वार्षिक रीपोर्ट

Annual Report

2012-2013

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राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)

National Institute of Cholera and Enteric Diseases

(Indian Council of Medical Research)

NICED Annual Report 2012-2013

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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

It's my privilege to witness the growth of the National Institute of Cholera and Enteric Diseases (NICED) over the last two decades. Journey of this Institute started from a rented house and as a four-room facility at central Kolkata which subsequently shifted to the northeastern part of the city named Beliaghata. The Institute has gradually established itself as one of the premiere research hubs of Indian Council of Medical Research (ICMR) under the Department of Health Research (DHR), Government of India. Presently, NICED is equipped with state of the art research facilities including a modern animal house. Mandate of NICED has also expanded, which currently includes research, training and services to society in the field of enteric diseases, vaccine research, HIV/ AIDS and other emerging viral diseases of national interest. Multidisciplinary approaches linking epidemiological findings with laboratory based analysis in the area of bacteriology, virology, parasitology, immunology, molecular biology and clinical medicine have been our major strength over all these years. Identifying strategies for prevention of infections as well as developing management guideline for care of those who are already infected have been our focus.

Apart from conducting research, NICED has been instrumental in generating trained human resources in health service sectors. A good number of Ph.D. students come out every year, trained in the field of diarrheal disease or HIV/AIDS research and with the capacity to conduct independent research by themselves in future. Further to this, a good number of doctors, health practitioners, and Government officials attached with various State Health Systems also get trained through workshops and seminars - events that this Institute conducts on regular basis. Recently, we have started collaborating with the Indian Medical Students' Association (IMSA) in organizing workshops through which we offer orientation training for research methodologies and ethics as well as provide exposure to various laboratories of NICED that we believe will go a long way in creating a critical mass of medical researchers in the country.

I truly feel that sincere contributions from scientists, research fellows and the staff of NICED have made all these aforementioned achievements possible. Continuous support from the Council and able guidance from the Director General of ICMR has not only helped us in taking this journey but also to sustain good quality research. Last but not the least, it is my pleasant duty to acknowledge funding supports that we have received at NICED from national and international agencies to undertake various research projects at the bench as well as bedside and also in the communities.

Sekhar Chakrabarti
Ph.D., FNA, FNASc, FASc & T
Director-in-charge

निदेशक की मेज से

मुझे वर्ष 2012-2013 के लिए वार्षिक रिपोर्ट प्रस्तुत करते हुए हर्ष हो रहा है। संस्थान की प्रगति के लिए यह समय बहुत ही चुनौती पूर्ण रहा है। यह मेरे लिए गर्व की बात है की मैं पिछले दो साल से नाईसेड के विकास का साक्षी हूँ। इस संसथान की शुरुआत एक चार कमरे के किराये के घर से हुई थी जो सेंट्रल कोलकाता में केन्द्रित था। बाद में यह शहर के उत्तरपूर्वी हिस्से में बेलियाघाटा नमक जगह पर चला गया। धीरे धीरे इस संस्थान ने अपने आप को भारतीय चिकित्सा अनुसन्धान परिषद का एक प्रमुख अनुसन्धान केंद्र बना लिया हैं जो कि स्वस्थ्य विभाग केंद्र, भारत सरकार के अधीन है। वर्तमान में, एन.आई.सी.ई.डी. एक आधुनिक पशु घर सहित अत्याधुनिक अनुसंधान सुविधाओं के राज्य के साथ सुसज्जित है। एन.आई.सी.ई.डी. के जनादेश विस्तार हो रहा हैं जिसमें फिलहाल अनुसंधान, आंत्र रोग के क्षेत्र में प्रशिक्षण और समाज सेवा, वैक्सीन अनुसंधान, एचआईवी/एड्स और राष्ट्रीय सूचि के अन्य उभरते वायरल रोग शामिल है। इन वर्षों में बक्टेरीयोलॉजी, वायरोलॉजी, पैरासाईटोलॉजी, इम्यूनोलॉजी, मॉलिक्यूलर बायोलॉजी और क्लिनिकल दवा के क्षेत्र में प्रयोगशाला आधारित विश्लेषण के साथ महामारी विज्ञान के निष्कर्ष हमारी प्रमुख शक्ति रहा है। हमारा ध्यान संक्रमण को दूर रखने के लिए नीतियों की पहचान करने के साथ ही पहले से ही संक्रमित लोगों की अच्छी तरह से देखभाल के प्रबंध करने पर भी रहा हैं।

