the present prescription practice of the treating physicians in three levels of health care service delivery by the government among children less than 5 years of age in West Bengal and

b) to review culture sensitivity results of organisms isolated from under 5 children with diarrhea through stool culture during the current study. The study has received necessary approval from the institutional ethics committee and scientific advisory committee of NICED. The Director of Health services and ex-officio secretary of the Government of West Bengal have issued necessary instructions to the study health centers for extending co-operation in this regard. Currently data collection forms, field and laboratory based logistics, data entry platform and study execution plan are being finalized.

Oncogenic HPV among female population with and without HIV infection in West Bengal, India

Principal Investigator	: K. Sarkar
Co-Principal Investigators	: S. Chakraborti, B. Saha and S. Sengupta

Introduction & objective: Prevalence of both cervical cancer and Human Immunodeficiency Virus (HIV) infection are very high in India. Natural history of Human Papilloma Virus (HPV) infection is known to be altered in HIV positive women and there is an increased possibility of persistence of HPV infections in this population. Therefore, this study was conducted to understand the epidemiology and circulating genotypes of oncogenic HPV among HIV positive and negative female population in West Bengal, India.

Method: In this hospital-based cross-sectional study, 93 known HIV positive females attending a pre-ART registration clinic and 1106 HIV negative females attending a Reproductive and Child Health Care Clinic were subjected to study. Cervical cell samples collected from the study population were tested for the presence of HPV 16, 18 using specific primers. Roche PCR assay was used to detect other specific HPV genotypes in the cervical cells specimens of HIV positive cases only.

Results: Prevalence of HPV 16, 18 among HIV positive females (32.2%; n = 30) was higher than HIV negative females (9.1%; n = 101). About 53% (23/43) of cases with oncogenic HPV were

infected with genotypes other than 16, 18 either as single/multiple infections. HPV 18 and HPV 16 were the predominant genotypes among HIV positive and HIV negative subjects respectively. Oncogenic HPV was not found to be associated with age and duration of sexual exposure. But the presence of HIV was found to a statistically significant predictor oncogenic HPV.

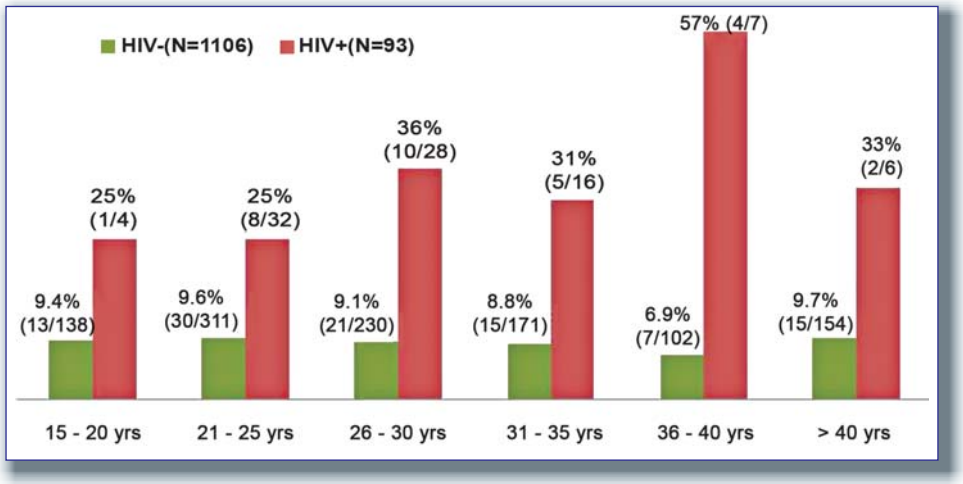


Figure-1: Age-wise distribution of oncogenic HPV among HIV positive and negative subjects

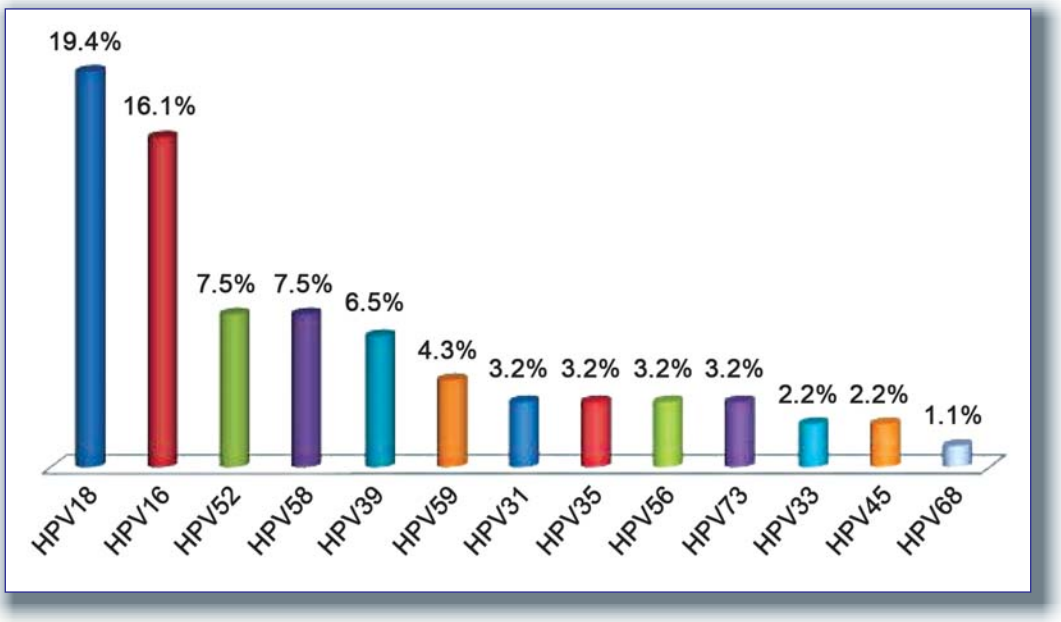


Figure-2: Distribution of HPV genotypes among HIV infected study subjects

Comprehensive population-based diarrheal disease surveillance program among under-five children in rural West Bengal, India

Principal Investigator	:	A. K. Deb
Co-investigator	:	S. Ghosh

The primary objectives of this study are to determine:

(a) overall incidence of diarrheal illnesses in the study population,
(b) drug sensitivity pattern of the major isolated pathogens,
(c) risk factors for development of diarrhea and dehydration, and
(d) cause-specific mortality and proportionate mortality due to diarrhea.

Children aged 0-4 years residing within the Langalberia PHC area in Sonarpur block of South 24-Parganas district are eligible for participation if their parents give consent. The study consists of three inter-related components:
(a) surveillance for diarrhoeal diseases (both community- and hospital-based),
(b) case-control study to determine risk / preventive factors, and
(c) verbal autopsies.

The required study instruments for collection of relevant data were developed and finalized after necessary pre-testing, which was conducted during March-April, 2009. Local community volunteers were recruited and trained on different aspects of the study and data collection processes. The baseline data collection for the households and for eligible children was started on July 01, 2009 and this was completed on February 28, 2010. These data have been computerized using the EpiInfo software. The preliminary findings indicated that there was a total population of approximately 27000 residing in 6275 households in 20 villages covering health sub-centre areas. The approximate birth rate was 13.6 per 1000 population. The striking features regarding diarrhea included very low level of awareness and highly prevalent misconceptions about different aspects of diarrhea. Data collection regarding other aspects of the study, including ongoing recruitment of new households and newly born infants are going on.

Mr. Gerardo Sanchez Martinez,
Technical Officer,
WHO Kobe Centre, Japan,
visiting one of the field offices
for the rural diarrheal diseases
surveillance project of NICED

Safety and immunogenicity of a Killed oral cholera vaccine among infants 10 weeks to less than 12 months of age when given concomitantly with EPI vaccines

Principal Investigator : A. K. Deb
Co-investigators : D. Sur, B. Manna, S. Kanungo, S. K. Niyogi

This phase-II clinical trial is an individually randomized, double-blinded, placebo-controlled trial in 300 healthy infants aged 10 weeks to 12 months allocated to receive either a bivalent killed oral cholera vaccine or a placebo. The primary **objectives are –**

(a) to determine the safety of the two-dose killed oral cholera vaccine among infants, and
(b) to determine immune responses to one and two doses of killed oral cholera vaccine among infants, when given concomitantly with EPI vaccines.

This trial has already been registered with the WHO and the Clinical Trials Registry, India. The study instruments, including the CRFs and consent forms have been prepared and approved for use; all necessary supplies, including the EPI and the study vaccines have also been procured / supplied. Institute of Child Health (ICH), Kolkata has been identified and already approved as a new collaborator for the study. Most of the study staff have been hired and trained (including training on GCP) according to study guidelines. However, due to a delay in obtaining the DCGI approval we have not yet initiated the actual study activities.

Award and Honours

D. Sur

- N. C. Sen Memorial Oration- Calcutta Medical College 2011

Conferences/ Seminars/Workshops/Trainings Attended/Organised

D. Sur

- Attended "Multi-country typhoid fever surveillance programme in sub-Saharan Africa" and spoke on "Experiences on typhoid surveillance in Kolkata, India" at Nairobi, Kenya, 27-29 April 2010
- Invited to International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) as an expert in relation to cholera vaccine trial, for its future use in Bangladesh. 27-29 July 2010
- Participated in investigators' meeting of 'Global Enteric Multicentric study (GEMS)' in Maputo, Mozambique and presented study data during 28-30 September 2010
- Attended Yakult Shirota Conference and Intestinal flora Symposium and presented on "Use of a probiotic strain in prevention of childhood diarrhoea" in Tokyo, 25-29 Japan October 2010
- Invited as member of Data Safety Monitoring Board meeting of a typhoid vaccine trial by Novartis Vaccine for Global Health at Dubai 9-11 December 2010

S. Panda

- Presented paper as an invited speaker in the 'Conference on Emerging Frontiers and Challenges in HIV/AIDS Research' in Mumbai organized by the National Institute of Research on Reproductive Health in February, 2011.
- Worked as a resource person in the 'Capacity Building Workshop on Operational Research for Northeast Region under NACP-III' in collaboration with NACO and CDC-India at North East Indira Gandhi Regional Institute of Health & Medical Sciences (NEIGRIHMS) in September 2010.

K. Sarkar

- Attended an international conference titles ``The 14th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim", held in Penang, Malaysia during 4-6 October 2010 and presented a paper.

A. Deb

- Delivered a presentation on "Implementation of generic research protocol on retrospective studies: Experience from India" in the "Informal Consultation on Research to Assess Impact of Climate Change on Communicable Diseases", organized by WHO/SEARO during 15-17 September 2010 at WHO-SEARO office, New Delhi.
- Delivered a presentation on "Climate Change and Diarrheal Diseases: With Special Emphasis on Cholera" as an invited speaker in the "NASI Symposium on Climate Change" organized by the National Academy of Science, India during December 2-4, 2010 at Jaipur.
- Rotavirus Symposium organized by the Rotavirus Vaccine Policy Unit as an invited participant on March 26, 2011 at National Institute of Immunology, New Delhi.
- A faculty in the "Informal Consultation for Development of Research Proposal on Communicable Diseases" organized by the WHO-SEARO during 23-24 December 2010 at National Institute of Cholera & Enteric Diseases, Kolkata.
- CME titled "Ethical Issues in Clinical Research & Healthcare" organized by the Drug Information Dissemination Center (DIDC), Government of West Bengal, during 28-29 March 2011 at School of Tropical Medicine, Kolkata

S. Kanungo

- Presented Data in 6th PDVI Field Site Consortium, organized by Paediatric Dengue Vaccine Initiative, Seoul, Korea, during 3-4 June 2010, in Santa Monica, USA
- Attended the expert group Meeting in New Delhi CDSA in September 2010
- Attended the training course on "Good Clinical Practices" in NICED, Kolkata on 12 November 2010
- Attended as faculty in "Informal Consultation for development of Research Protocol on Communicable diseases" organized by SEARO Office, WHO, New Delhi, in collaboration with National Institute of Cholera and Enteric Diseases in Kolkata 23-24 December 2010
- Attended the training course "Advanced Vaccinology Course 2010 (ADVAC2010)" from 10-21 May, Annecy, France.

IMMUNOLOGY

The Division of Immunology is exploring the regulation of mucosal immune cells by two proteins: porin, the major outer membrane protein with pore-forming activity of *Shigella dysenteriae*, and hemolysin, a pore-forming toxin of *Vibrio cholerae*. The major focus of the Immunology group centres on understanding how the two proteins are recognized by the cells that steer the signaling machinery either towards activation or apoptosis. The study of porin is aimed at establishing it as a potential adjuvant in vaccine strategies, while the work with hemolysin reveals the putitive mechanism of how the two forms of the exotoxin differentially interact with the cells of the mucosal immune system.

Scientist	:	T. Biswas, Scientist E
Staff	:	S. K. Shaw, Technician B
		N. C. Mondal, Attendant Services
Research Scientist	:	R. Biswas
Fellows	:	K. Sasmal (JRF)
		S. Mukherjee (JRF)

Porin-induced TLR mediated signaling of marginal zone and follicular zone cells to elicit cytokine and antibody response

Principal Investigator : T. Biswas

The murine splenic B cell sub-populations, marginal zone (CD19+ CD21hi CD23lo, MZ) and follicular zone (CD19+ CD21lo CD23hi, FO) B cells were sorted from total splenocytes of C57BL/6 mice using FACSAria II. The sorted cells were devoid of T cells due to absence of co-receptor CD3ε and TCRβ. However, the cells were positive for B cell marker B220. The percentage of FO and MZ cells in the splenocyte population was analyzed to be 45.9% and 4.5%, respectively. FO cells were of 97.4% purity whereas MZ were 90.2%.

In order to decipher the regulation of FO and MZ B cells by porin of *Shigella dysenteriae*, the cells in presence of the adjuvant up-regulated toll-like receptor (TLR) 2 and TLR6, implicates role of the two TLRs in associative induction of downstream signaling. Although the naïve MZ B cells poorly expressed TLR at 5 µg/ml porin, it got up-regulated at lower concentrations of protein.

Up-regulation of CD40 following that of the early activation markers CD69 and CD25 showed FO B cells were capable to receive cognate or by standing T cell help for amplification of adaptive responses. Furthermore, FO B cells showed a selective up-regulation of CD86 but not of CD80. MHCII (I-Ab) was also over-expressed indicating that FO B Cells were APC. Thus porin can effectively activate and prime FO B cells without T cell help for action.

Award and Honours

T. Biswas

- Invited reviewer for European Journal of Immunology (2009a & b); The Journal of Immunology (2009); Journal of Leukocyte Biology (2010).

Conferences/ Seminars/Workshops /Trainings Attended/Organised

T. Biswas

- Presented poster "Marginal zone anti-inflammatory and follicular zone pro-inflammatory cytokine expressing B cells are promoted by porin of *Shigella dysenteriae* type 1" at International Symposium on Molecular and Pathophysiological Research on Enteric Pathogens, 27-29 January, 2011.

PARASITOLOGY

The Division of Parasitology actively integrates research into the mechanisms of parasitic diarrhoeal diseases at the molecular and cellular levels with epidemiological investigations of parasitic diagnosis from hospital and community patients. While ensuring an increasing understanding of human parasitic diseases, like amoebiasis, giardiasis, cryptosporidiasis, it also provides the foundation for further development in diagnosis and future therapeutics

Research efforts are built upon understanding the mechanism of ribosome biogenesis in giardia, macro molecular interactions, mechanisms of macro molecular complex formation and its use as a drug target in giardiasis. Genomic DNA microarray chip of giardia has been constructed in this division and is utilized for studying the effects of oxidative stress regulation in micro-aerophilic giardia at the transcryptomic and proteomic level. A surveillance of enteric parasites from stool samples collected from different hospitals are regularly done in this laboratory to understand the current scenario of parasitic diarrhoea in Kolkata as well as to establish the prime aetiology with parasitic co-infections.

This division is the eastern node as well as the central unit of parasitic network under Indo-US Joint collaboration for training and manpower generation and quality control of parasitic diagnosis across India.

This division has strong collaborations with Okayama University, Japan, NIID, Japan, CDC, USA, City University of New York, USA, Childrens' International, USA, ICDDR, Bangladesh, Amsterdam Medical University, Netherlands etc.

This division offers PhD. And Post-Doctoral training programme in different aspects of enteric parasitology. Besides its PhD. And Post-doctoral programme, this department organizes workshops and training for scientists, students and technicians.

Different prestigious grants and awards from national and international levels have enriched this department from time to time.

Scientist	:	S. Ganguly, Scientist C
Staff	:	T. N. Boral, Technical Officer A S. L. P. Singh, Technician B P. Chowdhury
Students	:	A. Ghosh (SRF) A. K. Mukherjee (SRF) K. Das (RA) D. Raj (JRF) S. Karmakar (JRF)

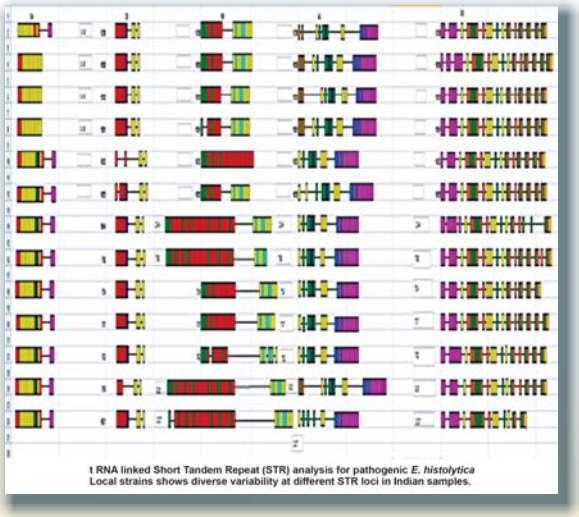
Exploring the biology of enteric parasites.

Investigator : S. Ganguly

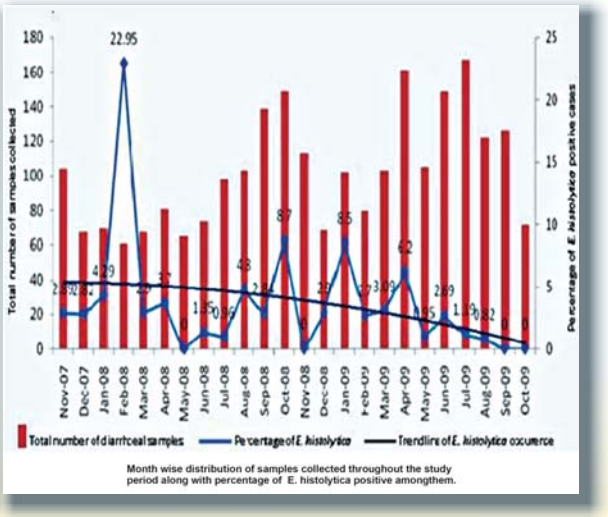
It is reported for the first time that the infection status of the Amoebiasis was changed in tropical country and also have identified new pathogenic strains of *Giardia* and *Cryptosporidium* in Kolkata and submitted the sequence to Genebank. Zoonotic transmission of *Giardia* and *Cryptosporidium* in Kolkata between human and other mammals are also reported as well as enormous genetic diversity of giardiasis among diarrhoeagenic population of Kolkata has been identified. A new high resolution genotyping of *Entamoeba histolytica* has been developed to assess its virulence and pathogenicity. Based on this genotyping new Indian pathogenic isolates have been identified. Parasite and helminth burden among different age groups in West Bengal with special emphasis on children of low socio- economic class has been identified and evaluation of a new TriCombo ELISA kit (Techlab) for the identification of *E. histolytica, G. lamblia* and *Cryptosporidium* directly from stool samples has been done.

In the present studies on oxidative stress response of *G. lamblia*, it has been observed for the first time that trophozoites at high oxygen environment produce higher reactive oxygen species (ROS) in a time dependent manner. It is also evident from transcriptomic and proteomic analysis that mitochondrial remnant proteins are not the key proteins from stress regulations, rather a cascade of other biochemical pathways and proteins are involved in stress relief. RT-PCR results clearly highlight the genes that are differentially expressed in high oxygen environment exposed cells and a possible link in survival of the parasite in high oxygen environment during its pathogenesis.

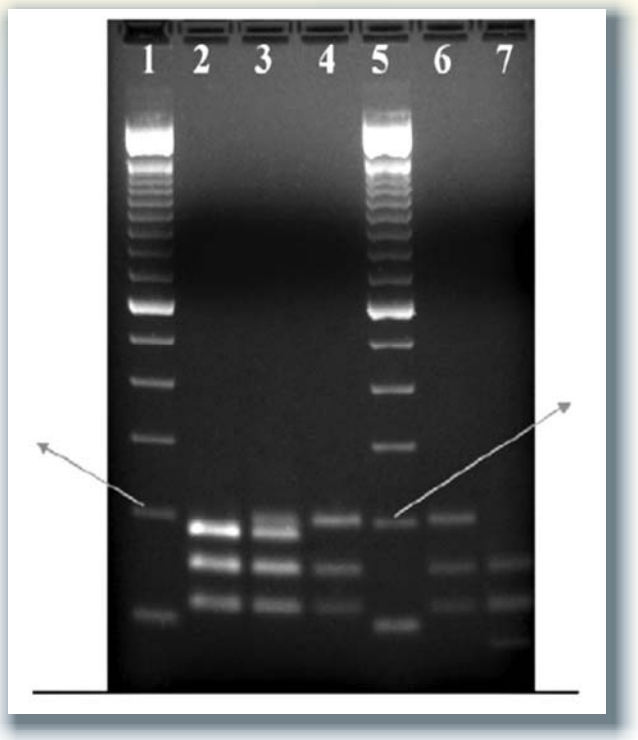
It is also reported for the first time that these early branching eukaryotes (EBE) undergo Programmed Cell Death (PCD) using a novel pathway other than known proteases dependent pathways.



t RNA linked Short Tandem Repeat (STR) analysis for pathogenic *E. histolytica* Local strains shows diverse variability at different STR loci in Indian samples.