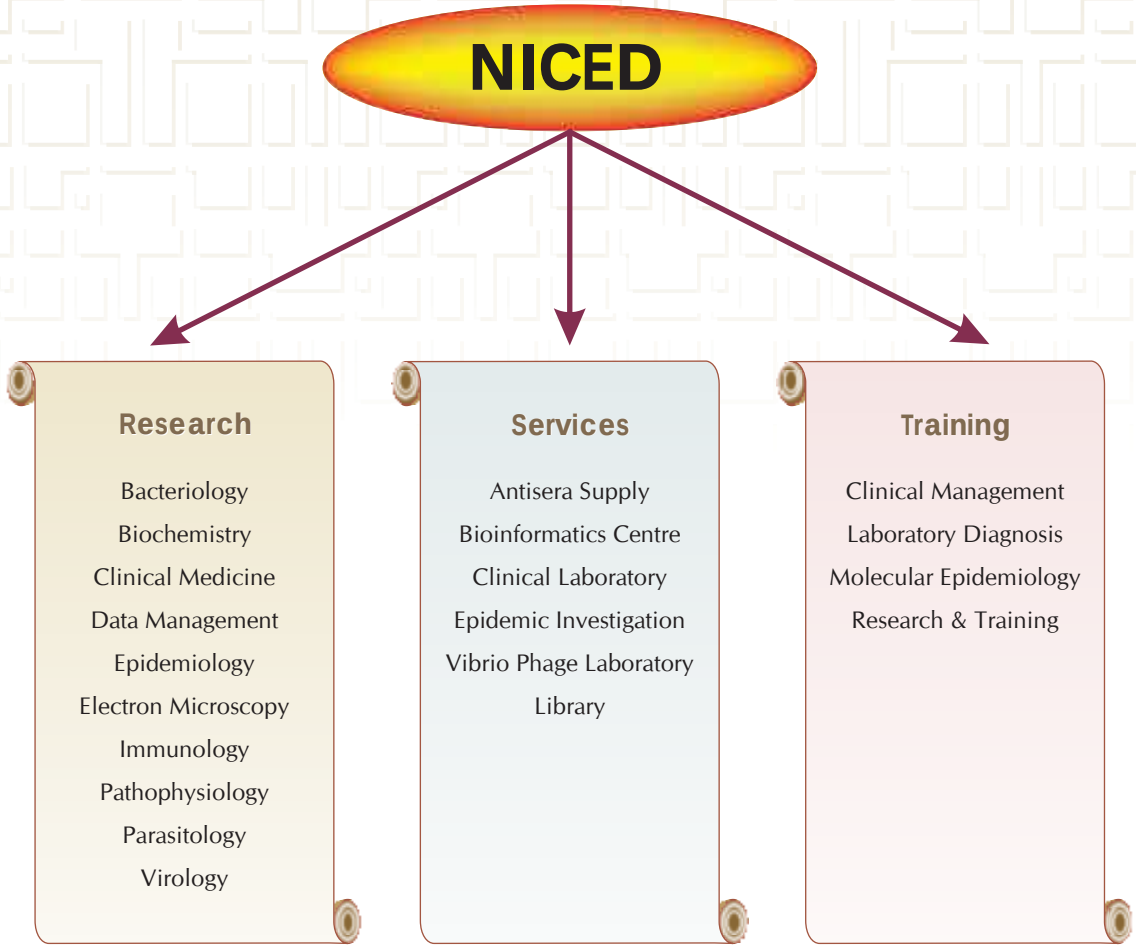
शोध कार्यों के अलावा, एन.आई.सी.ई.डी. स्वास्थ्य सेवा क्षेत्र में प्रशिक्षित मानव संसाधन पैदा करने में महत्वपूर्ण भूमिका निभाई है। अच्छी संख्या में पीएच.डी. छात्र डायरिया की बीमारी या एचआईवी/ एड्स अनुसंधान के क्षेत्र में प्रशिक्षित हो कर और भविष्य में स्वयं द्वारा स्वतंत्र अनुसंधान का संचालन करने की क्षमता के साथ हर साल बाहर आते हैं। इसके अलावा अच्छी संख्या में, विभिन्न स्वास्थ्य प्रणालियों के साथ संलग्न डॉक्टरों, स्वास्थ्य चिकित्सकों, और सरकारी अधिकारिया, इस संस्थान द्वारा नियमित रूप से आयोजित कार्यशालाओं और सेमिनार द्वारा प्रशिक्षित हो रहे हैं। हाल ही में हम ने भारतीय चिकित्सा स्टूडेंट्स एसोसिएशन (IMSA) के सहयोग से कार्यशाला का आयोजन शुरू किया हैं जिसमें अनुसंधान के तरीके और नैतिकता के साथ एन.आई.सी.ई.डी. की विभिन्न प्रयोगशालाओं की शरुआती प्रशिक्षण देते है, जिससे हम देश में चिकित्सा शोधकर्ताओं का महत्वपूर्ण समूह बना सकते है।

मुझे वास्तव में महसूस होता है की, वैज्ञानिकों के सच्चे योगदान, रिसर्च फेलो और एन.आई.सी.ई.डी. के प्रशासनिक और तकनीकी कर्मचारियों ने सभी पूर्वकथित उपलब्धियों को संभव बना दिया है। परिषद के सतत समर्थन और भारतीय चिकित्सा अनुसंधान परिषद के महानिदेशक/स्वास्थ्य सचिव (स्वास्थ्य विभाग, भारत सरकार) के कुशल मार्गदर्शन से न केवल अब तक की यात्रा में मदद मिली है अपितु अच्छी गुणवता अनुसंधान को बनाए रखने के लिए भी हमें सहयोग मिला है। एन.आई.सी.ई.डी. में राष्ट्रीय और अंतरराष्ट्रीय एजेंसियों से आर्थिक समर्थन और योगदान रहा है ताकि हम विभिन्न अनुसंधान परियोजनाओं पर काम कर सके।