Month wise distribution of samples collected throughout the study period along with percentage of E. histolytica positive amongthem.



Genotyping of Giardia isolates by RFLP analysis based on digestion of-giardin gene PCR products by HaeIII. Lanes1 and 5:100 bp plus DNA ladders, lane2:AssemblageE, lane3:mixed Assemblage A and E, lane4:Assemblage A(bovinesource), lane6:Assemblage A (human source) and lane7 : Assemblage B

Award and Honours

S. Ganguly

- Top Reviewer Honor (2009-10) from Parasitology International journal published by Elsevier.
- Reviewer of different peer reviewed International Journals *viz*. Parasitology International, Epidemiology and Infection, Chemotherapy, Indian Journal of Gastroenterology etc.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

- Presented a paper in XIIth International Congress of Parasitology (ICOPA)" held in Melbourne, Australia, from 15-20 August 2010.
- Presented two papers in 45th Annual Japan-U.S. Joint Conference on Parasitic Diseases" held in NIID, Shinjuku, Tokyo, Japan, from 10-15 January 2011.
- Presented a paper in International Conference on Opportunistic Pathogens" held at All India Institute of Medical Sciences, New Delhi, India from 27-30 September 2010.
- Presented a paper in National Congress of Parasitology at Kalyani, West Bengal, India from 30th October to 1t November, 2010.

PATHOPHYSIOLOGY

The research interest of the Division of Pathophysiology is related to the understanding of pathogenesis and signal transduction mechanism of different darrhoeagenic bacteria, development of candidate vaccine, Super ORS and use of proper antibiotics against diarrhoea.

This Division is involved in the purification and characterization of different toxins and virulence factors secreted by diarrhoeal pathogens and in-depth study of these signaling mechanisms. The Division is well conversant in identification, purification and characterization of receptors, bacterial adhesions, toxins and proteases.

The involvement of different intracellular signal molecules in the induction of intestinal secretion by *E. coli* heat-stable toxin (STa), non-01 *V. cholerae* (NAG-ST), *Yersinia enterocolitica* heat-stable toxin (Y-STa) have been evaluated. Moreover, calcium sensing receptor mediated downregulation of colonic carcinoma cell proliferation by thermostable direct hemolysis (TDH) has also been studied. It has been reported that COLO-205 cell line might be used as a model cell line to study the mechanism of action of *E. coli* STa. Furthermore, a significant rearrangement of actin cytoskeleton has been shown after *E. coli* STa treatment in COLO-205 cells.

The pathogenic mechanism of nonO1, nonO139 *V. cholerae* is not yet known clearly. In course of our studies, two forms of Hemagglutinin Protease (HAP), viz. a mature 45-kDa and another processed 35-kDa form has been purified from non01, non0139 strain and subsequent studies suggest that HAP may be an important virulence factor of these strains. A novel 59 kDa serine protease was indentified from Δ hap A *V. cholerae* O1 strain and shown to cause hemorrhagic response in rabbit ileal loop assay.

A study on vaccine development revealed that oral administration of heat-killed *Shigella flexneri* 2a could give 100% protection against homologous challenge which may lead to develop a simple, practical and effective vaccine against shigellosis. 34 kDa OMP was identified as the responsible immunogen which is cross reactive, surface exposed and antigenically conserved among the *Shigella* spp. The protein has high potential to be a candidate vaccine and / or diagnostic kit.

The studies undertaken by the division are important for the development of vaccines and other therapeutic agents which can stop the signaling mechanisms of diarrhoeagenic pathogens at a particular stage which ultimately may prevent diarrhoeal diseases.

Scientist	:	M. K. Chakrabarti, Scientist F A. Pal, Scientist E
Staff	:	S. Sen Senior Technical Assistant J. Ram Technician C [Retired on 31 October 2010]
DBT Ramalingaswami Fellow	:	K. M. Hoque
Junior Research Fellows	:	P. Karmaker R. Tapadar R. Bhowmick Sk. Irshad Ali

Characterization of the 34kDa outer membrane protein of *Shigella flexneri* 2a and study of its immune response

Principal Investigator : M. K. Chakrabarti

Our previous study has revealed that the 34 kDa outer membrane protein (OMP) of *Shigella flexneri* 2a is cross-reactive, antigenically conserved among *Shigella* spp., and the epitope is surface exposed on the intact bacterium as well as boost the induction of protective cytokines by macrophages, established itself as a highly immunogenic. The mechanism underlining the macrophage activation by 34 kDa OMP is not known so far. Therefore, in the present study the in-depth mechanism of macrophage activation in response to 34 kDa OMP has been evaluated. During this reported period TLR2 has been recognized as a receptor for 34 kDa OMP on macrophages. In addition to TLR2, 34 kDa OMP enhances the expression TLR6 in macrophages. Furthermore, it has been found that OMP up regulates the production of G-CSF, IL-1β, IL-6, TNF-α, IFN-γ and IL-12p70 that are involved in the antibacterial responses. Moreover, p38 MAP kinase has been found to be the important regulator of these cytokines. Our present findings reveal that pre-treatment of MΦ with SB203580, a specific inhibitor of p38 kinase, significantly inhibits the release of IL-6, TNF-α, IFN-γ and IL-12p70 by MΦ. Further studies are going on to identify the other intracellular signaling molecules involved in the 34 kDa OMP mediated immune response.

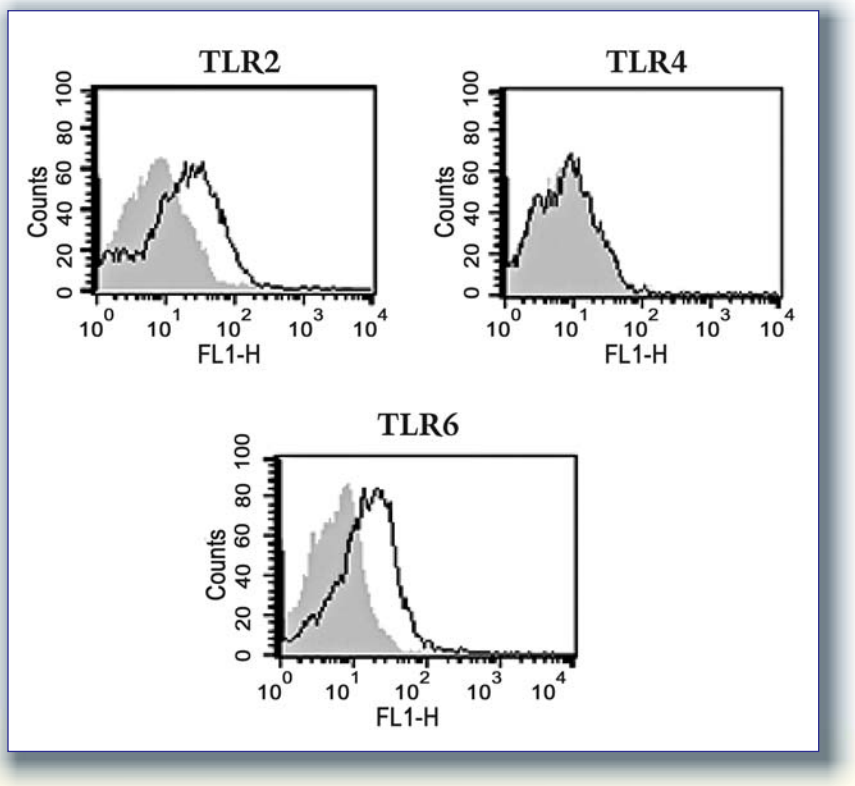


Fig. 1. Induction of TLRs on PerC MΦ of BALB/c mice in response to MOMP. MΦ were cultured with complete medium alone (shaded) and MOMP (open) for 4h and analyzed by flow cytometry for the expression of TLR2, TLR4 and TLR6. The data shown are representative of three independent experiments.

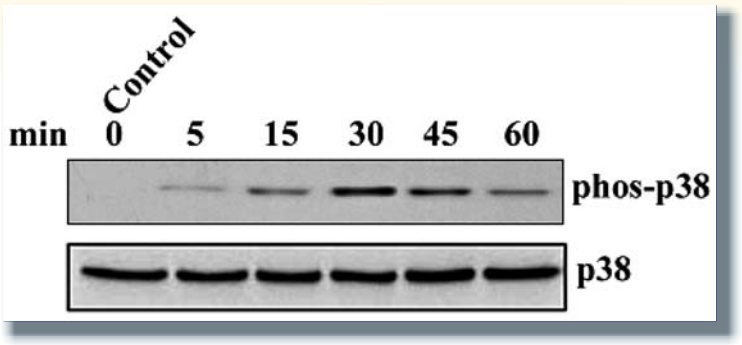


Fig. 2. Expression of phospho-p38 MAP kinase in MΦ treated with MOMP for different time periods as checked by immunoblotting. MΦ were incubated without and with 34 kDa MOMP for different time period. The cell lysates were then blotted with anti-p38 MAPK mAb (p-p38) or probed with anti-p38 MAPK (p38). The figures are representative of three independent experiments.

Studies on proteases of *Vibrio cholerae*

Investigator : A. Pal

Extracellular proteases may play an important role in the pathogenesis of diarrhoea caused by *V. cholerae*. The most well characterized protease in cholera is hemagglutinin protease. Microarray of the global transcription pattern of *V. cholerae* grown in vivo in the rabbit ileal loop model have shown that *hapA* gene is one of the 12 genes that belong to the pathogenic functional group and are highly expressed compared to their levels of expression under laboratory conditions. In an earlier study we have shown that HAP may play a role in the pathogenesis of ctx gene negative *V. cholerae* non-O1, non-O139 strain (Infection and Immunity, 2006). Two well-characterized proteases secreted by *Vibrio cholerae* O1 strains are hemagglutinin protease (HAP) and *V. cholerae* protease (PrtV). The *hapA* and *prtV* knock out mutant, *V. cholerae* O1 strain CHA6.8Δ*prtV*, still retains residual protease activity. We initiated this study to characterize the protease present in CHA6.8Δ*prtV* strain and study its role in pathogenesis in rabbit ileal loop model (RIL). We partially purified the residual protease secreted by strain CHA6.8Δ*prtV* from culture supernatant by anion-exchange chromatography. The major protein band in native PAGE was identified by MS peptide mapping and sequence analysis showed homology with a 59-kDa trypsin-like serine protease encoded by VC1649. The protease activity was partially inhibited by 25 mM PMSF and 10 mM EDTA and completely inhibited by EDTA and PMSF together. RIL assay with culture supernatants of strains C6709 (FA ratio 1.1 +/- 0.3 n=3), CHA6.8 (FA ratio 1.08 +/- 0.2 n=3), CHA6.8Δ*prtV* (FA ratio 1.02 +/- 0.2 n=3) and partially purified serine protease from CHA6.8Δ*prtV* (FA ratio 1.2 +/- 0.3 n=3) induced fluid accumulation and histopathological studies on rabbit ileum showed destruction of the villus structure with hemorrhage in all layers of the mucosa. RIL assay with culture supernatant of CHA6.8Δ*prtV*ΔVC1649 strain (FA ratio 0.11 +/- 0.005 n=3) and with protease incubated with PMSF and EDTA (FA ratio 0.3 +/- 0.05 n=3) induced a significantly reduced FA ratio with almost complete normal villus structure. Our results show the presence of a novel 59-kDa serine protease in a Δ*hapA*Δ*prtV V. cholerae* O1 strain and its role in hemorrhagic response in RIL model (PloS ONE.2010)

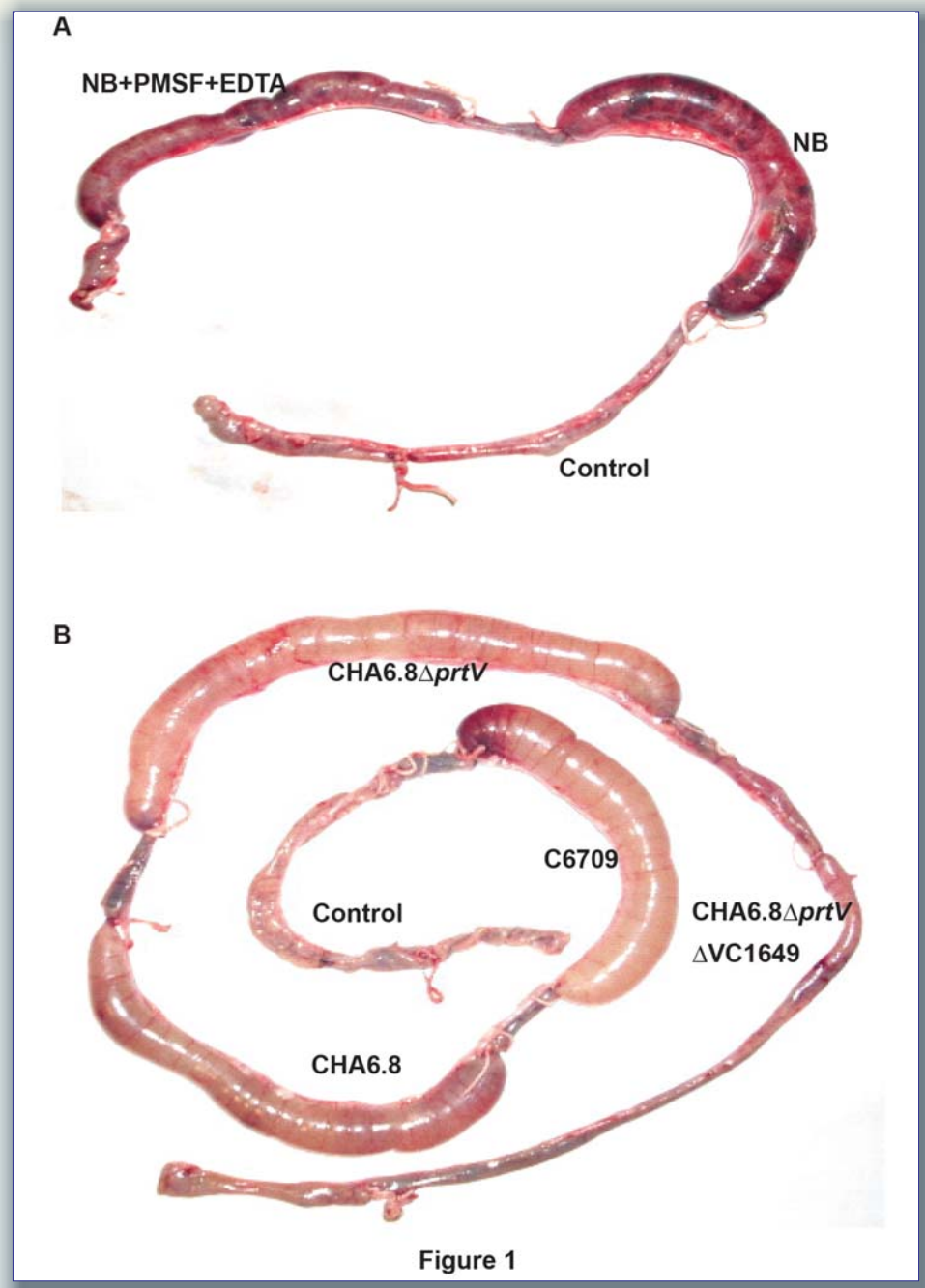


Figure 1. Rabbit ileal loop assay. A) RIL response of partially purified protease (50 µg, NB) showing significant hemorrhagic fluid accumulation (FA ratio 1.2 +/-0.2 n=3) and its effect after inhibition with 25 mM PMSF and 10 mM EDTA (NB+PMSF+EDTA) shows significant decrease in fluid accumulation (FA ratio 0.3 +/-0.05 n=3). Twenty five mM Tris-HCl with 25 mM PMSF + 10 mM EDTA was used as a negative control (FA ratio=0.12 +/-0.002, n=3). B) RIL response with culture supernatants of C6709 (FA ratio 1.1 +/- 0.3, n=3), CHA6.8 (FA ratio 1.08 +/- 0.2, n=3), CHA6.8Δ*prtV* (FA ratio 1.02 +/- 0.2, n=3), CHA6.8Δ*prtV*ΔVC1649 (FA ratio 0.11 +/- 0.005, n=3) and Tryptic soy broth as negative control (FA ratio 0.09 +/- 0.002, n=3).

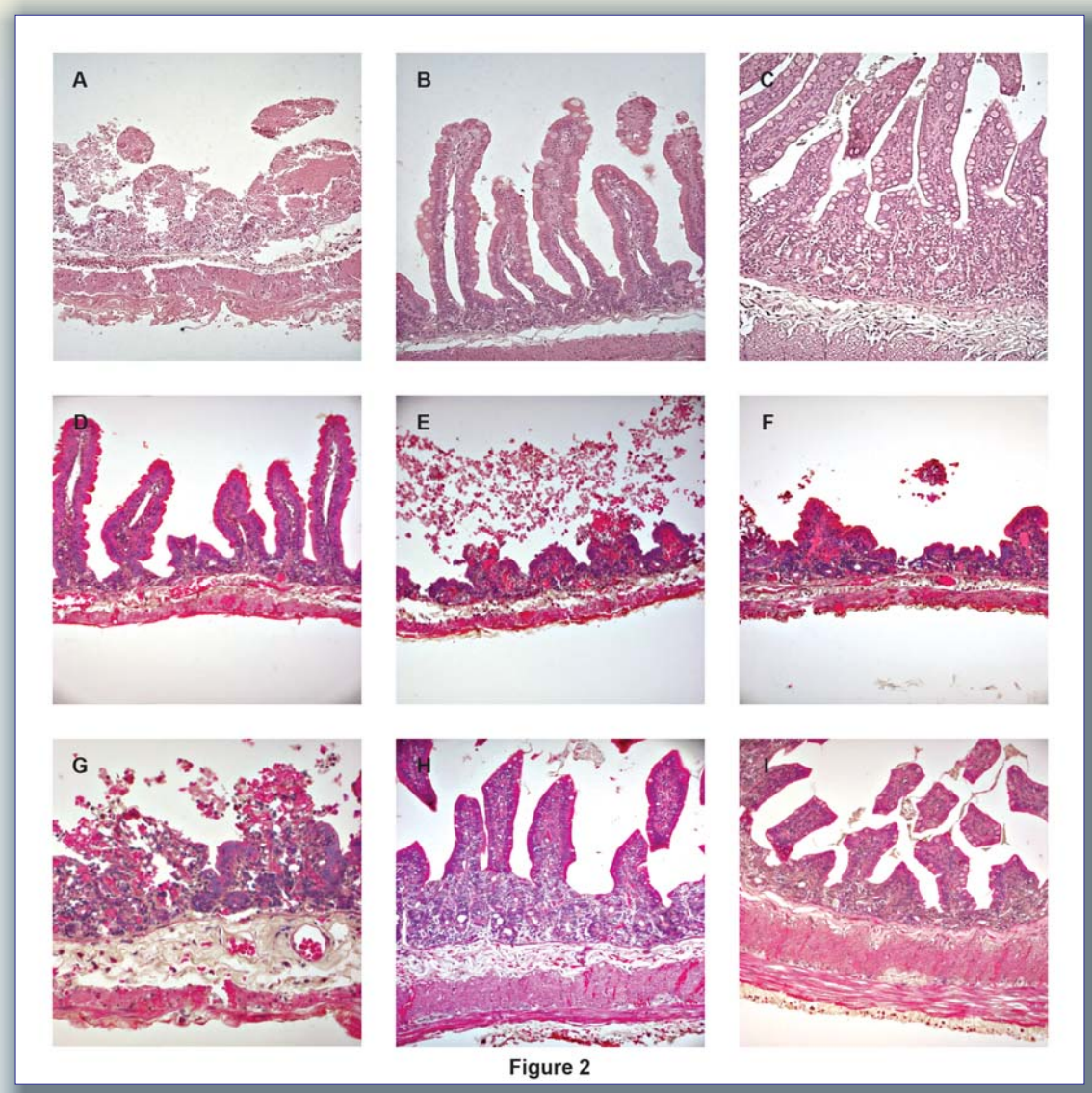


Figure 2. Histopathological study of ileal tissues. Panels show photomicrographs of histology of rabbit ileal loop tissue after treatment with (A) Partially purified serine protease from *V. cholerae* strain CHA6.8Δ*prtV* showing hemorrhagic fluid accumulation (Fig. 4A, NB). Gross damage of the villus surface structure was observed with hemorrhage in all layers of the mucosa. Magnification, 20X. (B) Almost normal villous architecture observed in ileal tissues treated with 50 µg of partially purified protease inhibited with 25 mM PMSF and 10 mM EDTA (Fig. 4A, NB+PMSF+EDTA). This photomicrograph shows no gross alteration in villus structure but villus lamina propria are slightly dilated and RBC have accumulated at a few places in the basal area. Magnification, 20X. (C) Ileal tissues treated with 25 mM Tris-HCl buffer with PMSF and EDTA (Fig. 4A, control) showed normal villus structure. Magnification 20X. (D) ileal tissues treated with culture supernatant from C6709 strain showed presence of hemorrhage in all layers of the gut mucosa specially in the submucosal layer, Magnification 20X. (E) ileal tissues treated with culture supernatant from CHA6.8 strain showed widely dialated villi with rupture at places with gross hemorrhage and inflammatory cells in mucosa and submucosa, Magnification 20X. (F) Ileal tissues treated with culture supernatant of CHA6.8Δ*prtV* strain also showing dilated villi with gross hemorrhage in all layers of the mucosa. Magnification 20X. (G) The same section in higher magnification 40X showing ruptured villi with hemorrhage and inflammatory cells in mucosa and submucosa. (H) ileal tissues treated with culture supernatant from CHA6.8Δ*prtV*ΔVC1649 strain showing villous architecture almost normal with minimum hemorrhage in mucosa and submucosa. (I) TSB treated ileal tissue showing normal gut mucosa.

Award and Honours

M. K. Chakrabarti

- Secretary /Convener. Medical Science Section, West Bengal Academy of Science and Technology, 2009-2011.
- General Secretary (Membership Affairs), Indian Science Congress Association, 2010-2013.
- Vice-President, The Physiological Society of India, 2010-2014.
- Member of the Editorial Board of Indian Journal of Physiology and Allied Sciences, Asian Journal of Experimental Sciences and Al Ameen Journal of Medical Sciences.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

M. K. Chakrabarti

- Delivered a lecture as Chief Guest at the inaugural programme of National Science Day at ISCA headquarters, organized by ISCA Kolkata Chapter on 28 February, 2011.
- D.Pore, N.Mahtata and M.K.Chakrabarti. "An outer membrane protein of Shigella flexneri 2a: Potential subunit vaccine candidate against shigellosis" at International conference on "Molecular and Pathophysiological research on enteric Pathogens" held at Kolkata, India on 27-29 January, 2011.
- N. Mahtata, D.Pore and M.K.Chakrabarti. "Actin cytoskeleton reorganization and translocation of PKC-α in the mechanism of action of Escherichia coli heat stable enterotoxin (STa) in COLO-205 cells" at International conference on "Molecular and Pathophysiological research on enteric Pathogens" held at Kolkata, India on 27-29 January 2011.
- Chaired a session on Microbiology and Immunology, Section of Medical Sciences including Physiology, 98th Session of Indian science Congress, SRM University, Chennai, 5 January 2011
- "Impact of Global Warming on the Spread of Infectious Diseases" Key note address delivered at the UGC sponsored National Seminar on Aftermath of global changes on biological world organized by D.G. College, C.S. J.M. University Kanpur on 20-22 November, 2010.
- "Vaccines Against Enteric Pathogens" Invited lecture delivered at UGC Refresher course for College and University Teachers, Academic Staff College, Department of Pharmaceutical Engineering, Jadavpur University, Kolkata on 17 July, 2010

A. Pal

- "International Symposium on Molecular and Pathophysiological Research on Enteric Pathogens" 27-29 January 2011 Kolkata, India

VIROLOGY

The Division of Virology focuses on Enteric Viruses and Human Immunodeficiency Virus (HIV) with three basic components namely, service, training and research.

For service, the division plays a key role in the surveillance studies undertaken by the institute to understand the etiological role of different diarrhoeagenic viruses in and around Kolkata to gather information with relation to the disease burden. The division provides laboratory diagnostics for viral pathogens like rotaviruses, noroviruses, sapoviruses, astroviruses, adeno viruses and picobirna viruses during the diarrhoeal outbreaks in the state or country. In addition, epidemiological and molecular characterization of HIV strains among high risk groups in West Bengal and Manipur is done in collaboration with epidemiology division.

The Division also serves to impart training to graduate and doctoral students and staff so as to improve the human resources capable of studying viral diarrhoeal diseases across the country.

The research programs in the division include intramural projects and extramural projects with national and international funding and collaborating scientists. The current programs are associated with DBT, ICMR, CDC Atlanta, Sapporo Medical University, Okayama University etc. The division is involved in basic research involving studies on genetic diversity, vaccine development, host-virus interactions related to enteric viruses and human Immunodeficiency virus (HIV).

The Division has extended its activities to include studies on influenza viruses and has organized a routine surveillance program in collaboration with World Health Organization and Centers for Disease Control and Prevention, Atlanta, USA for close monitoring of genetic diversity among circulating strains. The division also maintains Biosafety Level 3 laboratory to carry out investigations during an outbreak of suspected highly pathogenic viral diseases such as SARS or avian influenza.

Objectives of Division :

1. Molecular characterization of crucial HIV encoded genes with focus on understanding immunogenicity for developing vaccine candidates.
2. Surveillance and disease burden of diarrhoea induced by Enteric Viruses.
3. Molecular phylogenetic analysis of the circulating enteric viruses in and around Kolkata with focus Rotaviruses, Caliciviruses (Norovirus and Sapovirus), Astroviruses, Picobirnaviruses and Adenoviruses.
4. Analysis of the signaling mechanisms during Rotavirus-host cell interactions: Study of host cellular proteins required for viral replication and pathogenesis.

Scientist	:	S. Chakrabarti, Scientist G
		T. Krishnan, Scientist E
		M. Chawla-Sarkar, Scientist C
		B. Ganesh, Scientist B
Staff	:	S. Omesh, Technical Officer A
		M. Mallick, Technical Assistant
		K. Sen, Technician C
		P. De, Technician B
		B. K. Bera, Technician B
		Md. M. Hossain, Technician B
Research Scientist/Pool Officer	:	R. Dey
Young Woman Scientist	:	M. Sarkar
Senior Research Fellows	:	A. S. Agarwal
		D. Dutta
		R. Mullick
		R. Sarkar
		S. M. Nataraju
		M. S. Pativada
		A. Mukherjee
		P. Bagchi
		S. Chattopadhyay
Junior Research Fellows	:	N. Biswas
		U. C. Halder
		R. Kumar

Molecular Characterization of *tat* and LTR region of HIV-1 among the IDUs in Manipur

Investigator : S. Chakrabarti

The main objective of our study was to characterize the tat and LTR regions of HIV-1 among the IDU populations of Manipur,a north-eastern state in India. Our study revealed interesting findings on both tat and LTR genomic regions of HIV-1 among IDU populations from this part of India.

Blood samples were collected in EDTA coated vacutainer tubes (Beckton Dickinson) and peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll – Hypaque gradient centrifugation . DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany) according to the manufacturer's protocol. Subtyping analysis of all the samples were done by gag and env heteroduplex mobility assays followed by sequencing analysis.The exon1 region of the tat gene was amplified by nestedpolymerase chain reaction (PCR) from PBMC DNA in a thermal cycler (Geneamp PCR system, 9700, Perkin Elmer) using primer pairs TAT OF1 : 5_ACAGGAGTCGAAGCTATAATAAG3_
and TATOR1: 5_TTCTATATATACTATGGTCCACACAATTAT3_ in 1st round and TATINF1: 5_GACTACTGCAACAACTACTGTTTAT3_,
TATINR1 : 5_ ATTAATGCTACTACTATCAATGCTCCTACTCC3_ in 2nd round. A total of 35 samples of Manipur IDUs were amplified using the PCR protocol described earlier.The exon2 region of the tat gene was amplified by nested PCR using primer pairs TATEX2OF : 5_AAATAGAGTTAGGCAGGGATACTCACCT3_ ,
TATEX2OR : 5_ATCGTCCCAGGCAAGTGCTAAGA3_ in first round and
TATEX2IF : 5_ACCCTTACCCCGAACCCGAGGGGA3_,
TATEX2IR : 5_TTACTAATCGAATGGATCTGTCTTGTGCTTG3_ in second round. About 1.0–10_g of template was used for PCR amplification in the presence of 1× PCR Gold Buffer, 1.5mM MgCl2, 0.2mM dNTPs (Perkin Elmer), 10 pmol of each primer, 2.5U of Taq DNA polymerase (Ampli Taq gold, 5U/_l, Perkin Elmer), in a total volumeof 50_l. Conditions followed in the first round PCR was: 94 °C for 15 min, 35 cycles consisting of 94°C - 30s, 50°C - 30 s and 72°C for 1min, with a final extension at 72 °C for 7min. 1_l of the first round PCR product was used as a template for the second round PCR and the condition followed in the second round was: 94°C for 15 min, 35 cycles consisting of 94 °C - 30 s, 55 °C - 30 s, 72 °C for 1min; with a final extension at 72 °C for 7min. The LTR region of all the samples was amplified through nested PCR. Primer pairs used in the first round was

LTR5OF: 5_TGGAAGGGTTAATTTACTCCA3_ , LTR5R: 5_CCTCTCGCC

TCTTGCCGAGT3_ and LTR5IF(N) : 5_TCCTTGATTTGTGGGTCTAT

CACA3_, LTR5IR: 5_TGCTAGAGATTTTCCACACT3_ in the second round. About 1.0–10_g of template was used for amplification using 1× PCR buffer, 1.5mM MgCl2, 0.2mM dNTPs (Perkin Elmer), 10 pmol of each primer, 2.5U of Taq DNA polymerase (Ampli Taq gold, 5U/_l, Perkin Elmer), in a total volume of 50_l. PCR condition followed in both the rounds were 94 °C for 15 min, 35cycles consisting of 94 °C - 30s, 50°C - 30s, 72°C for 1min, with afinal extension at 72 °C for 7min. All the 35 samples were amplifiedfor the LTR region.

Alignment and phylogenetic analysis of the tat gene (exon1) of Manipur IDUs with subtype C reference sequences from India and other outgroup references available in the database

(http://www.hiv.lanl.gov/content/index) clearly showed that 31 samples from Manipur out of 35 belonged to subtype C, forming a strong cluster with subtype C reference strains from India including China and Africa (Fig. 1). Four samples, mnp47, mnp84, mnp78 and mnp27 were more close to subtype B references, however forming a visibly separate subgroup from the other B strains. This impelled us to do the similarity plot analysis of these 4 samples for further confirmation with the C and B reference strains where in each case it showed a mosaic pattern between subtypes B and C. Simplot analysis showed that majority of the exon1 region (region spanning from 35 to 152 bp) showed strong similarity with subtype B and exon2 with subtype C (Fig. 2a). Moreover recombination breakpoints in all the 4 sequences were found to be identical. Further Simplot analysis of other samples was also done in order to confirm the subtype C nature (Fig. 2b). Phylogenetic and simplot analyses of the exon2 region of the corresponding 4 samples along with few other samples showed similarity (>85%) with subtype C sequences (data not shown). Interestingly, mnp47 and mnp84 showed subtype C as their prominent subtype as analysed by gag and env HMA analyses, reported earlier.. However mnp78 and mnp27 showed subtype B for the gag p24-p7 region and subtypes C and B respectively for the env C2-V3 region.

Sequencing and phylogenetic analyses of the LTR region of the 35 samples from Manipur showed that, all the sequences form a strong cluster with subtype C sequences from India (Fig. 3). Interestingly, subtype C sequences from Africa (Zambia, South Africa, Botswana and Ethiopia) formed a separate cluster altogether with the outgroup reference sequences of other subtypes and was distinctly separated from the Manipur IDU cluster. Discrete sequence polymorphisms was found between the Manipur, Indian and African LTR sequences. Analysis of the transcription factor binding sites (TFBS) of the LTR sequences showed presence of three NF-kB sites in all samples as found in subtype C. Number of AP1 andSp1 binding sites were also conserved. MFNLP (most frequent naturally occurring length polymorphism), a common polymorphism found within the LTR region located upstream of the NF-kB site at position −120 of HXB2 was found in case of Manipur IDU samples mnp29, mnp18, mnp41, mnp26, mnp15, mnp60, mnp72, mnp83, mnp25, mnp07, mnp30, mnp52, mnp58, mnp44, mnp14 including the recombinant samples mnp78, mnp84 and mnp27. Next alignment of the TAR of all the LTR sequences were studied in order to find out any possible variation among the TAR binding region of the recombinant samples, including others. It was observed that in spite of the recombination event, there were no major variations in the loop or bulge portion of the TAR of the corresponding four samples including others.

This is the first report of characterisation of HIV-1 based on tat gene and LTR region of HIV-1 samples from IDUs of Manipur, India. It showed that subtype C is prevalent in Manipur as studied earlier Here, subtype B was mainly found as BC recombinant rather than as a mother subtype. Sequencing and simplot analysis of the tat gene suggests that the recombination event has a tendency to concentrate in the exon1 region of the tat gene. Recombination lead to an almost 9:1 ratio of subtype C to BC recombinant of tat gene while the sequences analysis of LTR region showed a total

subtype C prevalence. Interestingly though the LTR sequences of Manipur IDUs formed a distinct group among themselves and thus were characteristically unique from other global C types. The presence of discrete sequence polymorphisms in the Manipur including Indian sequences might be responsible for the separation of the Manipur and Indian cluster from the African LTR cluster. Earlier studies on HIV-1 3_-LTR sequences from India also confirmed the prevalence of HIV-1 subtype C in India with increased promoter diversity which revealed

increasingly complex phylogeny of HIV-1 subtype C within India . Analysis of those 3_-LTR sequences with the Manipur IDU 5_-LTR sequences showed close similarity with each other and formed same cluster (data not shown). Studies from the LTR sequences from West Bengalalso showed close similarity with subtype C sequences. Though subtype C is prevalent in India, recent studies from North India detected both B and BC recombinants for the LTR region in few samples. The HIV-1 TAR RNA element is positioned immediately downstream of the transcription start site of 5_-LTR forming a stable hairpin structure and has a wide natural variation. The loop region (CUGGGA) of the TAR is responsible for Tat-CyclinT1/CDK9 interaction for activating transcription elongation from HIV-1 LTR. Changes in the relevant nucleotide position in this region have been attributed to variation in its interaction with CyclinT1 while changes at the bulge region (UCU) interfere with its interaction with the Tat protein. Further studies are in process to investigate the genetic and functional characterization these genes.

Genbank accession numbers

The Genbank accession numbers of the tat gene sequences of 35

Manipur IDUs are available in the database (EU810162–EU810196).

Accession nos. of the mnp78 tat exon2 region and NRS2 full-length tat gene (subtype C) are also available in the same (EU921439 and

EU921440) respectively. Accession nos. of 35 Manipur IDU LTR

sequences are EU835818–EU835852.

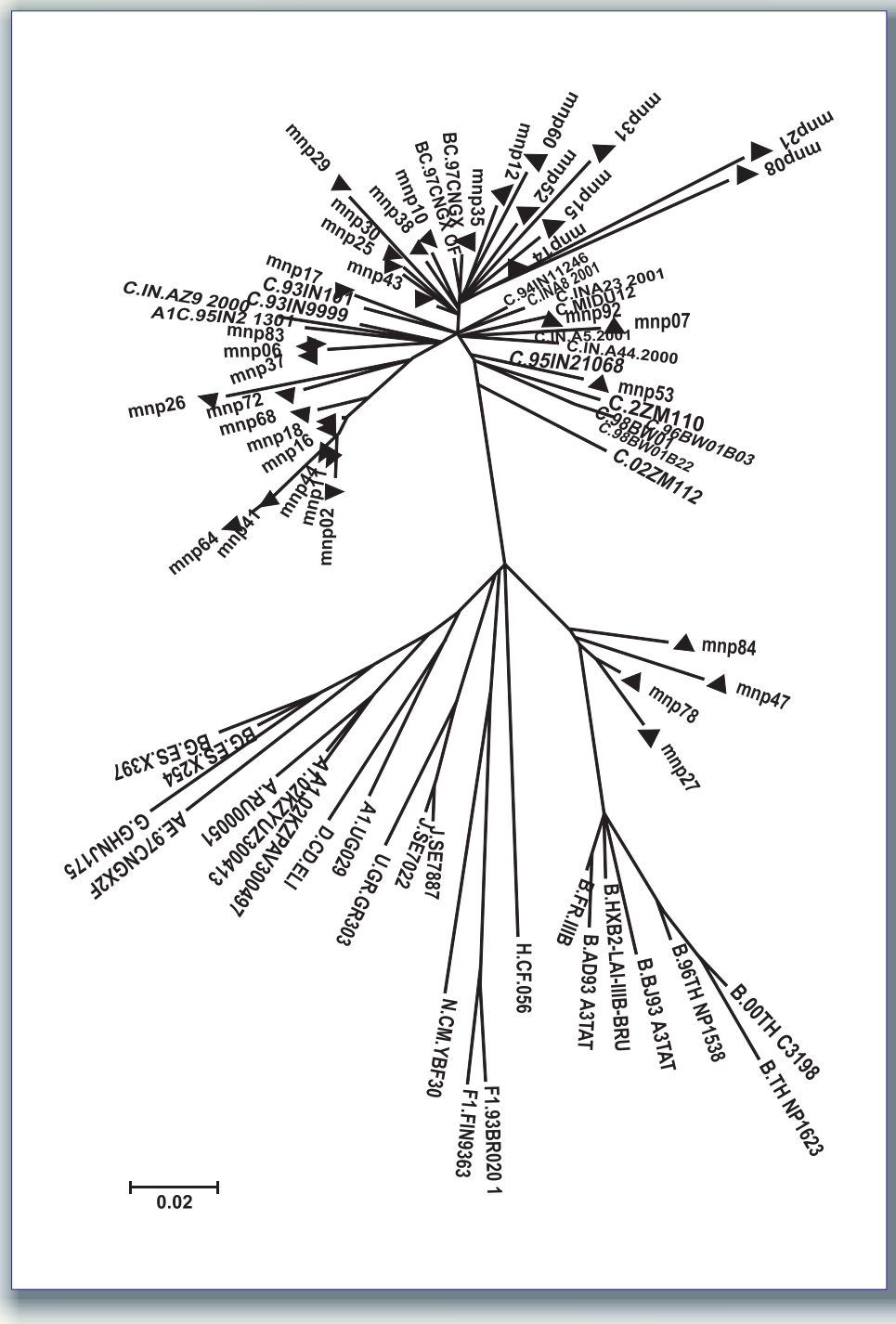
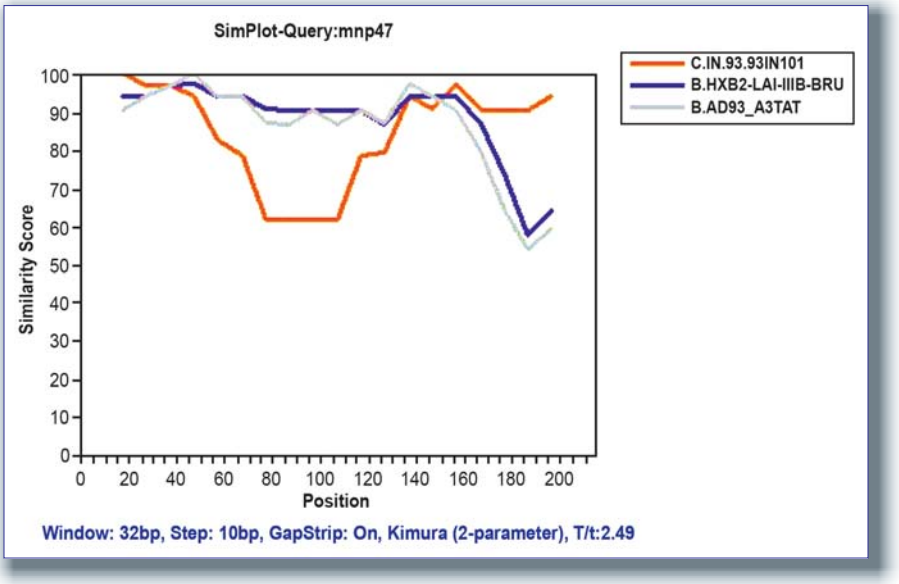
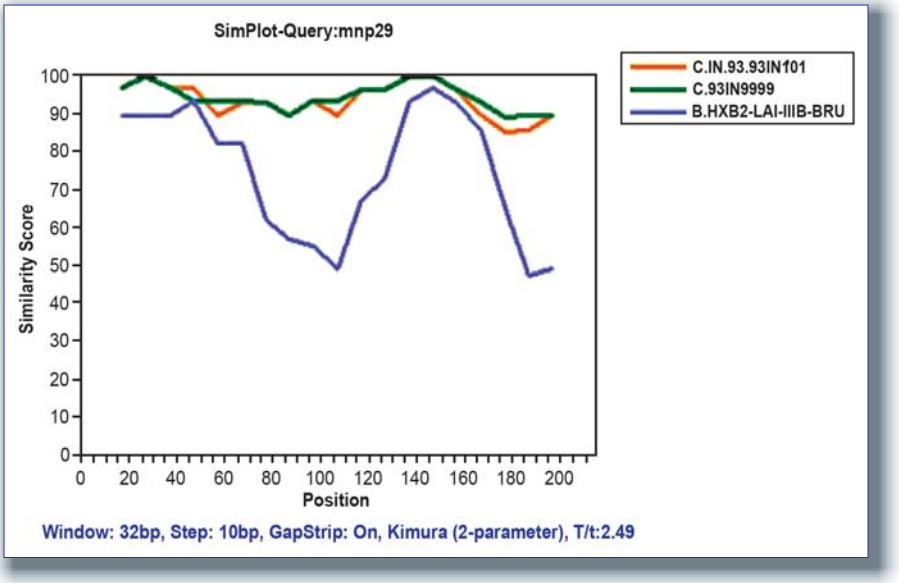


Fig.1. Phylogenetic analysis of HIV-1 *tat* sequences



(a)



(b)

Fig. 2. Simplot analysis of (a) BC*tat* (mnp47) and (b) C*tat* (mnp29) genes from Manipur.