डॉ. शेखर चक्रवर्ती

पीएच.डी., एफ.एन.ए, एफ.एन.ए.एस.सी, एफ.ए.एस.सी & टी

प्रभारी निर्देशक



ADMINISTRATION

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

Contents

Research
Activities

BACTERIOLOGY

One of the major activities of the Division included extending laboratory supports in diarrhoeal outbreak/epidemic investigations which were and are being carried out in West Bengal and other parts of the country. In addition, surveillance on diarrhoeal pathogens is being conducted among hospitalized diarrhea cases at Kolkata. This division generates weekly report on the prevalence of diarrhoeal pathogens and that are being sent to State Govt. and other agencies for control and improvement for better patients care and management. Prevalence of bacterial enteropathogens among hospitalized diarrhoea cases included *Vibrio cholerae* O1, *Shigella spp., V. fluvialis, Campylobacter jejuni*, and diarrhoeagenic *Escherichia coli*. Enteroaggregative *E. coli* (EAEC) was the most common followed by enterotoxigenic (ETEC) and enteropathogenic *E. coli* (EPEC). Vibrios remained susceptible for most of the fluoroquinolones but highly resistant to cotrimoxazole and furazolidone. *Shigella* spp. were highly resistant to ciprofloxacin, norfloxacin and ofloxacin. Most the *Shigella* strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone. Associations of *V. fluvialis* and *V. parahaemolyticus* were identified in several outbreaks in West Bengal which is a new trend detected in recent years. A good percentage of *V. cholerae* non-O1, non-O139 strains mediated cholera-like diarrhoea was also detected among hospitalized patients at Kolkata. *Salmonella enterica* serovar Weltevreden and *V. fluvialis* were identified as etiological agents of foodborne gastroenteritis outbreaks. In the pulsed-field gel electrophoresis, *S. Weltevreden* was identified as a single clone but the *V. fluvialis* strains were heterogonous. The existing surveillance system has helped in the timely identification of causative pathogens in these diarrhoeal outbreaks. Non typhoidal *Salmonella* (NTS) isolates are important for understanding of their antimicrobial resistance phenotypes. Transferable resistance to fluoroquinolones and cephalosporin groups were detected in the NTS isolates from both clinical (blood and stool) and environmental (food, animals) samples in Kolkata and were considered as a threat for spread of drug resistance.

This division took an initiative for a holistic surveillance system for tracking the mode of global dissemination of the *V. cholerae* O1 El Tor variants. As a part of this initiative molecular characterization on the *V. cholerae* strains isolated form different parts of the world were made. This characterization revealed that recent Indian *V. cholerae* O1 and strains isolated in Zanzibar were capable to produce more cholera toxin as compared to the prototype El Tor and belonged to a single cluster different from prototype strains. Newly developed *ctxB* allele specific PCR assay helped in identifying existence of novel Haitian types of *ctxB* allele in recent *V. cholerae* O1 strains. Retrospective analysis on strains also demonstrated that this Haitian *ctxB* first appeared in Kolkata during April 2006. Studies on pathogenic mechanisms revealed involvement and importance of the Entner Doudoroff pathway among *V. cholerae* O1 strains.

Work form this division also helped in successful development of a modified "cost effective" technique to quantify coliform and *E. coli* in different potable water sources. Presence of multidrug resistant diarrhoeagenic *E. coli* was identified in potable waters which

raised the concern on the potentiality of potable water sources as a vehicle and a potent diarrheal inducer in diarrhoea prone area.

Molecular epidemiology studies as well as assessment of risk factors associated with neonatal septicemia showed prevalence of ESBL-producing *Klebsiella pneumoniae* and *E. coli* with gene encoding the $bla_{CTX\text{-}M\text{-}15}$. The PFGE analysis of these isolates demonstrated a vast diversity of genotypes. Tigecycline was identified as a potent drug against most ESBL- or carbapenemase-producing *K. pneumoniae* and *E. coli*. Therapeutic usefulness of orally administered "phage cocktail" against *V. cholerae* infection in oral rabbit cholera model has been established in recent days. This was first time reported results of combatting *V. cholerae* infection in an animal model by cholera phages administered through the oral route. Studies focused on potential of the ethno-medicinal plant extracts as anti-typhoid and anti-diarrhoeal activates came out with some level of success. Analysis is in progress for isolation of active compound(s) responsible for anti-typhoid activity. Safety and efficacies of multi-serotype outer membrane vesicles (OMV) of *Shigella* spp. as potential vaccine against shigellosis has also been established by the work carried out in this division.

Worldwide actual occurrence of typhoid remains underreported due to absence of disease surveillance in resource poor settings and lack of suitable point of care diagnostic test. Therefore, development of rapid diagnostic test (RDT) for typhoid became the research priority. One indigenous serology based point of care dipstick test has been validated at NICED in field situation as a component of translational research, which showed promising result with 51.2% sensitivity, 85% specificity and 58% efficiency. Further improvement of the kit is under way.

Work on *Helicobacter pylori* as carried out in this Division at the molecular level helped in characterization of the strains isolated from 30 clinical cases. Results showed infections were caused by more than one strain and sometimes with 5 to 6 different types of genetic variants. Analyses of genetic loci helped in establishing micro-diversity among circulating strains isolated from single patient. All these pointed towards plausible events of recombinations during long-term carriage of the pathogen within the host. These results also suggested that most of the patients have acquired *H. pylori* due to repeated exposure to the pathogen with different genetic make-up and that may be responsible to increase severity and the possibility of super infections as well.

Work form this Division has also been taken further by the application to ICMR patents. These included i) A Herbal formulation for treatment of typhoid fever and preparation thereof and ii) A multiple outer membrane vesicles (MOMV) of shigellae as a novel candidate vaccine.

Considering the fact that huge numbers of gastrointestinal tract pathogens are being characterized routinely at this Division, the Gastrointestinal Tract Pathogens Repository (GTPR) has been established as National Facility through special support from Indian Council of Medical Research (ICMR), New Delhi. Goals included for this facility are to archive, maintain and providing scope for retrospective analysis on medically important pathogens. A web based support and dissemination of information is available from www.gtpr.org.in.

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Dr. S. Dutta, *Scientist 'F'*
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Dr. B.L. Sarkar, *Scientist 'E'*
Dr. R.K. Nandy, *Scientist 'D'*
Dr. A.K. Mukhopadhyay, *Scientist 'D'*
Dr. S. Basu, *Scientist 'D'*
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Prachetash Ghosh
Prasenjit Batabyal
Priyanka Ghosh
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Ritam Sinha
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Saswati Datta
Shravani Mitra
Soma Mitra
Somdutta Chatterjee
Subham Mookerjee
Sucharita Guin
Surojit Das
Taniya Golder
Tanusree Nag

Awards :

Mr. Tapas Patra, Mr. Goutam Chowdhury and Mr. Parijat Das received Ph. D. degree in Science of Jadavpur University.