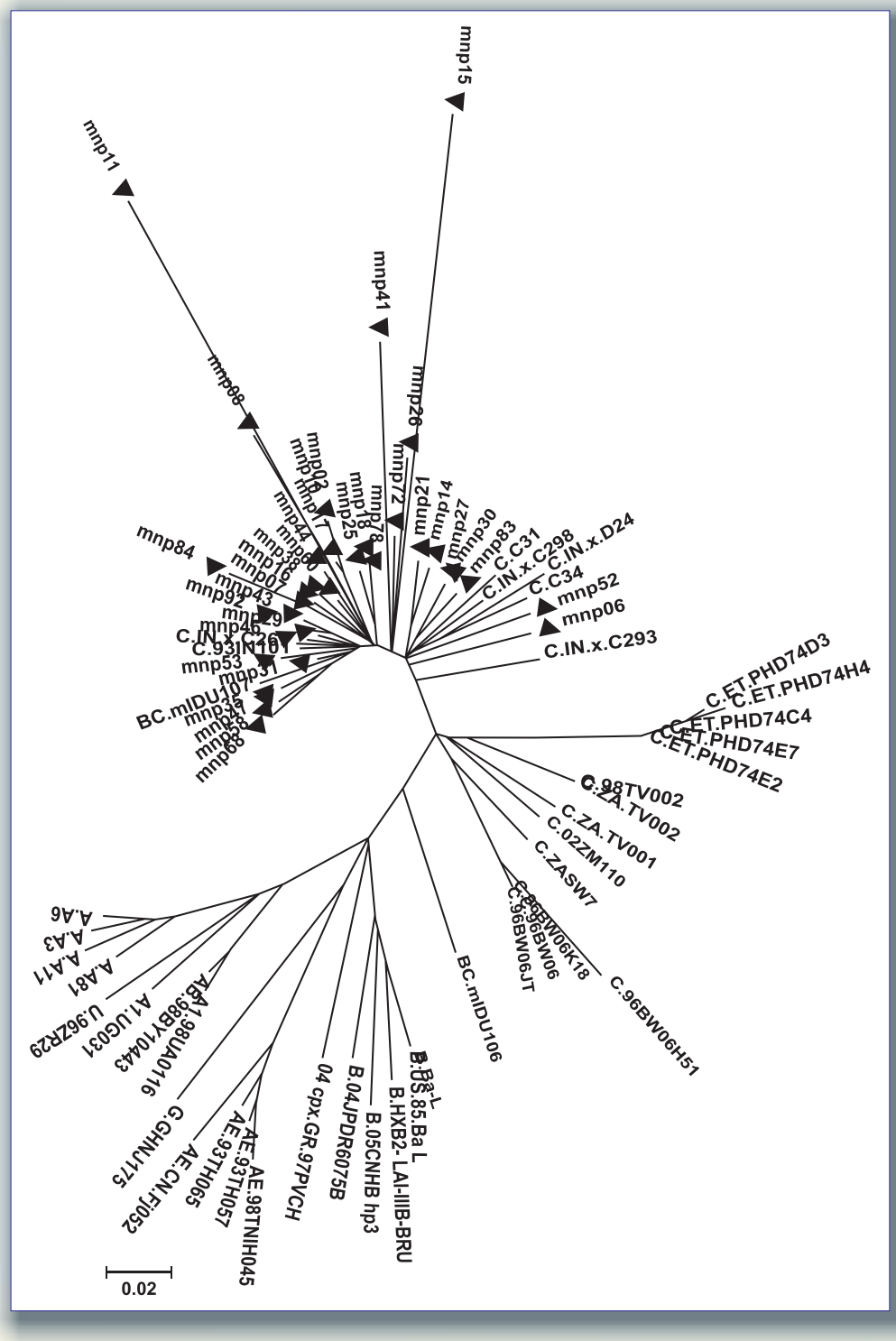


Fig. 3. Phylogenetic analysis of the LTR sequences of Manipur IDUs

Detection and molecular characterization of viral etiological agents causing acute watery diarrhoea in Kolkata

Principal Investigator : T. Krishnan

Viral pathogens viz. Rotavirus, Adenovirus, Norovirus, Sapovirus and Astrovirus were characterized following their detection in faecal specimens of diarrhoea cases received in Division of Virology of National Institute of Cholera and Enteric Diseases Kolkata from ongoing surveillance programme at Dr B.C. Roy Memorial Hospital for Children, Infectious Diseases and Beliaghata General Hospital and from periodical outbreaks in Eastern India. The data generated from diagnostic virology experiments is useful for assessment of disease burden owing to different viral pathogens viz. Rotavirus, Norovirus [Genogroup I & II], Sapovirus Astrovirus, Adenovirus and Picobirnavirus from acute watery diarrhoea cases. The early diagnosis of viral pathogens and monitoring the genetic nature of circulating strains has imparted useful information for effective maintenance of the health system.

The molecular epidemiological studies provided important information about the genetic diversity amongst strains of different Noroviruses associated with infections causing acute watery diarrhoea necessitating hospitalization, viz. Norovirus: classisfication into Genogroup I or II; partial RNA dependent RNA polymerase gene (RdRp) sequences corresponding to six Norovirus GII positive samples shown homology to the sequences of Djibouti (horn of Africa), Brazil, Italy, Japan and US norovirus strains [Fig 1]. This study showed the detection of Norovirus strains among diarrheic and non diarrheic children in Kolkata. The constant monitoring of Noroviruses indicated the occurrence of recombinant strains and their phylogenetic relationships [Fig 2]; Astroviruses were also characterized for genotype and phylogentic analysis of the major ORFs viz. ORF1a, ORF1b encoding RNA-dependent RNA polymerase and ORF2 encoding capsid.

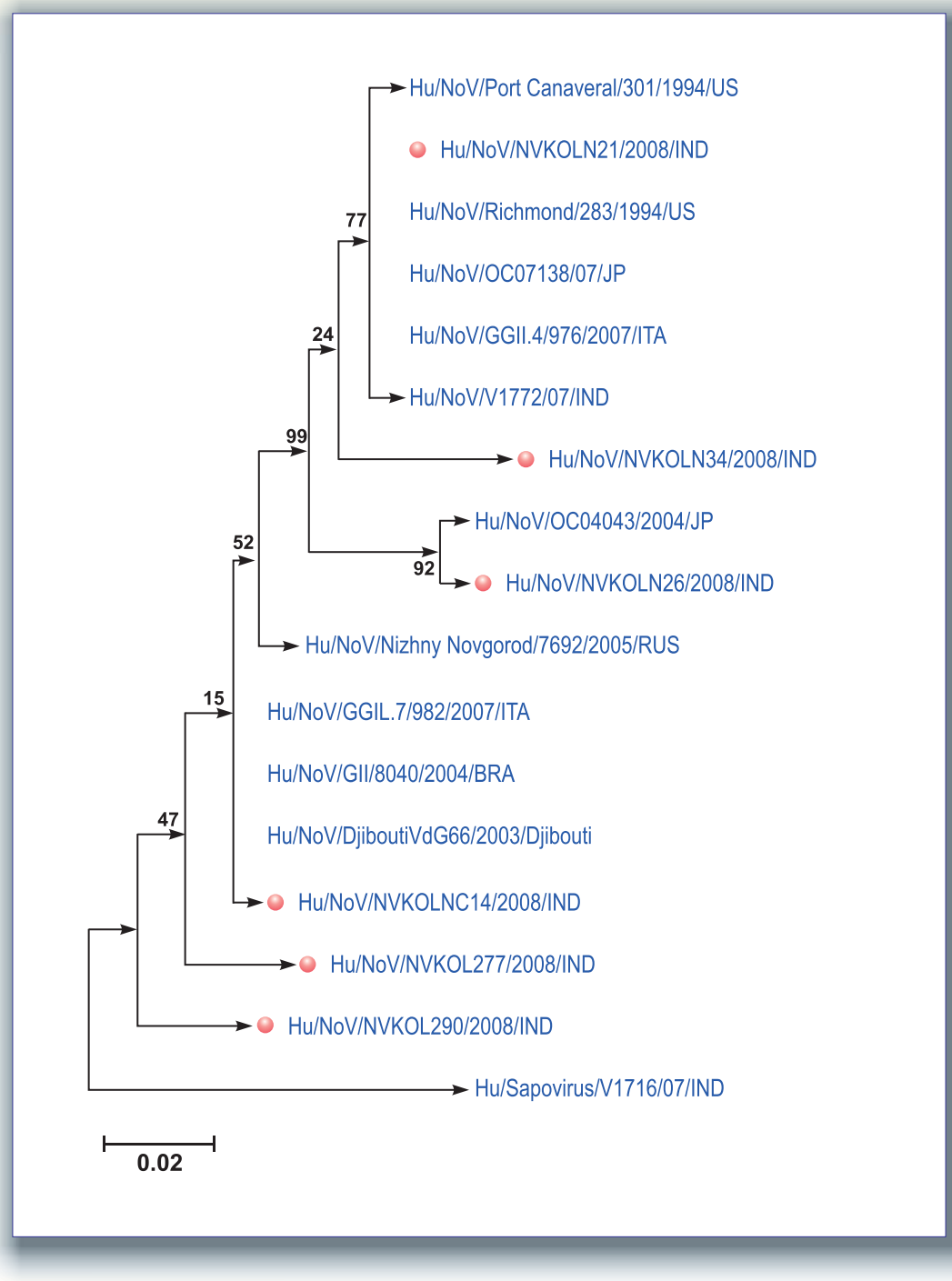


Figure. Phylogenetic analysis based on deduced amino acid sequences corresponding to 172bp nucleotide fragment of the RNA dependent RNA polymerase gene (RdRp) of Kolkata NoV strains and other NoVs with Sapovirus defined as the outgroup strain.

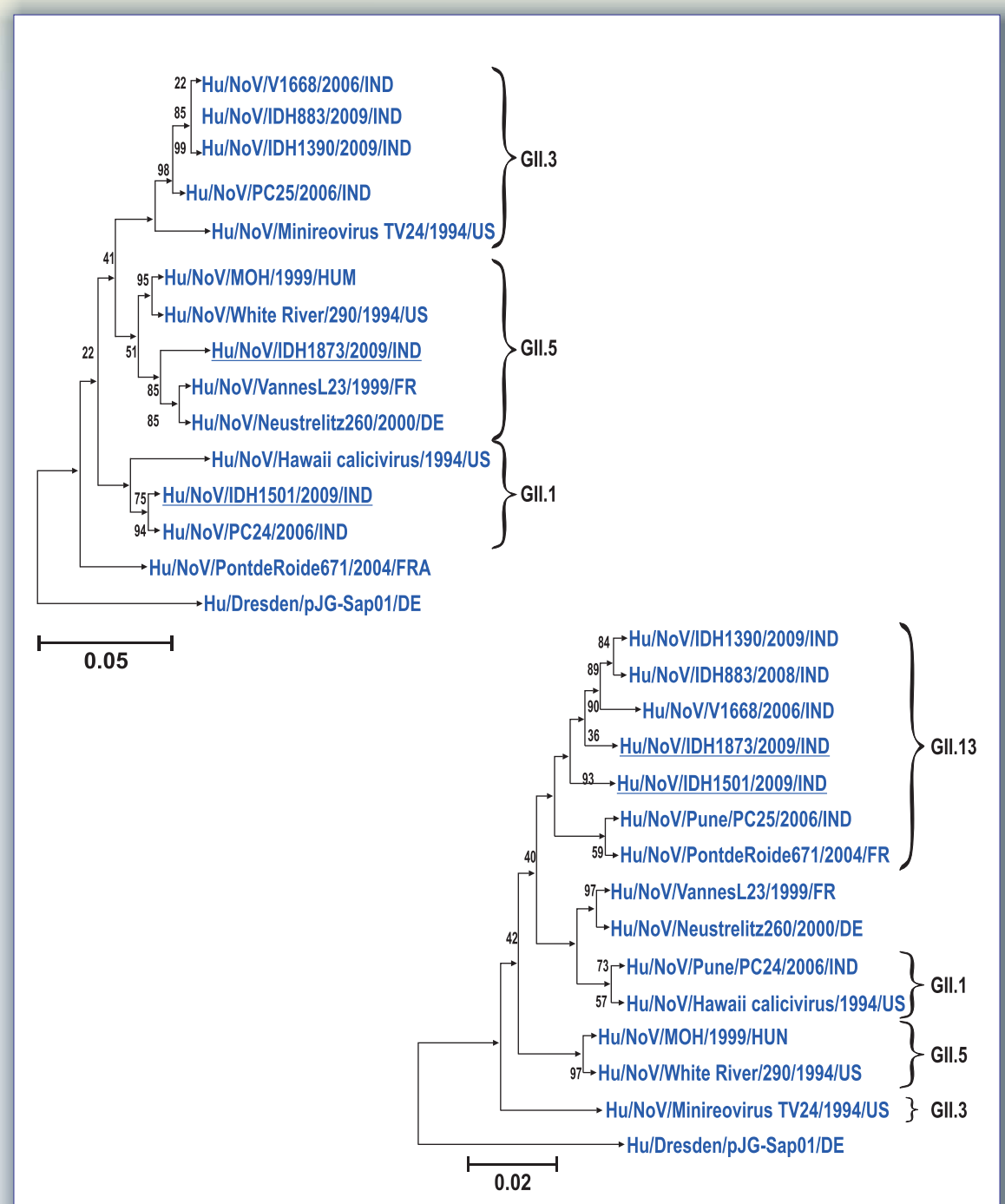


Figure Phylogenetic analysis of the nucleotide sequences of polymerase region and capsid region of Kolkata GII NoV strains in relation to other known GII NoV strains. The tree on the left shows the relationship of a 600bp region of the 3' end of the polymerase region and tree on the right shows the relationship of 282bp of the 5' end of the capsid sequence. The corresponding sequence of RdRp and capsid gene of sapovirus strain Dresden/pJG-Sap01 was selected as the outgroup strain, to root with an outgroup. Suspected recombinants are underlined to emphasize their different phylogenetic groupings.

Multisite monitoring of Influenza Virus strains in India, Phase II

Investigator : M. Chawla Sarkar

A total of 840 patients were enrolled during the study period. The sample collection was maximum during May-July due to higher load of various viral infections in Kolkata during Monsoon season. The clinical samples were classified based on sex and duration of fever though no direct correlation was observed with Influenza positivity. Maximum number of symptomatic patients were in the age group of 1-5 years. Very few adult cases were enrolled as few adults report to hospital OPDs with flu like symptoms.

Out of 840, 193 (22.9%) were positive by real time PCR whereas of 193, only 75 could be grown in culture (8.9%). Of 193, 139 were Influenza B, 52 pH1N1 and 2 H3N2. Influenza B was observed throughout 2010 with varying frequency, whereas pH1N1 circulated in July only.

Analysis of rotaviruses and their interactions with the host: A Viral Proteomics Approach

Investigator : M. Chawla Sarkar

To understand functional significance and mechanism of rotavirus induced activation of PI3K/Akt signaling, rotavirus encoded proteins were studied. In previous year we had reported that Nonstructural protein-1 (NSP1) activates PI3K/Akt during initial stages of infection, to delay virus induced apoptosis. To extend the work further, NFκB activation and translocation was confirmed, nuclear extracts were prepared at 2hpi and 6hpi from MA104 cells either mock infected or infected with A5-13 or A5-16 or SA11 strains. Immunoblotting with NFκB revealed significantly higher level of NFκB in nuclear extracts at 6 hpi in A5-13 and SA11 infected cells compared to A5-16 infected cells (Fig 1. A, B). To correlate activation of rotavirus induced PI3K/Akt with NFκB activation, NF-B-luc and pRL-TK were co-transfected in HEK293T cells followed by infection with A5-13 in presence or absence of PI3K (LY294002) or NFκB (SN50) inhibitors or infection with A5-16 alone. The activation of NFκB promoter was measured by luciferase reporter assay. A 3.0-3.5 fold higher NFκB driven luciferase activity was observed in A5-13 infected cells compared to A5-16 infection (moi 5). In presence of LY294002 (10μM), a 2.0-2.5 fold reduction in NFκB luciferase reporter activity was observed in A5-13 infected cells compared to the virus infected cells in absence of PI3K inhibitor, confirming correlation between PI3K and NFκB signaling during virus infection. In presence of NFκB inhibitor SN50 (5μM), the NFκB activation was completely inhibited (Fig 1 C, D).

Since cell survival markers such as NFκB and PI3K/Akt, were activated by NSP1, we analyzed role of NSP1 in apoptosis induction. NSP1 mutant virus induced apoptosis at very early stages of infection, resulting in complete viral replication cycle. Caspase 3 cleavage was observed as early as 4hpi in A5-16 infected cells. To analyze whether apoptosis is due to induction of IFN-β in NSP-1 mutant infected cells, induction of IFN-β and IRF3 genes was studied. IFN-β and IFN induced genes such as ISG56 were induced as early as 2hpi in both A5-13 and A5-16 infected cells, however in A5-16 infected cells, IFN-β expression increased in time dependent manner with 25 fold increase at 8 hpi, whereas it subsided in A5-13 infected cells at 4 hpi. This pattern was also observed with IRF3 and ISG56 genes. This indicates that, NSP1 suppresses virus induced IFN induction. At 2-3 hpi IFN induction was comparable in mutant and wild type infected virus, whereas apoptosis is induced in mutant virus by 4-6 hpi. Thus IFN does not seem to have direct role in apoptosis induction (*Bagchi P et al 2010*).

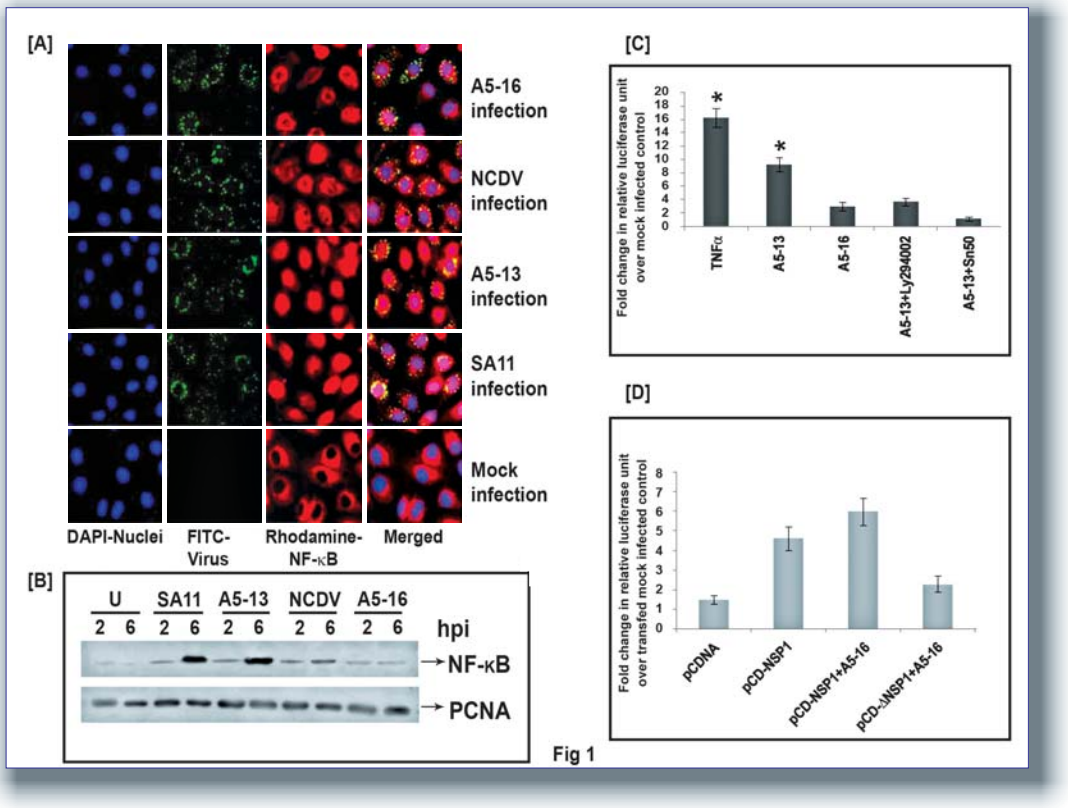


Fig 1

Figure Legend:

FIGURE 1. Sustained activation of prosurvival protein NFκB during rotavirus infection is NSP1 dependent. (A) Nuclear translocation of NFκB following rotavirus infection by immunofluorescence microscopy. MA104 cells either mock infected or infected with wt A5-13 or SA11 or NCDV or mutant A5-16 (moi: 3) strains were fixed 6hr post infection with paraformaldehyde and incubated with both viral rabbit polyclonal NSP5 and NFκB specific mouse monoclonal antibody followed by FITC-labeled anti rabbit and RRX-labeled anti mouse secondary antibodies. Nuclei were stained with DAPI. Poor nuclear translocation of cellular NFκB was observed upon infection with NSP1 mutant A5-16 compared to wild type bovine A5-13 or simian SA11 strains. Bovine strain NCDV also showed relatively lower nuclear localization compared to SA11 and A5-13. (B) Western blot analysis of NFκB from nuclear lysates of MA104 cells either mock infected or infected with SA11 or A5-13 or NCDV or A5-16 (moi 3) for 2h and 6h. Blots were reprobed with anti-mouse PCNA antibody to analyze expression of nuclear protein PCNA as internal expression control. (C) & (D) Relative increase in NFκB promoter activity measured by NFκB luciferase reporter assay in HEK293T cells reveals 3-3.5 fold higher activity in A5-13 infected cells compared to A5-16 infection (moi 5). LY294002 (10μM) and SN50 (5μM) significantly inhibit NFκB activation in A5-13 infected cells. TNFα (10nM) was used as positive control for NFκB activation. Relative fold change in NFκB promoter activity in pcD-NSP1 transfected HEK 293T cells showed 2.5-3.2 fold increase over vector control or the truncated NSP1 (pcD-ΔNSP1). NFκB activation was restored in cells expressing wt NSP1 followed by A5-16 mutant virus infection. The data is presented as fold change in luciferase unit (mean ± SD; n=4) relative to mock infection or untransfected control and was normalized with Renila luciferase activity.

Detection and molecular characterization of complete nucleotide sequence of human picobirnavirus causing acute watery diarrhoea among children in Kolkata.

Principal Investigator : **B. Ganesh**

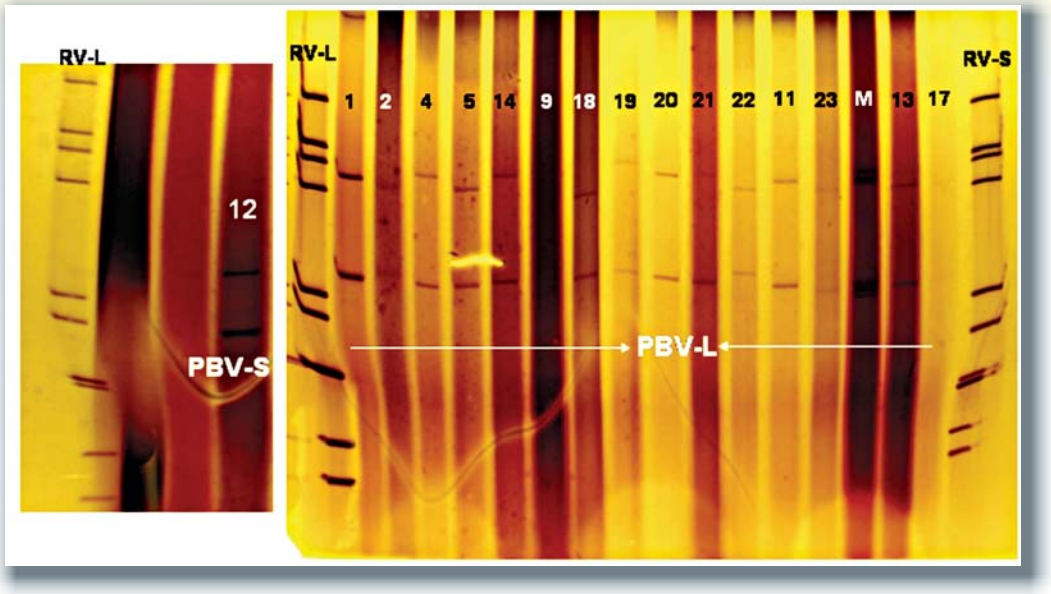
Co-Investigator : T. Krishnan, M. Chawla-Sarkar, U. Mitra, M. K. Bhattacharya

Picobirnavirus is currently the only genus in the family "*Picobirnaviridae*". The name is derived from the Spanish "pico" meaning 'small' with birna for 'bipartite RNA'. The virus particles are 35-41nm in diameter and non-enveloped. The genome consists of two dsRNA segments of 2.3-2.6 and 1.5-1.9 kilobase pairs (kbp), respectively. Segment 1 codes for capsid protein and segment 2 encodes RNA-dependent RNA polymerase. Picobirnaviruses [PBVs] have recently been shown to cause acute watery diarrhea among children in Kolkata [Bhattacharya et. al., 2007].

Detection of viral RNA by PAGE and silver staining showed that large and small profile PBVs were circulating among children and adults in Kolkata. The role of small profile PBVs as a cause of acute watery diarrhoea among children was established in Kolkata (Bhattacharya et. al., 2007).

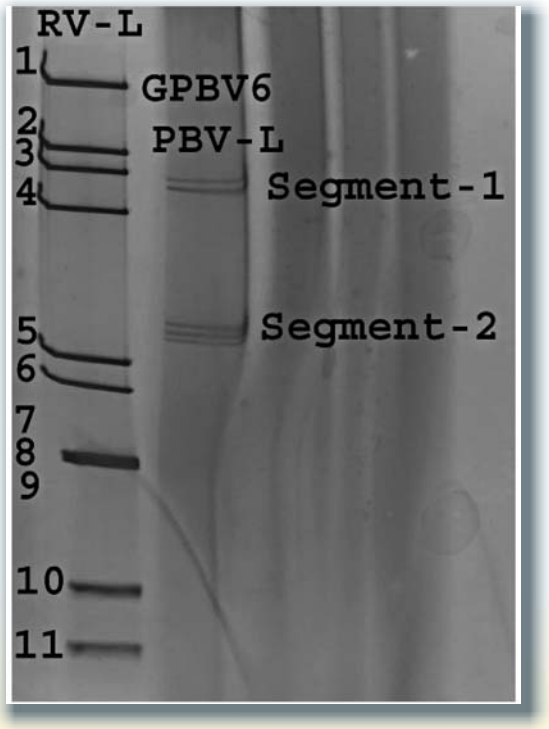
To date, molecular biology grade viral RNA was extracted and RT-PCR was carried out using specific primers to amplify small fragments of the bisegmented genome of genogroup I and genogroup II picobirnaviruses represented by the Chinese strain 1-CHN-97 and US strain 4-GA-91 respectively. The amplicons were sequenced and sequence data was analysed to understand the phylogenetic relationship of the picobirnaviruses. There are few reports of human picobirnaviruses from different countries as well as occurrence of PBVs in faeces of different animals. The information that has been gathered shows remarkable genetic diversity among picobirnavirus from Kolkata and other PBVs hitherto reported uptil now.

The partial molecular characterization study has shown interesting results of detecting 4 genogroup I picobirnaviruses from children suffering from acute watery diarrhoea in Kolkata showed very close match with porcine PBV strains reported from Hungary, 3 isolates showed match with PBV strains reported from USA and a Kolkata strain reported earlier. Partial molecular characterization and sequence analyses of the Kolkata strains showed that there exists distinct sequence heterogeneity among human PBVs warrants stringent surveillance of newly emerging variants of PBVs.

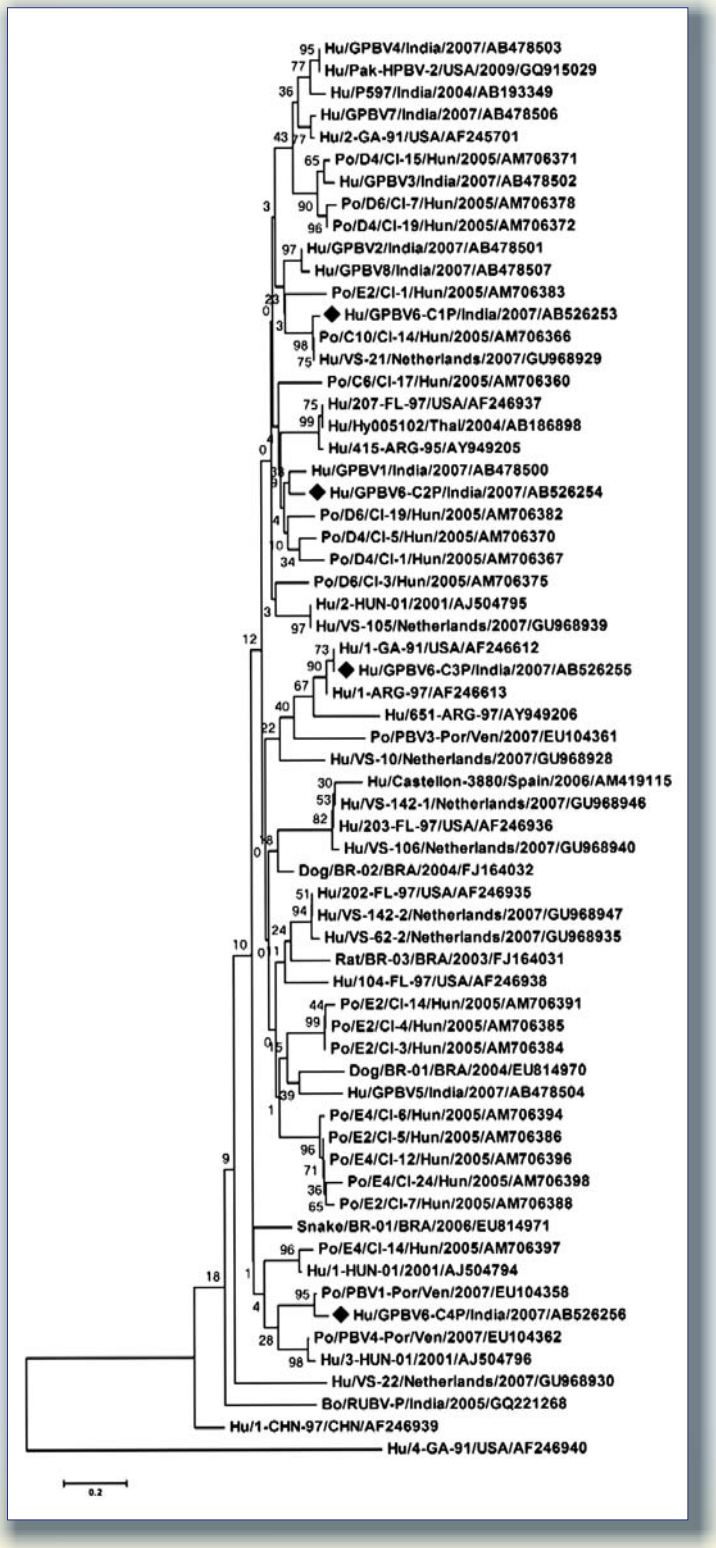


Picobirnavirus positives in silver stained PAGE gel with large and small genome profile

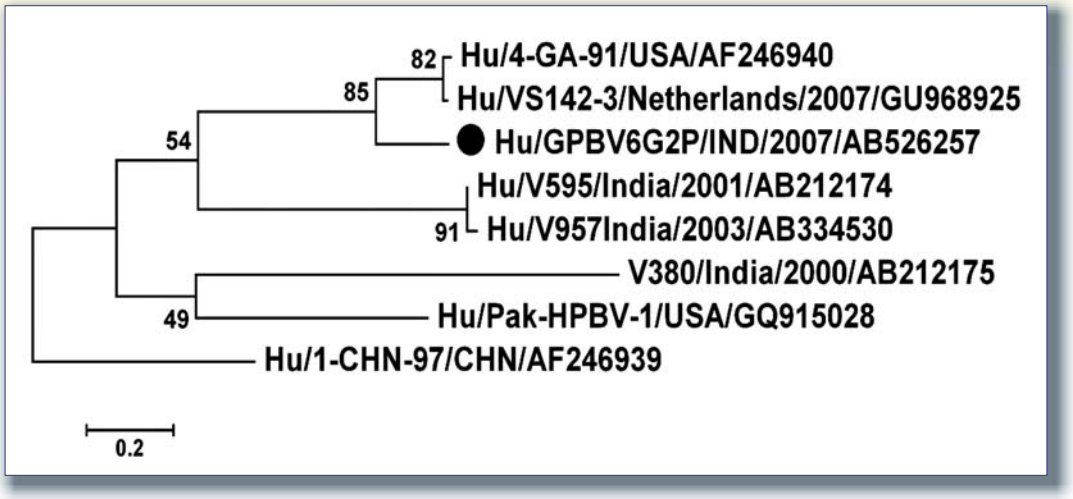
Picobirnaviruses detected in children were genetically related to the porcine strains reported from Hungary, Venezuela, Argentina and human strains reported from USA, Netherlands, etc., Also detected multiple strains of picobirnavirus infection in a diarrheic child with both genogroup I and II PBVs. The genogroup II PBV strain detected in our study was genetically related to the PBV prototype strain 4-GA-91 (USA).



PAGE profile of PBV multiple strain infection



Phylogenetic analyses of genogroup I PBV multiple strains detected and sequenced



Phylogenetic tree of PBV genogroup II strain detected and sequenced

Award and Honours

S. Chakrabarti

- Elected as the Fellow of National Academy of Science (FNASc)
- Elected as the Fellow of Academy of Science & Technology, West Bengal (FASc & T)

T. Krishnan

- Appointed as a member of the Board of Examiners in Microbiology of Vidyasagar University, Midnapore, West Bengal.
- Acknowledged by American Journal of Tropical Medicine and Hygiene (American Society of Tropical Medicine and Hygiene) as reviewer 82(1):pp168-172. [2010]
- Ten assignments as Associate Editor for the journal BMC Infectious Diseases.
- Reviewed fourteen research publications for scientific journals viz. The Pediatrics Infectious Disease Journal; Archives of Virology; Letters in Applied Microbiology; Infection Genetics and Evolution; African Journal of Biochemistry Research; Journal of Bacteriology Research; Future Virology; BMC Pediatrics.

B. Ganesh

- Received Travel Grant to participate in the 14th US-Japan Cooperative Medical Sciences Program (USJCMSP) Regional Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim: Next Generation Diagnostics for Infectious Diseases" held at Penang, Malaysia during 4-6 October 2010.

Conferences/ Seminars/Workshops/Trainings Attended/Organised

S. Chakrabarti

- Delivered 101st Foundation Day lecture of National Centre for Disease Control, New Delhi on " Molecular Characterization of HIV-1 circulating in Eastern and North eastern India: Development of a candidate vaccine" on 30 April 2010

- Indo US Translational Research on AIDS and chaired one session on HIV Diagnosis at Goa, India on 15 January 2011
- Delivered the plenary lecture in International conference on Emerging Frontiers and Challenges in HIV/AIDS Research on " Development of an candidate vaccine against HIV/AIDS : Efforts' of ICMR on 8 February 2011 at Mumbai, India
- Resource person in Orientation programme of Academic staff college Burdwan University and deliverd a talk on "HIV/AIDS: Indian Scenario"
- Inaugural speech at Society of Tropical Medicine & Infectious Diseases On "Infectious Disease research spearheaded by NICED"

T. Krishnan

- "Phylogenetic analysis of etiological agents associated with viral gastroenteritis among children and adults in Kolkata, India" on 1 October 2010 at the International Conference on Frontiers in Biological Sciences held in Department of Life Sciences, National Institute of Technology, Rourkela from 1-3 October 2010.
- "Improved viral diagnosis by molecular detection of different viral etiological agents among acute watery diarrhoea cases, in Kolkata, India". Presented by colleagues at United States-Japan Cooperative Medical Science Program (CMSP) sponsored 14th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim held in Penang, Malaysia during 4-6 October 2010.
- "Norovirus surveillance among viral gastroenteritis cases in Kolkata, India: genetic diversity of Region C of capsid gene fragment" at the 58th Domestic Congress of Virology held in Tokushima, Japan from 7-9 November 2010
- "Viral Gastroenteritis: Emerging viruses" on 26th February 2011 at the National Seminar on Ecology and Environment Management: Indian Scenario organized as a part of Golden Jubilee Celebrations of the Sri Venkateswara College, University of Delhi [South Campus) from 24-26 February 2011

M. Chawla Sarkar

- IXth International Rotavirus Symposium, Johannesburg, South Africa, August 2nd-3rd 2010. Presented paper titled "Rotavirus Modulates The Host Innate Immune Responses By Activating PI3K/Akt And NFκB Pathways During Early Stages Of Infection" Parikshit Bagchi, Dipanjan Dutta, Shiladitya Chattopadhyay and Mamta Chawla-Sarkar.
- International symposium on Molecular and Pathophysiological Research on Enteric Pathogens, Kolkata, India, January 27-29, 2011. Presented paper titled "Comparitive analysis of full genome expression profile of host cells following infection with rotavirus strains from human and animal origin" P. Bagchi, S. Chattopadhyay, R. Bhowmick and M. Chawla-Sarkar.
- Annual Conference of International association of Medical and Pharmaceutical Virologists, University of Delhi, New Delhi, India, March, 3rd-March 5th 2011. Delivered Talk on "Hsp90 positively regulates Rotavirus Infection by Modulating the Function of Cellular Protein AKT and virus encoded Non structural Protein-3". D. Dutta, S. Chattopadhyay and M Chawla-Sarkar.

B. Ganesh

- Balasubramanian Ganesh, Shovan Das, Nataraju SM, Sourav Chowdhury, Mihir K Bhattacharya, Mrinmoy Ghosh, Rashmi Arora, Umesh D Parashar, Jan Vinje, Nobumichi Kobayashi, and Triveni Krishnan. "Comparative evaluation of a rapid immunochromatographic test (ICT), commercial enzyme linked immunosorbent assay (ELISA), agarose and polyacrylamide gel electrophoresis (PAGE) for detection of rotaviruses among children <5 years of age hospitalized for acute gastroenteritis in Kolkata, India". Presented a poster in the 14th US-Japan Cooperative Medical Sciences Program (USJCMSP) Regional Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim: Next Generation Diagnostics for Infectious Diseases" held at Penang, Malaysia during October 4-6, 2010 (B.Ganesh received Travel Grant to participate in the Conference).
- Rahul Kumar, Madhu Sudhan Pativada, S.M.Nataraju, B.Ganesh and Triveni Krishnan. "Improved viral diagnosis by molecular detection of differential viral etiological agents among acute watery diarrhoea cases, in Kolkata, India". Presented a poster as co-author in the 14th US-Japan Cooperative Medical Sciences Program (USJCMSP) Regional Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim: Next Generation Diagnostics for Infectious Diseases" held at Penang, Malaysia during October 4-6, 2010.
- Krishnan T, Nataraju SM, Kumar R, Pativada M, Ganesh B. "Norovirus surveillance among viral gastroenteritis cases in Kolkata, India: genetic diversity of Region C of capsid gene fragment". Paper presented (Poster) and Abstract published in the 58th Domestic Congress of Virology from 7-9, November 2010 held in Tokushima, Japan.
- Triveni Krishnan, Rakhi Dey, Ganesh B, M.Pativada, Nataraju SM and Rahul Kumar. "Phylogenetic analysis of etiological agents associated with viral gastroenteritis among children and adults in Kolkata, India". Paper presented (Poster) and Abstract published in the International Conference on Frontiers in Biological Sciences (InCoFIBS-2010) during 01-03 October, 2010 at National Institute of Technology, Rourkela, India.
- "Sequence Analysis and Protein Modeling workshop" organized by the Biomedical Informatics Center of NICED (ICMR) Kolkata during 18th-19th June 2010.

SERVICES WE OFFER

1. Active surveillance of enteric pathogens among hospitalized and outdoor diarrheal patients at Infectious Diseases Hospital and B. C. Ray Memorial Hospital for Children, respectively.

This is a hospital based surveillance program that monitors prevalence of 26 enteric pathogens and antimicrobial susceptibilities of *Vibrio cholerae* O1 and *Shigella* spp.

2. Cholera outbreak investigations:

From different parts of West Bengal, 87 stool specimens mostly from cholera outbreaks were processed. *Vibrio cholerae* O1 was identified from 30 cases (34.5%) and was informed to the respective hospitals and Health centers.

3. Global *Vibrio cholerae* data base using pulsed-filed gel electrophoresis (PFGE) profiles.

This program was initiated in 2009 to have PFGE profile collection of *V. cholerae* O1 and O139 serogroups. This program is a joint activity with CDC Atlanta, and PHL, Hong Kong. This data base helps in monitoring the global spread of *V. cholerae* O1 as the profiles were periodically compared and the new profiles are added. The GVD now has more than 30 PFGE profiles of *V. cholerae* O1 from 250 strains collected from India, Bangladesh, Africa and Haiti.

4. Identification & serotyping of Salmonella isolates

Confimed identification & serotyping of Salmonella isolates sent from other medical colleges & research institutes on regular basis by suitable conventional & serology based tests.

5. Microbial analysis and examination of samples of potable water sources

During the epidemic outbreaks (2010-'11) of diarrhoea spreading across different southern districts of West Bengal, microbial analysis and examination of samples of potable water sources, from different parts of West Bengal and reporting of results to the Govt. agencies, has been a routine activity of the environmental laboratory of the undersigned.

Water samples had been received from different PHCs of N. 24 Pargana, S. 24 Pargana, E. Midnapore, Howrah and Kolkata as well as from endemic and epidemic affected Municipal wards under the Kolkata Municipal Corporation and its adjoining areas. Results have been

TABLE 2

Sl No.	District	No. of samples received	Source					Culture Positive	PCR positive
			Tap	Tube well	Pond	Unknown	Stored		
1.	South 24 Parganas	4	-	3	-	-	1	2	1
2.	North 24 Parganas	41	19	13	2	2	5	10	4
3.	Kolkata	32	20	2	-	-	10	7	4
4.	East Midnapore	2	1	1	-	-	-	1	1
5.	Howrah	3	-	1	1	1	-	1	1
	Total	**82**	**41**	**19**	**4**	**2**	**16**	**21**	**11**

conveyed to the respective agencies with a copy of the same to State Health secretariat, Govt. of West Bengal. During the period under report, 82 samples had been received from various sources of which 39 had been found to be positive for faecal coliforms and 11 for presence of *V. cholerae* O1 (Table 2).

5. Vibriophage Reference Laboratory

As a WHO Collaborating Center for Diarrhoeal Diseases Research and Training, NICED is working as a Vibriophage Reference Laboratory and we receives strains of *V. cholerae* from all parts of India and abroad for biotyping, serotyping and phage typing since 1968. This year we received a total of 555 strains from different institutions from 7 different states of India. Of these, 493 (88.83%) representative strains confirmed as *V. cholerae* O1 biotype ElTor were included in phage typing study and reports have been sent to respective counterpart.