Cholera in the Zanzibar Archipelago of Tanzania, Africa : evidence for the emergence of high cholera toxin producing El Tor variant *Vibrio cholerae*

Investigator : A.K. Mukhopadhyay

Cholera infection still continues to be a substantial health burden in developing countries, due to lack of proper hygiene and sanitation infrastructure, especially in Africa and Asia. There was no published report of cholera in Africa for more than a century until the disease struck western regions in 1970. It quickly spread and became endemic across much of the continent, killing hundreds of people each year. Zanzibar, an archipelago, consists of two major islands, Unguja (also named Zanzibar) and Pemba. They are situated in the Indian Ocean about 40–60 km off the eastern coast of mainland Tanzania having population of about 1.1 million. During 2008, an increased number of cases occurred in the United Republic of Tanzania, with 7700 cases reported compared with 2911 in the previous year (WHO 2009). Cholera's new global incursion in Haiti after its absence of almost 100 years and the rapidly growing genetic diversity among toxigenic *V. cholerae* strains with epidemic potential provided the impetus for molecular characterization of strains collected in Zanzibar in 2009. Among the 1,180 samples collected from patients with acute diarrhea, 268 samples were positive for *V. cholerae*. Serotyping results established that 247 of the total *V. cholerae* isolates belonged to Ogawa serotype and the remaining 21 isolates were non-O1 non-O139. PCR and sequenced based analysis showed that all the *V. cholerae* O1 strains contained the classical *ctxB* (Fig 1). It was found that all the El Tor variant stains from Zanzibar produced

significantly higher amounts of cholera toxin (CT) *in vitro* than most strains of prototype El Tor (Fig 2A). Western blot study using CTB specific monoclonal antibody also showed that the Zanzibar isolates produced classical CTB (Fig 2B). Pulsed-field gel electrophoresis analysis showed that the Zanzibar strains formed a homogeneous banding pattern (except one strain) and this pattern is different from Indian and other African strains isolated in recent times (Fig 3). Dendogram analysis indicated that the Zanzibar strains formed a separate cluster predicting its different lineage (Fig 3). These new *V. cholerae* O1 El Tor variant strains not only replaced the *V. cholerae* O1 El Tor prototype strains, but also turned out to be genetically stable and spread rapidly even to remote islands in the east African continent as evidenced from our study. Moreover, the severity of the disease appears to be intensifying, and recent cholera outbreaks in various places, including Zimbabwe and Haiti, have followed protracted period. An active holistic surveillance system should be in place in order to track the dissemination mode of the *V. cholerae* O1 El Tor variant strains in the population using latest molecular diagnostic assays, as these strains possess all the potentialities and foundation for a new pandemic.

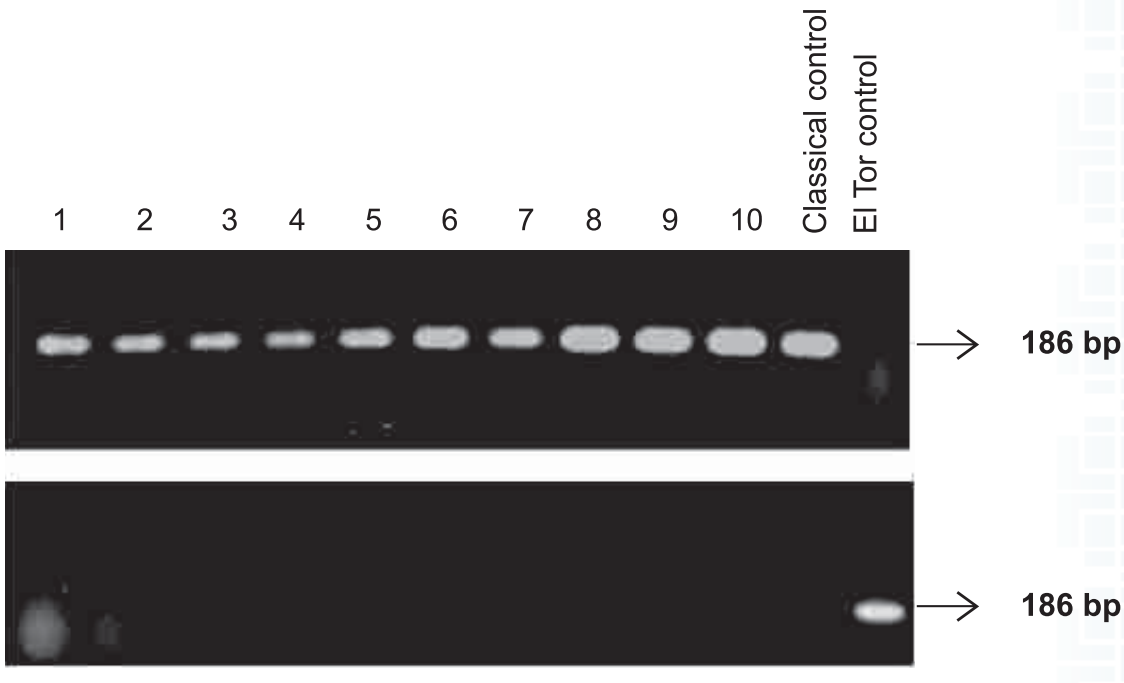

Fig. 1: PCR assay to detect the type of ctxB allele in representative Vibrio cholerae O1 strains isolated from Zanzibar, Africa along with the control strains, using primers (Fw-con/Rv-cla) for classical ctxB allele (Fig 1, upper panel) and Fw-con/Rv-elt for El Tor type ctxB allele (Fig 1, lower panel).