6. Outbreak of enteric parasites:

Two field studies have been performed during last fiscal year in Chakdah, Nadia, West Bengal for investigation of outbreak of different enteric parasites by improper hand wash. Investigation was carried out in Indore, Madhya Pradesh for identification of different parasites among rural populations.

7. Pandemic HINI outbreak in West Bengal:

Rapid diagnostics service was provided from NICED for the suspected cases during the pandemic H1N1 outbreak in West Bengal during July 2009 until Sept 2010.

8. Outbreak Investigation

A) Report of investigation of a cholera outbreak in Gujarat by team from National Institute of Cholera and Enteric Diseases, Kolkata, team from 31st May- 2nd June 2010

Acute Gastro Enteritis (AGE) cases were reported since 23rd May from different villages of a block in Gujarat.

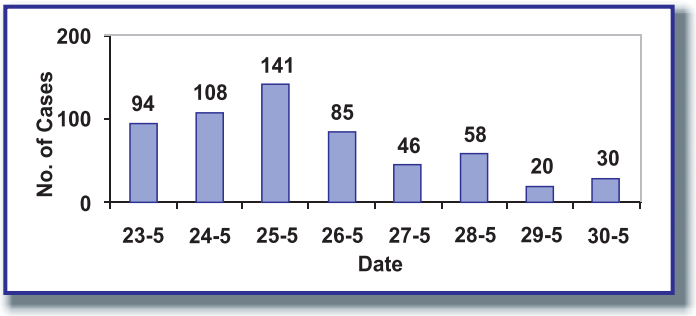
The week from 22nd May was marriage season during which time, there was large movements of groups of people from one village to another to attend the marriages. Most of the cases reported having attended one or other marriage ceremony. On returning to their homes, they fell ill and then spread the diseases to other members of the family. The temperatures had soared upto 48^{0}C and there was acute scarcity of water. Since large amounts of water are required during the marriage ceremonies, water was bought in tankers from 2 open wells in Jhalod town. The wells are privately owned wells. The wells were visited by the team and were observed to be in dire state. The inside linings of the wells were not continuous with gaps in the walls where trees and other fungi and algae had grown. The water was obviously contaminated and the municipal authorities had instructed the owners to chlorinate the wells. In spite of these instructions, there was no residual chlorine in the well waters when the team tested the water with chloroscope. Instructions had also been given to chlorinate the water in the tankers but these also showed negative results for residual chlorine. Considering that the causative organism had been identified to be *Vibrio cholerae,* (transmitted through water), the cases mostly presented with acute watery diarrhoea with severe dehydration and the contaminated water source, it is highly suggestive of a water borne outbreak (rather than food poisoning as suspected initially). The team visited the several affected villages and interviewed some family members of diarrhoea cases. As expected, most cases reported having attended a marriage ceremony. Interestingly, there were two ladies who had attended different marriages and had only taken water but no food. They also had diarrhoea and stool was positive for *Vibrio cholerae.* This probably clinched the issue of water borne outbreak.

Laboratory report

Of 98 samples collected, 32 samples were positive for *Vibrio cholerae.* Antimicrobial susceptibility pattern was also tested. Of the 84 water samples sent for testing (collected from different sources of water), 50 were declared microbiologically unfit for drinking.

Recommendations advised:

Most important recommendation was regular chlorination of wells and tankers supplying water to the community with routine monitoring with chloroscope for residual chlorine both at source and at the users' end.



B) Investigation of outbreaks of Acute Diarrhoeal Diseases (ADD)/Cholera in Rayagada and Kalahandi districts, Orissa, 17-21 September 2010

At the request of the Director, Emergency Medical Relief, (EMR), Govt. of India, a team of NICED scientists went to Orissa to assist the state/district health authorities in the investigation and control of outbreaks of acute diarrhoeal diseases (ADD)/cholera in Rayagada and other districts of Orissa along with other experts from NCDC, New Delhi. The team visited the affected areas of Rayagada and Kalahandi districts along with senior officers from the Directorate of Health Services, Orissa and district officials during the period from 17 to 21 September, 2010.

Observations

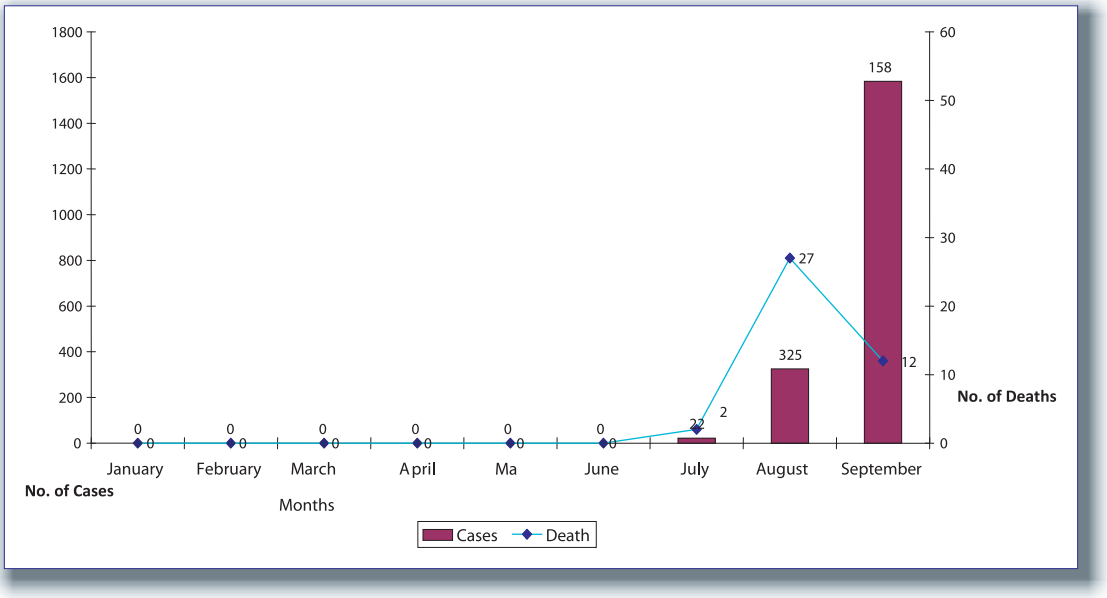
A total of 1930 cases and 41 deaths were reported from district Rayagada in 2010 (upto 21 September). These cases occurred in 420 villages in 93 GPs of 8 Blocks. Rayagada district has reported the maximum number of cases and deaths in the state due to severe diarrhoea in 2010 (Tables 1,2)

Eight of 11 Blocks in Rayagada reported cases of severe diarrhoea in 2010. The worst affected blocks are Kalayansinghpur (704 cases, 8 deaths), Kashipur (365 cases, 10 deaths), Bisamacuttack (318 cases, 11 deaths), Jemadeipentha (269 cases, 2 deaths), Gudari (74 cases, 6 deaths) and Jagannathpur (85 cases, 4 deaths)

Analysis of 41 deaths in district Rayagada revealed that 10 deaths occurred in 6 villages of three sub-centres in Kashipur Block (including 4 deaths in village Bahardulki), 8 deaths occurred in 6 villages of two sub-centres in Ksinghpur block (including 2 deaths each in village Lekapai and Ksinghpur), and 11 deaths occurred in 6 villages of three sub-centres of Bisamacuttack block (including 4 deaths in village Gadaba, 2 deaths in village Goilkana). Thus multiple deaths occurred in some very small villages. Virtually all deaths occurred in homes (Table 1).

People were not aware of ORS, where is it available in the village and when and how to use it. Halogens tablets have been distributed in the affected areas, but most in the remote rural areas do not seem to use them in the absence of a strong IEC campaign.

Month-wise Cases and Deaths due to severe diarrhoea in Rayagada district, Orissa, 2010 (up to 21st Sept)



Cases of severe diarrhoea in district Rayagada by age and sex, 2010							
Age (yrs)	Ksinghpur	Kashipur	Jemadeipentho	Bisamacuttack	Total	Male	Female
0-4	36	19	34	13	102 (6.4)	55	47
5-14	92	39	36	58	225 (14.2)	128	97
15-24	97	50	46	43	236 (14.9)	101	135
25-44	253	127	69	123	572 (36.1)	226	346
45 or more	203	78	70	99	450 (28.4)	215	235
All ages	681	313	255	336	1585 (100)	725 (45.7)	860 (54.3)

Deaths due to severe diarrhoea in district Rayagada by age and sex, 2010 (upto 21 Sept)				
Age (in yrs)	Male	Female	Total	% of deaths
<5	1	1	2	4.9
5-14	2	5	7	17.1
15-24	1	4	5	12.2
25-44	5	10	15	36.6
45 and above	6	6	12	29.3
Total	15 (36.6%)	26 (63.4%)	41	100

Analysis of age distribution of the deaths in Rayagada revealed that only 4.9% deaths occurred in under-five children and 78% deaths occurred in persons 15 years and above. Deaths in adults following dehydration usually indicate cholera. Male contributed to about 37% of deaths, the remaining 63% deaths occurred in females.

Clinical observations

The main presenting symptoms in outbreak associated cases are profuse painless watery diarrhoea, with or without vomiting, followed by dehydration. All these cases are managed with intravenous fluids (Ringer Lactate) and antibiotics like Norfloxacin, Ciprofloxacin, Ofloxacin and Tinidazole. None of them had received ORS at home; similarly none was given ORS solution in the treatment centre when they were being administered intravenous fluids.

Laboratory observations

During August-September 2010, 20 rectal swabs were sent to RMRC, Bhubaneswar from Rayagada district. 8 of 20 (40%) samples tested positive for cholera. The isolates were sensitive to Azithromycin, Norfloxacin, Ciprofloxacin, Chloramphenicol, Neomycin and Gentamycin. (later Ofloxacin and Doxycyclin were found to be sensitive), but were resistant to Ampicillin, Tetracycline, Nalidixicacid, Furazolidone, Streptomycin, Erythromycin, Co-trimoxazole. 14 water samples from various sources of Kashipur, Kalyansinghpur, Bisamacuttack and Gudari were also tested in RMRC, but there was no growth for Vibrio Cholerae.8 samples from district Kalahandi were also tested in RMRC Bhubaneswar during August-September 2010. 5 of them (63%) were found to be positive for Vibrio cholerae O1 biotype Eltor Ogawa. 15 samples were tested in the laboratories of NICED, Kolkata. 11 of 15 samples (73%) tested positive (all Vibrio cholerae O1 El Tor Ogawa except one which was Inaba, from Kalahandi) All the 11 isolates were found to be sensitive to Tetracycline, Doxycycline, Ciprofoxacin, Norfolxacin, Ofloxacin, Erythromycin and Azithromycin; and were found to be resistant to Ampicillin, Cotrimoxazole, Furazolidine, and Nalidixic acid.

Conclusions

Clinical, epidemiological and laboratory investigations confirm the outbreak of cholera in Rayagada and Kalahandi. Although the State/District officials are making efforts to provide safe water, making the facilities available for treatment of cases and increasing awareness to improve personal and domestic hygiene to control cholera, remoteness of the affected areas and poor transport and communication facilities make the task difficult for early detection and treatment of cases which is necessary to prevent mortality.

Biomedical Informatics Centre

The centre came into existence in 2006 and still run by an extramural grant from ICMR. Currently located at the top floor of the new NICED building, the center has become a valuable asset of the institute through its various activities (research, training, services and biological database development). It has developed collaborations with several national and international scientists and is actively publishing in peer-reviewed international journals.

Present infrastructure of the center includes 12 PCs, 2 laptops, one linux based server, one workstation, 2 printers (one colour), one digital scanner and several software (GCG, Discovery Studio, GOLD, SPSS and MatLab), in addition to a state-of-the-art Computer Laboratory. The center holds regular training and dissertation work for the postgraduate students and has conducted 2 workshops recently on "Sequence Analysis & Protein Modeling" and "Understanding Genome - A Bioinformatics Approach".

The activities of the center are coordinated and supervised by Dr. Santasabuj Das, Scientist D of NICED.

Present manpower

S. S. Das, Principal investigator and coordinator
S. Basak, Scientist II
R. Labala, Scientist I
R. Banerjee, PhD student of Calcutta University

Public Health Laboratory Division

Scientist : M. K. Saha, Scientist D

Staff : C. R. Pal, Technical Officer A
S. C. Bhunia, Technical Officer A
S. K. Sadhukhan, Technical Officer A
P. Bhaumik, Technical Assistant
C. Das, Attendant Services

NACO-National Reference Laboratory

National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India funds the HIV National Reference Laboratory of the Institute since 1992. According to the NACO programme design 5000+ ICTCs are supervised by 117 State Reference Laboratories which are under 13 National Reference Laboratories. An Apex Laboratory conducts EQAS for all the NRLs.

The activities of NACO-NRL at National Institute of Cholera & Enteric Diseases (NICED) comprises of the following:-

- EQAS and Panel Sera preparation for SRL of different states of Eastern and North-Eastern India.

- Confirmation of HIV testing results of the samples received from different SRLs.
- Training for Doctors, Lab/Program Supervisors and Medical Laboratory Technologists for HIV surveillance and laboratory diagnosis of HIV infection as and when requested by Institute of Serology, Govt. of India, State Health authorities, Hospitals etc.

Referral Services

Amongst the responsibilities allotted to the NACO-National Reference Laboratory at NICED, referral Service is of utmost importance. NACO-NRL, NICED has been entrusted with the responsibility of verifying results for all discordant samples sent by State Reference Laboratories, several Hospitals and sometimes requested for Foreign Nationals.

Source of Samples		No. of Tested	No. of Positive
A.	WEST BENGAL		
1.	Command Hospital	63	61
2.	Patients from Hospitals	29	27
3.	Miscellaneous	00	00
	Sub Total-	92	88
B.	OTHER STATES		
1.	Assam	00	00
2.	Meghalaya	00	00
3.	Orissa	00	00
4.	Sikkim	00	00
	Sub Total-	00	00
	GRAND TOTAL-	**92**	**88**

EQAS Programme of NACO

External Quality Assessment is a programme in which a laboratory participates and receives a blinded, composite panel of samples from another laboratory conducting the EQA. An EQA compares the performance and results among different test sites, and indicates areas that require improvement in participating laboratories by identifying the loopholes in the process. The general objective is to provide early warning for systematic problems associated with kits or operations. Apart from all these gains, an EQA serves as evidence to quality testing.

Table 2: External Quality Assurance (Apr 2010- January 2011) for SRLs under NACO NRL, NICED, Kolkata

Name of SRLs	Samples received by NRL, NICED from SRLs in four Quarter: Apr 2010-Jan 2011				Confirmed Result at NRL	No. of Discordant
	April 2010	July 2010	October 2010	January 2011		
G.B Pant Hospital, Andaman & Nicobar Islands	00	00	00	00	00	
Rajendra Institute of Medical Science, Ranchi, Jharkhand	00	00	00	00	00	
MGM Medical College, Jamshedpur, Jharkhand	13	07	07	00	27	
Patuliputra Medical College, Dhanbad, Jharkhand	20	12	02	10	39	
SCB Medical College, Cuttack, Orissa	09	22	20	15	66	1
VSS Medical College, Burla, Orissa	12	10	14	14	50	
MKCG Medical College, Beharampur, Orissa	05	14	12	17	48	
Guwahati Medical College, Guwahati, Assam	20	22	16	00	52	2
Assam Medical College, Dibrugarh, Assam	04	03	06	04	17	
Silchar Medical College, Silchar,Assam	04	07	08	05	24	
NEIGRIHMS, Meghalaya	40	26	13	10	89	

Table 3: HIV Sentinel Surveillance 2010 (ANC & STD): Quality Assurance for SRLs under NACO NRL, NICED, Kolkata

Sl. No	Name of SRL/Testing Centre	Samples sent by SRL		Samples rejected by NRL	Confirmed Result at NRL		No. of Discordant
		HIV -ve	HIV +ve		HIV -ve	HIV +ve	
1.	G.B Pant Hospital, Andaman & Nicobar Islands	68	04	3	66	3	Nil
2.	Rajendra Institute of Medical Science, Ranchi, Jharkhand	173	11	5	168	11	Nil
3.	MGM Medical College, Jamshedpur, Jharkhand	83	25	1	83	24	Nil
4.	Patuliputra Medical College, Dhanbad, Jharkhand	180	22	1	180	21	Nil
5.	Pasteur Institute, Shillong, Meghalaya	85	22	-	86	21	01
6.	SCB Medical College, Cuttack, Orissa	228	31	-	228	31	Nil
7.	VSS Medical College, Burla, Orissa	213	18	-	214	17	01
8.	MKCG Medical College, Beharampur, Orissa	202	41	-	203	40	01
9.	Guwahati Medical College, Guwahati, Assam	250	09	2	249	8	Nil
10.	Silchar Medical College, Silchar, Assam	75	09	7	72	12	03
11.	Assam Medical College, Dibrugarh, Assam	144	01	-	144	01	Nil

HIV Sentinel Surveillance 2010 (ANC & STD)

3118 samples (ANC & STD) were received from Murshidabad, Barddhaman, Nadia, Kolkata and North 24 Parganas districts of West Bengal. However only 3056 samples (98.0 %) could be processed for HIV testing, as 62 samples were rejected due to haemolysis, low volume and vial crack. Among these 88 samples were screened as HIV positive, amounting to 2.8% of the total volume.

Table 4: HIV Sentinel Surveillance 2010 (ANC & STD): Quality Assurance for Testing centre other than SRLs under NACO NRL, NICED, Kolkata:

Sl. No	Name of SRL/Testing Centre	Samples sent by SRL		Samples rejected by NRL	Confirmed Result at NRL		No. of Discordant
		HIV -ve	HIV +ve		HIV -ve	HIV +ve	
1.	School of Tropical Medicine, Kolkata, West Bengal	120	13	-	120	13	Nil
2.	RIMS, Imphal, Meghalaya	143	24	-	144	23	01
3.	Pasteur Institute, Shillong, Meghalaya	85	22	-	86	21	01
4.	Tura civil Hospital,Meghalaya	83	1	1	82	1	Nil

NABL Accreditation-

NACO National Reference Laboratory at NICED is in the process of NABL accreditation. Final assessment by NABL assessors is over. The NCs (Non Conformance) raised by lead assessor and technical assessors are addressed and closed. Awaiting for NABL Accreditation communication.

NACO- Early Infant Diagnosis (EID)

NACO conducted EID programme is the cornerstone in the efforts to significantly reduce HIV related morbidity and mortality in infants. The diagnosis of HIV infection in infants and children younger than 18 months is different from that in adults due to transplacental transfer of maternal antibodies from mother to child during pregnancy, childbirth and breast feeding. Hence HIV-1 DNA PCR testing is recommended for the babies less than 18 months of age.

NICED is one of the 7 Regional Reference Laboratories (RRL) under NACO performing HIV-1 DNA PCR from Dried Blood Spot (DBS) and Whole Blood Samples in NICED, EID program has been started from August,2010initially with three states West Bengal, Orissa and Chattishgarh. With gradual success of the program, the North Eastern states(Assam, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Jharkhand, Bihar) were also included from January, 2011 under NICED .

Presently, 70 ICTCs are involved in collection of DBS samples in 10 states under NICED and 27 ART centres are collecting Whole Blood Samples from infants reactive for DBS-HIV-1 DNA PCR. Different testing algorithms (algorithm A: for < 6months and algorithm B: for 6- 18 months)have been followed for two different age group of HIV exposed infants in this EID program for detection of HIV-1 DNA.

A total of 202 DBS and 22 Whole Blood Samples received at NICED from six states for the period of 01.08.2010 to 31.03.2011 and their status are depicted in Table 1 and Fig. 1.

Table 1. Status of DBS and Whole Blood Samples received at NICED from August'2010 to March'2011.

Name of state	No. of DBS samples received	No. of DBS samples accepted	No. of DBS samples rejected	HIV-1 DNA detected in DBS	Whole Blood samples tested	HIV-1 DNA detected in whole blood
West Bengal	118	113	05	31	18	12
Orissa	47	45	02	17	03	03
Chhattisgarh	18	15	03	03	01	01
Assam	04	04	-	01	-	-
Mizoram	13	13	-	05	-	-
Bihar	02	02	-	-	-	-

NACO-Regional Institute (EAST) for HSS

The Regional Institute (RI) has been functioning at NICED since its inception in early September-October 2008, as the sixth RI engaged for the purpose of HIV Sentinel Surveillance (HSS) for eastern region of the country.

Further to this, an RI team comprising of the Project Coordinator (Dr M. K. Saha, Head of the Public Health Laboratory Division, NICED), and two Senior Epidemiologists (Dr. S. Panda, Scientist 'E',NICED & Dr. A. K. Deb, Scientist 'D',NICED) was constituted.