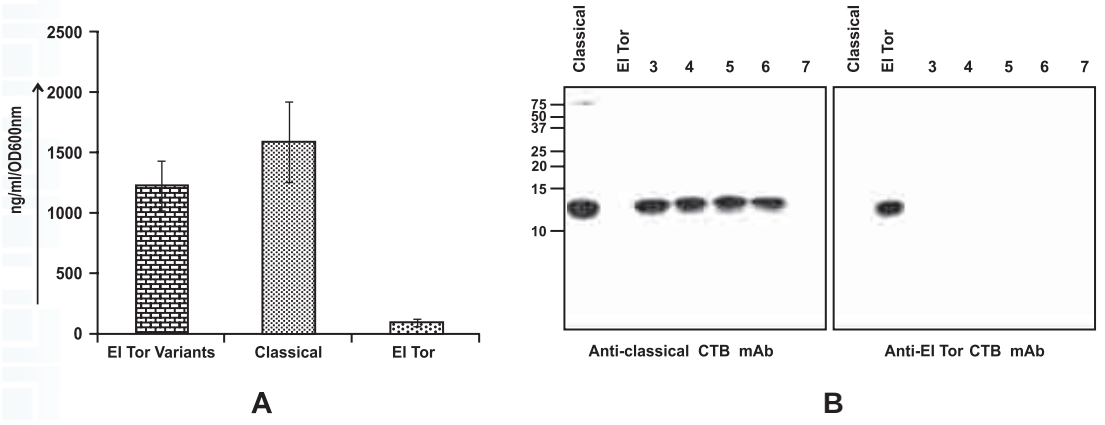

Fig. 2: (A) Amounts of cholera toxin production by Zanzibar variants, prototype El Tor strains and by classical strain. Error bars denote the standard error in taking each data in triplicate. (B) Western immunoblotting results of the culture supernatant of representative Zanzibar O1 isolates. 100 ng each of the purified classical CT (lane 1) and El Tor CT (lane 2) were used as positive controls for immunoblotting with the monoclonal antibody against classical and El Tor CTB, respectively. Lanes 3-6 represent Zanzibar strains, Lane 7: media (negative control). Numbers at left are molecular masses in kilodaltons.

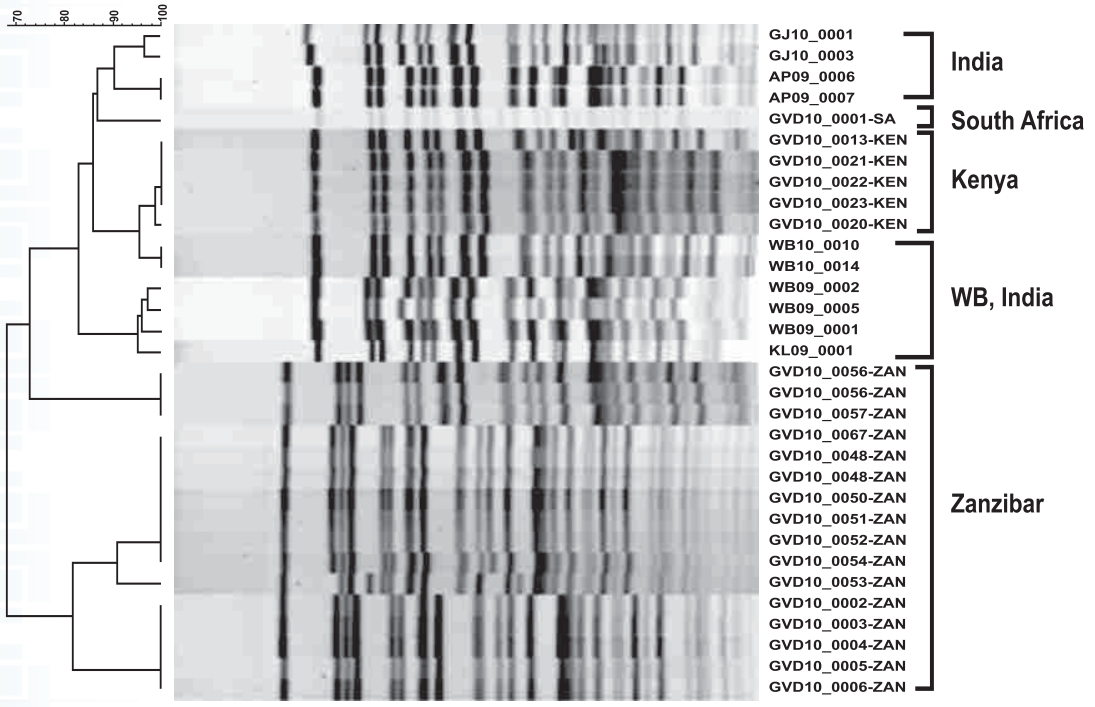

Fig. 3: PFGE patterns of the NotI digested V. cholerae strains from Zanzibar strains Dendogram analysis using Bionumeric software (Applied Maths, Sint-Martens-Latem, Belgium) shows three distinct clusters among the Zanzibar isolates tested. Sixteen representative strains were used for the study.

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

Physico chemical analysis

- Salinity varied between 28.5 ppt at the sea mouth to 0.1 ppt at 130 km inland with a seasonal as well as tidal variation. The total dissolved solids (TDS) ranges between 14.5–1380 mg/L. High pH value showed little oscillation between 7.8-8.2 at all the study sites. Water temperature and sediment re-suspension are two important physico-chemical parameters which can stimulate the bacterial growth and abundance in the aquatic environment.