Spectrum of Activity

- Technical support & guidance to SACS in overall planning & implementation of HSS activities in Andaman & Nicobar Islands, Chhatisgarh, Meghalaya, Nagaland, Sikkim and West Bengal, facilitating smooth implementation of HSS activities by liaisoning with the concerned state authorities and addressing specific problems at sentinel sites/ testing labs
- Technical Validation & approval of new sites through review of relevant data & site visits.
- Conduction of Regional Pre- & Post-surveillance co-ordination & planning meetings, Regional Trainings and Workshops for HSS
- Technical & Supervisory support for state level training of site personnel & lab personnel
- Monitoring & Supervision during HSS through site visits by RI team members

- Constitution of State Surveillance Teams (SST) and coordination of all their activities including Monitoring & Supervision by SST members
- Ensuring timely reporting & corrective action at sites/testing labs during the round
- Data Entry, matching & finalizing data collected during HSS
- Concurrent data monitoring and initiation of corrective action, as required
- Guide SACS in preparation of state surveillance reports after the round
- Undertaking special epidemiological or operational studies and in-depth analyses during the inter-surveillance period to validate or strengthen surveillance findings
- Technical review and approval of any other specific proposal from SACS related to HSS
- Submission of report of activities undertaken during surveillance and analysis of the surveillance findings in the allocated states

Other activities of RI (East) include, but are not limited to:

- Development of Database Management Systems to manage flow of information during Surveillance. Data Processing for Samples received at the NACO HIV National Reference Laboratory (NRL) at NICED during various Testing Programs supported by NACO.
- Providing Back-end and Technical Support for various Training Programs including Workshops held under the aegis of the Public Health Laboratory Division (PHLD), NICED.

Table 1. Number of sites at different states under RI (East)

Name of States	ANC	ANC (R)	STD	FSW	IDU	SMM	MSM	LDT	TOTAL
A&N Islands	4	0	1	0	0	0	0	0	5
Chattisgarh	18	0	4	3	1	0	1	0	27
Meghalaya	2	5	3	1	0	0	0	0	11
Nagaland	10	8	1	1	8	0	1	1	30
Sikkim	3	0	1	1	2	0	0	0	7
West Bengal	22	0	10	12	6	1	5	5	61
Site Type Totals	59	13	20	18	17	1	7	6	141

New site from HSS 2010 round:

Chhattisgarh

1. (FSW) Jankalyan Samajik Sansthan, Rajnandgaon
2. (FSW) Chetna Child & Women Welfare Society, Raipur
3. (MSM) Samta Mahila Mandal, Raipur
4. (FSW) Samarpit, Bilaspur
5. (IDU) Adarsh Navyuvak Mandal, Korba

Meghalaya

1. (IDU) Manbha Foundation, Shillong

Nagaland

1. (MSM) : Guardian Angel, Dimapur
2. (LDT): NEDHIV, Dimapur

West Bengal

1. (ANC)-ARANGHATA BPHC(Composite)
2. (IDU)-Sristy, Domkol
3. (IDU)-NIDS, Naxalbari
4. (MSM)-Swikrity, Shantipur,Nadia
5. (LDT) Ambuja Cement Foundation

Training Name	Venue	Date
Site Validation for the West Bengal	Nadia, Murshidabad, Howrah	23-30 Aug 2010
TOT for HSS 2010 (WBSAPCS, SIKKIM SACS, ANDAMAN SACS, CHHATTISGARH SACS, MEGHALAYA SACS,NAGALAND SACS)	NICED, Kolkata, West Bengal	21st to 23rd September,2010
A&N Islands Site Personnel Training for HSS 2010(ANC & STD Round)	GB Pant Hospital, Port Blair, A&N Islands	27th Sep 2010- 28th Sep 2010
Meghalaya Site Personnel Training for HSS 2010(ANC & STD Round)	Pasteur Institute, Shillong, Meghalaya	28th Sep 2010
Chhattisgarh Site Personnel Training for HSS 2010(ANC & STD Round)-1st Batch	CGSACS, Raipur, Chhattisgarh	30th Sep 2010
Chhattisgarh Site Personnel Training for HSS 2010(ANC & STD Round)- 2nd Batch	CGSACS, Raipur, Chhattisgarh	1ST Oct 2010
West Bengal Site Personnel Training for HSS 2010- 1st Batch	WBSAPCS, Kolkata, West Bengal	8th Oct 2010
West Bengal Site Personnel Training for HSS 2010(ANC & STD Round)- 2nd Batch	WBSAPCS, Kolkata, West Bengal	11th Oct 2010
West Bengal Site Personnel Training for HSS 2010 (ANC & STD Round)- 3rd Batch	WBSAPCS, Kolkata, West Bengal	12th Oct 2010
West Bengal Monitoring ANC/STD Visit		Jan' 11 to March' 11
Nagaland Site Personnel Training for HSS 2010 (ANC & STD Round)	NSACS, Kohima, Nagaland	21st Oct 2010-22nd Oct 2010
Nagaland Monitoring ANC/STD Visit	ANC & STD Sites	Nov' 10 to Jan' 11
Sikkim Site Personnel Training for HSS 2010(ANC AND STD Round)	STNM Hospital, Gangtok, Sikkim	25th Oct 2010-26th Oct 2010
Chhattisgarh Site Personnel Training for HSS 2010(HRG Round)	CGSACS, Raipur, Chhattisgarh	14th Feb 2011-15th Feb 2010
Nagaland Site Personnel Training for HSS 2010 (HRG Round)	NSACS, Dimapur, Nagaland	28th Feb 2011- 1st March 2011
Meghalaya Site Personnel Training for HSS 2010(HRG Round)	Pasteur Institute, Shillong, Meghalaya	2nd March 2011-3rd March 2011
Sikkim Site Personnel Training for HSS 2010(HRG Round)	STNM Hospital, Gangtok, Sikkim	17th March 2011-18th March 2011

Integrated Counselling & Testing Centre

NICED has been providing counseling and testing support for HIV lab diagnosis since the very beginning of HIV testing in India. The second Indian to be declared HIV positive (Tiklibai, a CSW, 1986) was tested at NICED. In addition, the first HIV positive case in Manipur was confirmed by Western Blot at NICED, Kolkata in 1990.

After more than two decades of uninterrupted service with institutional resources from ICMR, a formal Integrated Counseling and Testing Centre (ICTC) was setup under the Public Health Laboratory Division, NICED in September 2008 with the requisite financial, personnel and laboratory support from NACO through WBSAP&CS.

The Consolidation Phase: What started as a tiny room in the corner of the Clinical Laboratory (now re-designated Biochemistry and Hematology Laboratory), has now grown both in size and scope to include a multitude of activities and is the focal point of client-related HIV care at NICED.

The counseling room is now located at the ground floor of the NICED, New building and boasts of a dedicated client/volunteer waiting area. Testing facilities are, for the time being, conducted sharing with Hematology Laboratory.

Staff and Activity Plan : This ICTC unit caters to all HIV-testing needs of in-patients of the 680 bedded I.D. & B.G. Hospital as well as outpatients from various NGO's, CBO's and the general public. Health Camps organized by some NGO's were also successfully conducted during the tenure of the ICTC here.

Ably manned by Ms. Piyali Ghosh, Counselor and Mr. Debasis Chakraborty, Laboratory Technician, the ICTC runs from Monday through Saturday, 10:00 hrs to 18:00 hrs. Apart from counseling, testing and ART delivery (as Post exposure Prophylaxis) the ICTC also functions as an auxiliary unit for orientation and training of health-care personnel at NICED.

Achievements: The Integrated Counseling & Testing Centre of NICED was assessed by World Health Organization (WHO). This evaluation was done in two phase. The first phase was carried out on March 2010 and the second phase was completed on June 2010.

Future Directions: Projects in the pipeline include outreach visits to nearby TI sites and undertaking of operational research study in close collaboration with NACO Regional Institute (East), the implementing agency for HIV sentinel Surveillance at NICED.

TRAININIG ACTIVITIES

The Division of **Training and Extension (*a WHO collaborating center for research and training on diarrhoeal diseases)* is actively engaged in the following salient activities in the reported (2010-11) period:**

IA. Organize the following international/ national meeting/workshops/seminars/training:

1. Organized "Informal Consultation for Development of Research Proposal on Communicable Diseases" NICED/KOLKATA, 23-24 December, 2010 (WHO-SEARO-APW).
2. Organised & participated as joint Principal Investigator in **"Kick off" meeting** and subsequent programme specific field and local visits for conduction of project work in the DST-DFG project "Role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal: relation with cholera incidence" along with our international counterparts (German and Japan), 10-27 January,2011.
3. Organised & participated in ICMR, New Delhi assisted Public Opinion on "Knowledge Management Policy for Health – Service, Education and Research, 18 November, 2010, NICED, Kolkata
4. Organized & participated in ICMR, New Delhi assisted meeting on "National Health Research Policy", 18 November,2010,NICED, Kolkata
5. Organized as joint -organizer WHO assisted Global Food Nework (GFN) meeting for national and International participants in two phases at NICED,Kolkata, 14-18 March & 21-25 March 2011
6. Organized as joint –organizer and participated in Indo - Swedish sponsored "International Symposium in Molecular & Pathological Research on Enteropathogens" jointly sponsored at Kolkata, NICED, 27-29 January 2011.
7. Participated and presented paper in 22nd National Congress of Parasitology, at Department of Zoology, Kalyani University, Kalani, India, 30 October-1 November 2010.
8. Organised & participated as Local Principal Coordinator for ICMR assisted "Public Consultation" (Eastern region) of ICMR-DBT Guidelines for Stem Cell Research and Therapy at CGCRI, Kolkata, India, 17 April 2010.
9. Organized as joint–organizer and participated in International CME-2 on Tropical & Infectious Diseases, jointly orgnised by School of Tropical Medicine, Kolkata and NICED at NICED Kolkata, 5-6 March 2011.

I.B. Organize the following meetings of the Institute:

- SAC meetings on 27-28 August, 2010 at NICED, Kolkata
- Biosafety committee (IBSC) meeting
- Organizing meeting for GCLP training 2010.
- IVI training programmes
- Meeting of NICED scientific forum

- Meeting of NACO from time to time through the year viz. induction training programmes for Medical Technologists
- Observance of National Science Day on 2 March,2011
- 50th Anniversary celebration of NICED, 18 February,2011
- Alumni Day celebration of NICED,18 February,2011
- Seminars and oration lectures organised by ISCA, Kolkata chapter & NICED, Kolkata
- Oration lecture of Indian Science Congress Association, Kolkata chapter
- Observation of National Science day by ISCA, Kolkata chapter on March, 2011
- NACO Regional Institute meeting for HIV surveillance
- WHO/TDR La. Survey,2010
- Arrangement of meeting for Dr. Rita Colwell, Dr. Colin Stein, Dr. Nozaki, DR. Alessandro Craviato
- NICED Scientific forum
- Bacteriology student seminar
- Assistance for Annual session meeting of Calcutta University and National Academy of sciences, Allahabad.

II. Prepared the following documents for the institute:

- Preparation, compilation and submission of 4 (four) years' **performance appraisal report** for WHO for redesignation of the Institute as a WHO collaborating center for research and training on diarrhoeal diseases.
- The redesignation has been granted by WHO for a four (4) year period from April, 2010-April, 2014.
- Compilation and submission of Annual report of WHO Collaborating Center for research and training on diarrhoeal diseases (2009-10).
- Project report book (2009-10)
- Compilation of minutes of SAC,2010
- Reports for Institutional Biosafety Committee (IBSC)
- Minutes of Institutional Biosafety Committee Meeting
- Highlight of the Institute for DBT
- Highlight of the Institute for ICMR Centenary celebration (Publication & Information directorate)
- History of NICED (Compilation of documents) for publication of 100 years of Memoirs of ICMR
- Training modules of different workshops
- Document for the Institutional Scientific Audit

- Workshop Planning & Budget
- Report of the Training Programme
- Preparation of documents for Scientific audit.

III. Prepared following documents for ICMR Head Quarter:

- Highlights of the Diarrhoeal Diseases Research carried over by the Institute
- Highlights of the Institutional activities for ICMR Annual Report
- Highlights of the Institutional activities for ICMR SAG Meeting
- Power point presentation for SAG meeting
- Reports of celebration of National Science day for ICMR
- Reports of celebration of Technology day for ICMR
- Reports of celebration of ICMR day for ICMR
- Documents for foreign participation in R&D activities of the Institute
- Documents for Annual Report for Department of Health Research (DHR)
- Documents for National Institute of Science Communication & Information Research
- WHO training details with prospective budget estimate conducted by NICED
- Documents on activities undertaken by NICED in the context of global climate changes on diarrhoeal diseases and cholera.
- Highlight of the Institute salient activities for ICMR Centenary celebration (Publication & Information directorate) –write up and POSTER
- History of NICED (Compilation of documents) for publication of 100 years of Memoirs of ICMR

III. Submission of Annual report, administrative and A/C related documents for WHO, providing Institutional profile.

IV. Training programmes:

- Training for M.Sc. Biotechnology of students from Bishwabharati University
- Training programme for students of National Institute of Homeopathy, 6 August 2010
- Training programme for Microbiology students of Calcutta University.
- Training programme for Microbiology students of Kalyani University
- Training programme for M.Sc.(Zoology) students of Kalyani University, 2010.
- Training programme on Clinical Tropical Medicine, Optional Module for Master's programme in International Health.

- Coordinating training programme for WHO nominated International trainee in the Institute.
- NACO workshops.
- Training Programmes of Immunization strengthening Project for mid level managers of the districts of Eastern states Andaman & Nicobar islands, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Orissa, Sikkim, Tripura, West Bengal as per programme schedules.
- Coordination for Video shooting for Institute activities by ICMR technical group, 2010.

VI. Organization of workshops/meeting for professional bodies at this Institute:

- Organized as joint–organizer and participated in International CME-2 on Tropical & Infectious Diseases, jointly orgnised by School of Tropical Medicine, Kolkata and NICED at NICED Kolkata, 5 & 6 March, 2011.
- For Indian Science Congress Association, Kolkata chapter for observance of Science Day, 2011
- For "Sparsh", an NGO, HIV study sensitization programme,2011
- Meeting public health officials with Dr. Eric Mintz, Dirrhoeal disease Epidemiology from CDC, Atlanta, West Bengal State AIDS prevention and control Society meeting on DBS and whole blood sample collection at NICED, Kolkata, coordinated by WBSAP&CS.
- For Indian Science Congress Association, Kolkata chapter for observance of Environment Day.

VII. Preparation of other popular documents:

1. History of NICED (ICMR) : Document prepared as a part of ICMR centenary celebrations for publication by ICMR
2. Achievements & contributions of NICED for public health care since its inception : Document prepared as a part of ICMR centenary celebration and NICED's 50th year anniversary for publication by ICMR.

Staff : A. Palit, Scientist "E" and Head
R. J. Mukherjee, Technical Officer A
A. Jana, Technician B
A. Roy, Technician B
S. Adhikary, Attendant Services

EVENTS

ICMR-DBT Guidelines for Stem Cell Research & Therapy (2007): ICMR-DBT Guideline for Stem Cell Research and Therapy (2007) for providing ethical and scientific directions to scientists and clinicians working in this field was held at Dr. Meghnad Saha Auditorium of Central Glass and Ceramic Research Institute Kolkata. Dr. G. Jotwani from ICMR and Dr. A. Palit from NICED were joint coordinators of the meeting.

Bioinformatics Workshop: The 5th Bioinformatics Workshop was held at the NICED during 18-19 June 2010. 22 Participants from all over India attended the workshop. The topic of the workshop was "Sequence analysis and protein modeling". Dr. S. S. Das, NICED Scientist, was the coordinator of the workshop

Scientific Advisory Committee (SAC) Meeting: 38th SAC meeting was held at the NICED on 27-28 August 2010. Prof. N. K. Ganguly was the chairperson of the meeting.

Diarrhoeal Disease Repository Meeting : Meeting regarding establishing of Diarrhoeal Disease Repository was held at the NICED on 17 September 2010. Dr. V. I. Mathan chaired the meeting. Dr. R. Arora from ICMR was present in the meeting.

Capacity Building Workshop of NACO: Capacity Building Workshop of NACO Leading to Proposal Development was held at Shilong on 21-24 September 2010. Dr. S. Panda, NICED Scientist was one of the resource persons.

External Quality Assessment Programme for State Reference Laboratories: External Quality Assessment Programme for State Reference Laboratories of Andaman, Assam. Jharkhand, Meghalaya and Orissa was conducted at the National Reference Laboratory at NICED on 29 October 2010.

WHO / SEARO-NICED Meeting: WHO/SEARO-NICED Meeting on Informal Consultation for Development of Research Proposal on Communicable Diseases was held at the NICED on 23-24 December 2010. The meeting was attended by 12 participants nominated from different SEAR countries. NICED scientists Dr. A Palit, and Dr. A. K. Deb were local facilators. Dr. S. K. Bhattacharya and Dr. A. P. Dash were faculties from SEARO office, New Delhi. Dr. G. B. Nair and Dr. S. Kanungo were faculties from NICED. Dr. D. Mahalanabis was guest lecturer

2nd International CME: 2nd International CME on 'Tropical and Infectious Diseases' was co-hosted by NICED at the NICED campus on 5-6 March 2011. It was organized by Society of Tropical Medicine & Infectious Diseases, India. Dr. S. Panda, NICED scientist, was the organizing secretary of this meeting

NABL Quality Management Workshop: NABL Quality Management Workshop was held at the NICED on 29 July 2010. Mr. S. Chakraborty was the resource person

NACO Programme for HIV HSS-2010: NACO Programme for HIV HSS-2010 was held at NICED during 21-23 September 2010. This workshop "Training for Trainers" was organized by West Bengal State AIDS Prevention & Control Society.

SRL EQAS Training Workshop: The workshop was held at the NICED on 29 October 2010. 19 participants from Eastern & North eastern States attended the workshop

EXTRAMURAL PROJECTS

1. **Title:** Evaluation of anti-typhoid and anti-diarrhoeal activity of three ethnomedicinal plants of tribal use from different parts of India.

   P.I. : Dr. S. Dutta
   Fudnding agency : Funded by ICMR-NIF fund.
   Duration of Research Project : Two years from July 2008 - June 2010.

2. **Title:** Studies of the emerging El Tor variant *Vibrio cholera* in Asia and Africa.

   P.I : Dr. A. K. Mukhopadhyay
   Fuding agency : Okayama University
   Duration : 2010-2014.

3. **Title:** Elucidation and analysis of biological function(s) of *Helicobacter pylori* restriction-modification systems.

   P.I. : Dr. A. K. Mukhopadhyay
   Fuding agency : Departent of Biotechnology
   Duration : April 2009-March2012.

4. **Title:** Novel strategies to combat cholera

   P.I. : Dr. R. K. Nandy
   Funding agency : ICMR
   Duration : 2009-2012.

5. **Title:** Determine the immune response to novel conserved Shigella protein antigens in patients with recent onset of Shigellosis.

   P.I. : Dr. R. K. Nandy
   Funding agency : Internation Vaccine Institute(IVI), Korea
   Duration : 2010-2011.

6. **Title:** A Randomized controlled trial (Phase III) of the bivalent killed whole cell oral cholera vaccine in eastern Kolkata, West Bengal, India.

   P.I. : Dr. D. Sur.
   Fundian agency : International Vaccine Institute (IVI), Korea.
   Duration : 5 years.

7. **Title:** Pediatric HIV-1 infection and childhood immunization coverage: a study to investigate whether pediatric HIV infection is an independent risk factor for incomplete childhood immunization.

   P.I. : Dr. S. Das Bhattacharya.
   Funding agency : IIT Kharagpur

8. **Title :** Development and evaluation of a heat killed multi-serotype oral Shigella vaccine.

P.I.	:	Dr. H. Koley
Funding agency	:	Okayama University.

9. **Title :** Studies on emerging and reemerging infectious diseases.

P.I.	:	Dr. G. B. Nair
Funding agency	:	Okayama University.

10. **Title :** A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in Eastern Kolkata, West Bengal, India.

P.I.	:	Dr. D. Sur.
Funding agency	:	Bill and Melinda Gates Foundation, USA.

11. **Title :** Global Enteric Multicentric Study (GEMS) Microbilogy.

P.I.	:	Dr. D. Sur.
Funding agency	:	Bill and Melinda Gates Foundation, USA.

12. **Title :** Studies on the effect of arsenic on the pathophysiology of bacteria.

P.I.	:	Dr. S. Mazumder.
Funding agency	:	DST, Government of India.
Duration	:	3 years.

13. **Title :** Surveillance for dengue fever in Eastern Kolkata.

P.I.	:	Dr. S. Chakrabarti
Funding agency	:	Bill and Melinda Gates foundation.
Duration	:	2 years.

14. **Title :** A randomized controlled trial (Phase-II/III) of the live recombinant oral cholera vaccine (VA1.4) in eastern Kolkata.

P.I.	:	Dr. D. Sur.
Funding agency	:	Dept. of Biotechnology, Govt. of India.
Duration	:	18th Months.

15. **Title :** Diarrhoeal disease in infants and young children in developing countries .

P.I.	:	Dr. D. Sur.
Funding agency	:	Bill and Melinda Gates Foundation.
Duration	:	3 years.

16. **Title:** A randomized controlled trial to evaluate the immunogenicity of two doses of the modified killed whole cell oral cholera vaccine (WC-OCV) under two alternative vaccination schedules.

P.I.	:	Dr. D. Sur.
Funding agency	:	Bill and Melinda Gates Foundation.
Duration	:	12 months.

17. **Title:** A community based epidemiological study of Rotavirus in children below 2 yrs of age.

P.I.	:	Dr. S. Panda.
Funding agency	:	Serum Institute India Limited.
Duration	:	5 and ½ months starting in December 2010.

18. **Title:** Technical assistance support to NACO – baseline/impact assessment study on HIV in IDUs in Punjab.

P.I.	:	Dr. S. Panda.
Funding agency	:	Futures Group/DFID.
Duration	:	14 months starting in August 2010.