Bacteriological analysis

- Total bacterial count (TBC) ranges between 1×10^2 to 5×10^9 at all the study sites. Cultivable *Vibrio* count (CVC) seems to be very high at Basonti (0.6×10^1 to 6×10^3cfu/ml) in comparison to Namkhana (0.3×10^1 to 3×10^3cfu/ml). Coliform contamination has been observed at all the sites (TCC) ranging between (200cfu/mL to 6500cfu/mL) with the highest value at Kolkata. Likewise, prevalence of TFCC could only be noticed in Kolkata site.

- At Diamond Harbour, during high tide, salinity as well as halophillic bacterial community increased while at low tide the lowest prevalence of all type of bacterial community except coliform bacteria.

- *V. cholerae* have been identified of which 93% of the isolates are found to be *V. cholerae* non O1. Only 7% *V. cholerae* O1 isolates have also been identified with the predominance of *V. cholerae* O1 Ogawa serotype. 33% of the isolates possess both *ctx* and *tcp* gene. Among those *ctx/tcp* positive *V. cholerae* isolates, other toxin regulatory genes *toxR, zot, RJ, LJ, ald* etc. have also been detected. El Tor type *ctx*B is predominant among the *ctx* positive *V. cholerae*.

- While isolation of *V. cholerae* O1 from higher saline zone (≥10ppt) was rare, isolation of toxic *V. cholerae* O1 during summer from Diamond Harbour (0.5-5ppt), in absence of fecal contamination, demarcates $>30^{\circ}$C water temperature, >600NTU turbidity and 2-5ppt salinity as core inducing factors enhancing genetic modification in the riverine ecosystem.

- Except a few *V. cholerae* O1 isolates (isolated from Howrah site), most of the *Vibrio cholerae* isolates were sensitive towards the conventional antibiotics. Although, 60% of total *V. cholerae* isolates were found to be β-lactam resistant. Multidrug resistant *V. cholerae* O1 isolates were identified to carry SXT integron.

- Apart from SXT integron (among *V. cholerae* O1), AmpC (*blaDHA*), *blaCIT* genes have also been identified among environmental *V. cholerae* O1, which can facilitate

gene transfer in a flowing aquatic environment.

- Except some non-O1 isolates, most of *V. cholerae* were devoid of plasmids which signifies the role of chromosomal DNA in β-lactamases production.
- Multidrug resistant *V. cholerae* in reivrine-estuarine sources reveal the natural gene transfer mechanism and transferable third generation drug resistant *V. cholerae* in flowing aquatic sources.
- Persistence of toxic *V. cholerae* O1 in chitinous exoskeleton surface of market crabs but neither in riverine crab nor in riverine-estuarine water sample highlights the possibilities of chitin induced horizontal gene transfer during dry condition under the continuous exposure of sun light, which has further been validated by in-vitro experiment.
- While more than 75% *V. cholerae* O1 showed biofilm formation capacity, only 20% *V. cholerae* non-O1/O139 showed their efficiency to the formation of biofilm.
- Surprisingly, most of the *V. cholerae* O1 strains isolated from Howrah showed higher bile salt tolerance capacity (< 15%) than that of other *V. cholerae* O1 and non-O1 isolates (< 10%).
- When all of the *V. cholerae* isolates were subjected to protease activity test, only the *V. cholerae* non-O1/O139 showed positivity than that of *V. cholerae* O1 isolates.
- DNA fingerprinting (PFGE and ERIC PCR) reveals that the *V. cholerae* non-O1/O139 have similarities with *V. cholerae* O1 isolates while they themselves are closely related.

Interpretation

- From this study it can be interpreted that prevalence of *V. cholerae* of riverine-estuarine ecosystem depends on physico-chemical (pH, turbidity, salinity etc.) hydrological (water current, tidal pressure etc.) and seasonal (water temperature, seasonal variation etc.) influences.
- While earlier studies have showed the seasonality of cholera incidence in Indian part of Gangetic delta with a peak in October, our present observation of abundance of toxic *V. cholerae* O1 at Diamond Harbour as well as at Howrah in summer followed by Kolkata during rainy season significantly tallies with the established cholera peaks.

Vibrio dynamics in riverine-estuarine ecosystem in West Bengal : cholera paradigm

Investigators : A. Palit and B. L. Sarkar

Results Obtained so far :

- Higher water temperature (31°C ±1.6°C), alkaline pH (≥7.5) are observed as the favorable conditions for *Vibrio* as well as Vibriopghage proliferation.

- 66 (45.2%) samples were found to be positive for *V. cholerae* O1 phage, of which 42 were from Howrah and 24 were from Diamond Harbour.
- Altogether 56/146 (38.3%) were harbouring *V.cholerae* O1, comprising of 49 (87.5%) Ogawa and 7(12.5%) Inaba, out of which, 34 samples were from Howrah and 22 from Diamond Harbour.
- Altogether, 19.8% samples have been identified along with the presence of both *V. cholerae* O1 and their phages. Simultaneously, 42.4% samples have been detected with the presence of either *V. cholerae* O1 or *V. cholerae* O1Φ.
- It has been well observed that the predominance of *V. cholerae* O1 and its phage varied inversely. While the preponderance of *V. cholerae* O1Φ was higher during the summer and winter months, disposition of *V. cholerae* O1 increased gradually to reach at its peak in rainy season followed.

Interpretation :

- Most of the vibriophages were identified from flood tide samples at both the sampling sites which signify the impact of tidal effect on the preponderance of *V.cholerae* as well as their phage community.
- Prevalence of *V. cholerae* O1Φ in riverine flow seems to control/restrict over the preponderance of *V. cholerae* O1.
- High turbidity seems to be an influential factor for growth and abundance of *Vibrio* sp. at a very low saline region.
- Late summer onwards, higher abundance of *V. cholerae* O1 than that of *V. cholerae* O1Φ is the key factor for the seasonal cholera out breaks.
- During the monsoon period the preponderance of *V. cholerae* O1 can easily be extrapolated on seasonal cholera outbreaks in this part of Indian subcontinent especially in and around the endemic belt of Kolkata metropolis, West Bengal.

Entner Doudoroff (ED) pathway and gluconate utilization system in *V. cholerae*

Investigators : R. K. Nandy and H. Koley

The Entner Doudoroff (ED) pathway is constituted by functional activities of the two genes *edd* and *eda*, encoding 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase. Involvement of this pathway has recently been shown to be most important in *V. cholerae* pathogenesis *in vivo* and its obligate involvement in gluconate (Gnt) utilization *in vitro*. Comparative genomic analysis predicted that *V. cholerae* Gnt utilization system is composed of genes for the ED pathway along with Gnt transporter (*gntU*) and regulatory element (*gntR*) including specific kinase (*gntK*). Bioinformatics approaches indicated that the *eda* and the *gntP* are in a single operon while other the *edd* and the *gntK* are in another operon, transcribe in opposite orientation and linked to each other with *gntR* in

vicinity. Such organization is unique in *V. cholerae* as compared to *E coli* and other enteric pathogens. Prediction also indicated regulatory role of the GntR to control expression of these operons.

Conditional gene silencing as well as in-frame deletion mutagenesis confirmed that the *gntP* functions as sole Gnt transporter in *V. cholerae*. Studies were extended further by characterization of the role of *gntR* of the predicted Gnt utilization system in *V. cholerae*. Deactivation of *gntR* by in frame deletion did not affect Gnt utilization by *V. cholerae* in M9 minimal media supplemented with Gnt. However, over expression of *gntR* from promoter-reporter fusion construct caused suppression of growth of *V. cholerae* in M9 medium with Gnt. All these suggested that GntR possessed negative regulatory function and this was initially predicted.

Characterization of pandemic and non-pandemic strains of *Vibrio parahaemolyticus* from an outbreak of diarrhoea

Investigators : T. Ramamurthy and A. Mukhopadhyay

Vibrio parahaemolyticus belonging to pandemic serovars have been associated with several outbreaks of diarrhoea all over the world. This pathogen has caused a large foodborne outbreak in West Bengal on June 22, 2011 due to food contamination. The outbreak peaked during the morning of June 22, 2011. All of the admitted diarrhoeal cases were treated intravenously or with oral rehydration fluids and, depending on severity, some patients were given divided dozes (400mg each) of norfloxacin. There were no fatalities associated with this outbreak. The three rectal swabs collected from the patients were positive for *V. parahaemolyticus* and no other enteric pathogen was detected. The *V. parahaemolyticus* isolates exhibited haemolytic activity on sheep blood agar but were urease negative. Serotyping determined by slide agglutination showed that one *V. parahaemolyticus* strain was identified as an O3:K6 serovar and the other two strains belonged to the O4:K8 serovar. The O3:K6 strain was PCR positive for the pandemic GS target gene (*toxRS*), confirming its similarity with the pandemic strains, but the serovar O4:K8 strains were negative for *toxRS*. All three strains harboured the *tdh*, but not the *trh* gene. Antibiotic susceptibility analysis showed that all the strains were resistant to ampicillin and streptomycin but susceptible to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, norfloxacin, and nalidixic acid. To determine the genetic relatedness among *V. parahaemolyticus,* O3:K6 (NICED 459) and O4:K8 strains (NICED 458 and NICED 460) isolated in this outbreak, four O3:K6 strains from the Kolkata outbreak that occurred in 2003 (SC 188, SC 189, SC 192, SC193), two strains of O3:K6 (IDH 03525), and O4:K8 (IDH 03062) from diarrhoeal patients from the Infectious Diseases Hospital, Kolkata isolated during in 2011 were compared in the pulsed-field gel electrophoresis. Cluster analysis showed that the recent outbreak O3:K6 *V. parahaemolyticus* strain possessed an identical profile with that of the IDH 03525 (O3:K6) strain, but its banding pattern differed from the 2003 outbreak strains. The current O4:K8 strains, on the other hand, were closely related but distinct from IDH 03062 (O4:K8) isolate. The O3:K6 and O4:K8 strains formed distinct serovar-related clusters (A and B); hence they are genetically different (Fig. 4).