19. **Title:** Study the prevalence and genetic characterization of *Entamoeba histolytica* reference strains from Kolkata, India.

P.I.	:	Dr. S. Ganguly.
Funding agency	:	Japan health Sciences Foundation
Duration	:	3 years.

20. **Title:** To study the presence of common enteric parasites found during regular hand washing.

P.I.	:	Dr. S. Ganguly.
Funding agency	:	Research Foundation of City University of New York, USA.
Duration	:	3 years.

21. **Title:** Multisite monitoring of Influenza virus strains in India, Phase II.

P.I.	:	Dr. M. Chawla-Sarkar.
Funding agency	:	ICMR, India and DHHS, USA.
Duration	:	2010-2015.

22. **Title:** Analysis of rotaviruses and their interactions with the host: a viral proteomics approach.

P.I.	:	Dr. M. Chawla-Sarkar.
Funding agency	:	Okayama University, Japan.
Duration	:	2010-2014.

23. **Title:** Biomedical Informatics Center of ICMR.

P.I.	:	Dr. S. S. Das.
Funding agency	:	ICMR.
Duration	:	2006 onwards.

24. **Title:** Studies on the regulation of antimicrobial peptide expression and their role in mixed and opportunistic infections of the gut.

P.I.	:	Dr. S. S. Das.
Funding agency	:	Okayama University, Japan
Duration	:	2010-2015.

25. **Title:** Role of Toll-like and NOD receptors in probiotics-induced mucosal tolerogenicity.

P.I.	:	Dr. S. S. Das.
Funding agency	:	Department of Biotechnology, Govt. of India.
Duration	:	2011-2014.

26. **Title:** Comparative analysis of luxO, the quorum sensing master regulator, among 01, 0139 and non-01, non-0139 *V.Cholerae* strains.

P.I.	:	Dr. R. K. Nandy.
Funding agency	:	Department of Biotechnology(DBT), Govt. of India.
Duration	:	Three and half years; 2007-2011.

27. **Title:** Hospital based surveillance system for diarrhoeal diseases.

P.I.	:	Dr. G. B. Nair.
Funding agency	:	Okayama University, Japan.

28. **Title:** Molecular mechanism of enterotoxigenic *Escherichia coli* adherence in the intestine: host-pathogen relationship.

P.I.	:	Dr. N. S. Chatterjee
Funding agency	:	Department of Atomic Energy.
Duration	:	2008-2011.

29. **Title:** Host intestinal response induced by *Vibrio Cholerae* chitin-binding protein GbpA and the subsequest effect on the pathogen.

P.I.	:	Dr. N. S. Chatterjee
Funding agency	:	Council of Scientific and Industrial Research
Duration	:	2010-2013.

PUBLICATIONS

1. **Agrawal, A. S., M. Sarkar, S. Ghosh, T. Roy, S. Chakrabarti, R. Lal, A. C. Mishra, M. S. Chadha, and M. Chawla-Sarkar**. 2010. Genetic characterization of circulating seasonal Influenza A viruses (2005-2009) revealed introduction of oseltamivir resistant H1N1 strains during 2009 in eastern India. Infect.Genet.Evol. **10**:1188-1198.

2. **Agrawal, A. S., T. Roy, S. Ghosh, and M. Chawla-Sarkar**. 2011. Genetic variability of attachment (G) and Fusion (F) protein genes of human metapneumovirus strains circulating during 2006-2009 in Kolkata, Eastern India. Virol.J. **8**:67.

3. **Alam, M., N. A. Hasan, M. Sultana, G. B. Nair, A. Sadique, A. S. Faruque, H. P. Endtz, R. B. Sack, A. Huq, R. R. Colwell, H. Izumiya, M. Morita, H. Watanabe, and A. Cravioto**. 2010. Diagnostic limitations to accurate diagnosis of cholera. J.Clin.Microbiol. **48**:3918-3922.

4. **Alam, M., S. Nusrin, A. Islam, N. A. Bhuiyan, N. Rahim, G. Delgado, R. Morales, J. L. Mendez, A. Navarro, A. I. Gil, H. Watanabe, M. Morita, G. B. Nair, and A. Cravioto**. 2010. Cholera between 1991 and 1997 in Mexico was associated with infection by classical, El Tor, and El Tor variants of Vibrio cholerae. J.Clin.Microbiol. **48**:3666-3674.

5. **Albert, M. J., H. M. Al Akbal, R. Dhar, R. De, and A. K. Mukhopadhyay**. 2010. Genetic affinities of *Helicobacter pylori* isolates from ethnic Arabs in Kuwait. Gut Pathog. **2** :6.

6. **Ali, M., D. Sur, A. L. Lopez, S. Kanungo, R. L. Ochiai, B. Manna, D. R. Kim, J. Deen, S. K. Bhattacharya, and J. D. Clemens.**2010. Community participation in two vaccination trials in slums of Kolkata, India: a multi-level analysis J. Health Popul. Nutr. **28**:450-457.

7. **Bagchi, A. K., A. K. Sinha, R. Adhikari, P. Maiti, J. Mukherjee, A. Panda, and D. R. Saha**. 2010. Selective deletion of CD8(+) cells upregulated by caspases-1 via IL-18 in mice immunized with major outer membrane protein of *Shigella dysenteriae* 1 following infection. J.Clin.Immunol. **30**:408-418.

8. **Bagchi, P., D. Dutta, S. Chattopadhyay, A. Mukherjee, U. C. Halder, S. Sarkar, N. Kobayashi, S. Komoto, K. Taniguchi, and M. Chawla-Sarkar**. 2010. Rotavirus nonstructural protein 1 suppresses virus-induced cellular apoptosis to facilitate viral growth by activating the cell survival pathways during early stages of infection. J.Virol. **84**:6834-6845.

9. **Bal, B., R. Mitra, A. H. Mallick, S. Chakraborti, and K. Sarkar**. 2010. Nontobacco substance use, sexual abuse, HIV, and sexually transmitted infection among street children in Kolkata, India. Subst.Use.Misuse. **45**:1668-1682.

10. **Banerjee, K. K.** 2010. Cholera: a biochemist's perception of an enterotoxin. Science and Culture160-165.

11. **Banerjee, M., G. B. Nair, and T. Ramamurthy**. 2011. Phenotypic & genetic characterization of *Bacillus cereus* isolated from the acute diarrhoeal patients. Indian J.Med.Res. **133**:88-95.

12. **Banerjee, S., K. Sarkar, S. Gupta, P. S. Mahapatra, S. Gupta, S. Guha, D. Bandhopadhayay, C. Ghosal, S. K. Paine, R. N. Dutta, N. Biswas, and B. Bhattacharya**. 2010. Multiplex PCR technique could be an alternative approach for early detection of leprosy among close contacts--a pilot study from India. BMC.Infect.Dis. **10**:252.

13. **Barman, S., S. Chatterjee, G. Chowdhury, T. Ramamurthy, S. K. Niyogi, R. Kumar, and H. Koley**. 2010. Plasmid-mediated streptomycin and sulfamethoxazole resistance in *Shigella flexneri* 3a. Int.J.Antimicrob.Agents **36**:348-351.

14. **Basu, S., A. K. Singh, and G. B. Nair**. 2010. Emerging carbapenem resistance in the context of a new metallo-beta-lactamase (NDM-1). Natl.Med.J.India **23**:261-262.

15. **Bhattacharjee, S., S. Bhattacharjee, B. Bal, R. Pal, S. K. Niyogi, and K. Sarkar**. 2010. Is *Vibrio fluvialis* emerging as a pathogen with epidemic potential in coastal region of eastern India following cyclone Aila? J.Health Popul.Nutr. **28**:311-317.

16. **Bhattacharya, K., S. Kanungo, D. Sur, S. B. Lal, B. Manna, A. L. Lopez, M. Bhattacharya, S. Nandy, and N. S. Kumar**. 2011. Tetracycline-resistant *Vibrio cholerae* O1, Kolkata, India. Emerg.Infect.Dis. **17**:568-569.

17. **Bhattacharya, M. K., S. Kanungo, A. Palit, S.Chatterjee, M. Ghosh, B. K. Seth, K. K. Dutta, P. Kundu and S. K. Bhattacharya.** 2010. Study on Socio-economic background of HIV/AIDS patients in West Bengal, India, Indian J. Physiol. Allied Sci. 64: 2-9.

18. **Bhowmick, T. S., M. Das, and B. L. Sarkar**. 2010. Evaluation of VcA VNTR as a strain-typing and phylogeny study method of *Vibrio cholerae* strains. Epidemiol.Infect. **138**:1637-1649.

19. **Bhowmick, T. S., M. Das, and B. L. Sarkar**. 2011. Genotypic characterization of *Vibrio cholerae* isolates using several DNA fingerprint techniques. Future.Microbiol. **6**:29-42.

20. **Biswas, A., A. Banerjee, P. K. Chandra, S. Datta, R. Panigrahi, D. Dutta, B. K. De, M. Pal, S. K. Guha, S. Chakrabarti, and R. Chakravarty**. 2011. Variations in the functional domain of basal core promoter of hepatitis B virus among Eastern Indian patients with prevalence of genotypes A, C, and D among the same ethnic population. J.Med.Virol. **83**:253-260.

21. **Chakrabarti, M. K**., 2010. Guest Column: A Simple approach towards the development of a shigella vaccine. Al Ameen J. Med. Sci. **3**:263-264.

22. **Chakraborty,N., S. Bhattacharya, C. De, A. Mukherjee, D. Bhattacharya, S. Santra, R. N. Sarkar, D. Banerjee, S. K. Guha, U. Datta and S Chakrabarti.** 2010. Incidence of multiple Herpesvirus infection in HIV seropositive patients, a big concern for eastern Indian scenario. Virol. J.**7 :** 147- 153.

23. **Chattopadhyay, S., P. Bagchi, D. Dutta, A. Mukherjee, N. Kobayashi, and M. Chawlasarkar**. 2010. Computational identification of post-translational modification sites and functional families reveal possible moonlighting role of rotaviral proteins. Bioinformation. **4**:448-451.

24. **Choi, S. Y., J. H. Lee, Y. S. Jeon, H. R. Lee, E. J. Kim, M. Ansaruzzaman, N. A. Bhuiyan, H. P. Endtz, S. K. Niyogi, B. L. Sarkar, G. B. Nair, B. M. Nguyen, N. T. Hien, C. Czerkinsky, J. D. Clemens, J. Chun, and D. W. Kim**. 2010. Multilocus variable-number tandem repeat analysis of *Vibrio cholerae* O1 El Tor strains harbouring classical toxin B. J.Med.Microbiol. **59**:763-769.

25. **Chowdhury, N., M. Asakura, S. B. Neogi, A. Hinenoya, S. Haldar, T. Ramamurthy, B. L. Sarkar, S. M. Faruque, and S. Yamasaki**. 2010. Development of simple and rapid PCR-fingerprinting methods for *Vibrio cholerae* on the basis of genetic diversity of the superintegron. J.Appl.Microbiol. **109**:304-312.

26. **Das, K, Das K, Mukherjee PS, Ghosh A, Ghosh S, Mridha AR, Dhibar T, Bhattacharya B, Bhattacharya D, Manna B, Dhali GK, Santra A, Chowdhury A.** 2010. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. Hepatology **51:**1593-1602.

27. **Das, P., S. Samuels, P. Desjeux, A. Mittal, R. Topno, N. A. Siddiqui, D. Sur, A. Pandey, and R. Sarnoff.** 2010. Annual incidence of visceral leishmaniasis in an endemic area of Bihar, India. Trop Med Int Health. 15 Suppl **2:**4-11

28. **Deb, A. K., S. Kanungo, G. B. Nair, and J. P. Narain**. 2011. The challenge of pneumonia & acute diarrhoea at global, regional & national levels: time to refocus on a top most priority health problem. Indian J.Med.Res. **133**:128-130.

29. **Degenhardt, L., B. Mathers, M. Guarinieri, S. Panda, B. Phillips, S. A. Strathdee, M. Tyndall, L. Wiessing, A. Wodak, and J. Howard**. 2010. Meth/amphetamine use and associated HIV: Implications for global policy and public health. Int.J.Drug Policy **21**:347-358.

30. **Dey, R. S., S. Ghosh, M. Chawla-Sarkar, S. Panchalingam, J. P. Nataro, D. Sur, B. Manna, and T. Ramamurthy**. 2011. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. J.Clin.Microbiol. **49**:500-505.

31. **Ganesh, B., S. M. Nataraju, K. Rajendran, T. Ramamurthy, S. Kanungo, B. Manna, S. Nagashima, D. Sur, N. Kobayashi, and T. Krishnan**. 2010. Detection of closely related Picobirnaviruses among diarrhoeic children in Kolkata: evidence of zoonoses? Infect.Genet.Evol. **10**:511-516.

32. **Ganesh, B., K. Banyai, G. Masachessi, Z. Mladenova, S. Nagashima, S. Ghosh, S. M. Nataraju, M. Pativada, R. Kumar, and N. Kobayashi**. 2011. Genogroup I picobirnavirus in diarrhoeic foals: Can the horse serve as a natural reservoir for human infection? Vet.Res. **42**:52.

33. **Ganesh, B., S. Nagashima, S. Ghosh, S. M. Nataraju, K. Rajendran, B. Manna, T. Ramamurthy, S. K. Niyogi, S. Kanungo, D. Sur, N. Kobayashi, and T. Krishnan**. 2011. Detection and molecular characterization of multiple strains of Picobirnavirus causing mixed infection in a diarrhoeic child: Emergence of prototype Genogroup II-like strain in Kolkata, India. Int. J. Mol. Epidemiol. Genet. **2**:61-72.

34. **Ghosh-Banerjee, J., M. Senoh, T. Takahashi, T. Hamabata, S. Barman, H. Koley, A. K. Mukhopadhyay, T. Ramamurthy, S. Chatterjee, M. Asakura, S. Yamasaki, G. B. Nair, and Y. Takeda**. 2010. Cholera toxin production by the El Tor variant of *Vibrio cholerae* O1 compared to prototype El Tor and classical biotypes. J.Clin.Microbiol. **48**:4283-4286.

35. **Ghosh, A. and T. Ramamurthy**. 2011. Antimicrobials & cholera: are we stranded? Indian J.Med.Res. **133**:225-231.

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38. **Ghosh, S., N. Kobayashi, S. Nagashima, M. Chawla-Sarkar, T. Krishnan, B. Ganesh, and T. N. Naik**. 2010. Full genomic analysis and possible origin of a porcine G12 rotavirus strain RU172. Virus Genes **40**:382-388.

39. **Ghosh, S., K. Chakraborty, T. Nagaraja, S. Basak, H. Koley, S. Dutta, U. Mitra, and S. Das**. 2011. An adhesion protein of *Salmonella enterica* serovar Typhi is required for pathogenesis and potential target for vaccine development. Proc.Natl.Acad.Sci.U.S.A **108**:3348-3353.

40. **Gleick, P. H., R. M. Adams, R. M. Amasino, E. Anders, D. J. Anderson, W. W. Anderson, L. E. Anselin, M. K. Arroyo, B. Asfaw, F. J. Ayala, A. Bax, A. J. Bebbington, G. Bell, M. V. Bennett, J. L. Bennetzen, M. R. Berenbaum, O. B. Berlin, P. J. Bjorkman, E. Blackburn, J. E. Blamont, M. R. Botchan, J. S. Boyer, E. A. Boyle, D. Branton, S. P. Briggs, W. R. Briggs, W. J. Brill, R. J. Britten, W. S. Broecker, J. H. Brown, P. O. Brown, A. T. Brunger, J. Cairns, Jr., D. E. Canfield, S. R. Carpenter, J. C. Carrington, A. R. Cashmore, J. C. Castilla, A. Cazenave, F. S. Chapin, III, A. J. Ciechanover, D. E. Clapham, W. C. Clark, R. N. Clayton, M. D. Coe, E. M. Conwell, E. B. Cowling, R. M. Cowling, C. S. Cox, R. B. Croteau, D. M. Crothers, P. J. Crutzen, G. C. Daily, G. B. Dalrymple, J. L. Dangl, S. A. Darst, D. R. Davies, M. B. Davis, P. V. De Camilli, C. Dean, R. S. DeFries, J. Deisenhofer, D. P. Delmer, E. F. DeLong, D. J. DeRosier, T. O. Diener, R. Dirzo, J. E. Dixon, M. J. Donoghue, R. F. Doolittle, T. Dunne, P. R. Ehrlich, S. N. Eisenstadt, T. Eisner, K. A. Emanuel, S. W. Englander, W. G. Ernst, P. G. Falkowski, G. Feher, J. A. Ferejohn, A. Fersht, E. H. Fischer, R. Fischer, K. V. Flannery, J. Frank, P. A. Frey, I. Fridovich, C. Frieden, D. J. Futuyma, W. R. Gardner, C. J. Garrett, W. Gilbert, R. B. Goldberg, W. H. Goodenough, C. S. Goodman, M. Goodman, P. Greengard, S. Hake, G. Hammel, S. Hanson, S. C. Harrison, S. R. Hart, D. L. Hartl, R. Haselkorn, K. Hawkes, J. M. Hayes, B. Hille, T. Hokfelt, J. S. House, M. Hout, D. M. Hunten, I. A. Izquierdo, A. T. Jagendorf, D. H. Janzen, R. Jeanloz, C. S. Jencks, W. A. Jury, H. R. Kaback, T. Kailath, P. Kay, S. A. Kay, D. Kennedy, A. Kerr, R. C. Kessler, G. S. Khush, S. W. Kieffer, P. V. Kirch, K. Kirk, M. G. Kivelson, J. P. Klinman, A. Klug, L. Knopoff, H. Kornberg, J. E. Kutzbach, J. C. Lagarias, K. Lambeck, A. Landy, C. H. Langmuir, B. A. Larkins, X. T. Le Pichon, R. E. Lenski, E. B. Leopold, S. A. Levin, M. Levitt, G. E. Likens, J. Lippincott-Schwartz, L. Lorand, C. O. Lovejoy, M. Lynch, A. L. Mabogunje, T. F. Malone, S. Manabe, J. Marcus, D. S. Massey, J. C. McWilliams, E. Medina, H. J. Melosh, D. J. Meltzer, C. D. Michener, E. L. Miles, H. A. Mooney, P. B. Moore, F. M. Morel, E. S. Mosley-Thompson, B. Moss, W. H. Munk, N. Myers, G. B. Nair, J. Nathans, E. W. Nester, R. A. Nicoll, R. P. Novick, J. F. O'Connell, P. E. Olsen, N. D. Opdyke, G. F. Oster, E. Ostrom, N. R. Pace, R. T. Paine, R. D. Palmiter, J. Pedlosky, G. A. Petsko, G. H. Pettengill, S. G. Philander, D. R. Piperno, T. D. Pollard, P. B. Price, Jr., P. A. Reichard, B. F. Reskin, R. E. Ricklefs, R. L. Rivest, J. D. Roberts, A. K. Romney, M. G. Rossmann, D. W. Russell, W. J. Rutter, J. A. Sabloff, R. Z. Sagdeev, M. D. Sahlins, A. Salmond, J. R. Sanes, R. Schekman, J. Schellnhuber, D. W. Schindler, J. Schmitt, S. H. Schneider, V. L. Schramm, R. R. Sederoff, C. J. Shatz, F. Sherman, R. L. Sidman, K. Sieh, E. L. Simons, B. H. Singer, M. F. Singer, B. Skyrms, N. H. Sleep, B. D. Smith, S. H. Snyder, R. R. Sokal, C. S. Spencer, T. A. Steitz, K. B. Strier, T. C. Sudhof, S. S. Taylor, J. Terborgh, D. H. Thomas, L. G. Thompson, R. T. Tjian, M. G. Turner, S. Uyeda, J. W. Valentine, J. S. Valentine, J. L. Van Etten, K. E. van Holde, M. Vaughan, S. Verba, P. H. von Hippel, D. B. Wake, A. Walker, J. E. Walker, E. B. Watson, P. J. Watson, D. Weigel, S. R. Wessler, M. J. West-Eberhard, T. D. White, W. J. Wilson, R. V. Wolfenden, J. A. Wood, G. M. Woodwell, H. E. Wright, Jr., C. Wu, C. Wunsch, and M. L. Zoback**. 2010. Climate change and the integrity of science. Science **328**:689-690.

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ANIMAL SECTION

G. Patra, *Technical Assistant*
K. C. Tudu, *Technician C*
S. Hari, *Technician B*
S. Balmiki, *Attendant Services*
P. Turi, *Attendant Services*
R. Hazra, *Attendant Services*

VEHICLE SECTION

D. Saha, *Driver (Gr-I, Per. Gr.)*
K. Ram, *Driver (Spl. Gr.) Retired on 31.1.2011*
S. Das, *Driver (Gr-I, Per. Gr.)*
D. K. Chowdhury, *Driver (Gr-II)*
H. P. Das, *Driver (Gr-II, Per. Gr.)*
C. Nayak, *Driver (Ord. Gr.)*
A. K. Dutta, *Driver (Ord. Gr.)*
S. Das, *Driver (Ord. Gr.)*
S. Ghosh, *Driver (Ord. Gr.)*
D. Dey, *Driver (Ord. Gr.)*
G. Mehboob, *Technician C (Eng. Support)*
Md. Ali Khan, *Technician C (Eng. Support)*

LIBRARY

K. Ray (nee Datta), *Asst. Library Information Officer (Expired on 15.2.2011)*
S. Samanta, *Library & Information Assistant*
T. Pal, *Library & Information Assistant*
M. Chakraborty, *Technical Assistant*

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