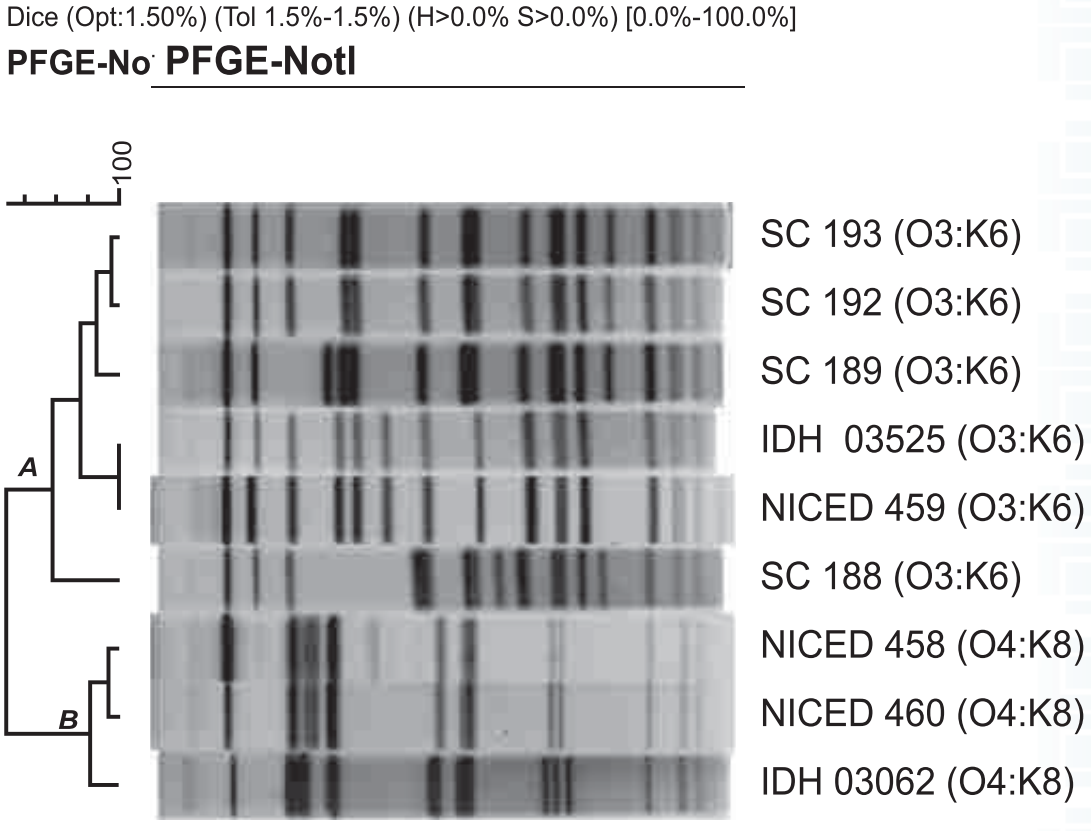

Fig. 4. NotI patterns of V. parahaemolyticus O3:K6 and O4:K8 strains. Strains SC188, 189, 192, and 193 were isolated during the outbreak in Kolkata, 2003 (cluster A). Strain NICED 458 and 460 was isolated during the outbreak in 2011, while IDH 03062 was the recent V. parahaemolyticus isolated from Kolkata in 2011 (cluster B).

Importance of *Vibrio fluvialis* as an enteric pathogen

Investigators : T. Ramamurthy, A. K. Mukhopadhyay, M. K. Bhattacharya, K. Rajendran and G. B. Nair

Vibrio fluvialis has caused sporadic cases and outbreaks of diarrhoea in several countries. The pathogenic mechanisms and the clinical importance of *V. fluvialis* are not well known. In this study, 400 strains identified as non-agglutinable vibrios (NAG) with *V. cholerae* O1 and O139 antisera that were collected during 2002–2009 from 11,904 stool specimens from patients with diarrhoea admitted to the Infectious Diseases Hospital, Kolkata. These strains were further confirmed as *V. fluvialis* by using a multiplex PCR targeting the *toxR* gene of *V. fluvialis* and the *ompW* gene of *V. cholerae*.

Among the 400 strains presumptively identified NAG vibrios, multiplex PCR confirmed 131 and 269 strains as *V. fluvialis* and *V. cholerae*, respectively. The overall prevalence rate of *V. fluvialis* among 11,904 hospitalized patients with diarrhoea was 1.1%. Abrupt appearance of *V. fluvialis* was identified in 2002. The isolation rate of *V. fluvialis* gradually increased from 0.7% in 2002 to 2.2% in 2009 (Table 1). Of the 131 strains of *V. fluvialis*, 43 (33%) were identified as the sole pathogen; the remaining 88 (67%) were isolated as a mixed pathogen with either *V. cholerae*, *V. parahaemolyticus*, *E. coli*, *Shigella* spp., parasites, or enteric viruses. *V. fluvialis* infection was more often detected in adults (73%) than in children <5 years of age (27%). Clinical symptoms of sole infection caused by *V. fluvialis* were similar to that of cholera: watery diarrhoea (86%), severe dehydration status (28%), and abdominal pain (12%) (Table 2). All the *V. fluvialis* strains were negative for the virulence genes commonly reported in *V. cholerae* and *V. parahaemolyticus*, but $>90\%$ were positive for genes encoding VFH and metalloproteases. More than 80% of the strains expressed haemolysin against rabbit and sheep red blood cells. These factors may increase the virulence of *V. fluvialis* and contribute to diarrhoea. When the culture filtrates were tested, cytotoxic effect was readily noticed in the Chinese hamster ovary and HeLa cell lines, i.e., cytoplasmic vacuolation, cell rounding, and destruction of the monolayer. In most strains isolated as a sole pathogen, the cytotoxic endpoint titer was 2–256.

In the antimicrobial susceptibility assay, *V. fluvialis* strains were highly resistant to ampicillin (92%), streptomycin (85%), furazolidone (85%), and sulfamethoxazole/trimethoprim (70%). About half the number of strains was resistant to ciprofloxacin and 45% to nalidixic acid. Although the *V. fluvialis* strains exhibited distinct *Not*I restriction profiles in the denrogroam analysis, at least 4 major clades were identified (Fig. 5). Clades A and B, with strains isolated during 2002–2007, exhibited less antimicrobial drug resistance than did clade C and D strains identified during 2008–2009; multidrug-resistant strains, especially those resistant to fluoroquinolones, were identified in higher numbers in clades C and D (Fig. 5).

Table 1. Prevalence of *V. fluvialis* among patients with diarrhoea, Kolkata

Year	No. of Samples	No. of V. fluvialis infection	Sole isolated (%)	Mixed infection
2002	2285	16 (0.7)	5(0.2)	11 (0.5)
2003	1673	8 (0.5)	1 (0.1)	7 (0.4)
2004	2430	19 (0.8)	6 (0.2)	13 (0.5)
2005	1472	17 (1.1)	7 (0.5)	10 (0.7)
2006	930	12 (1.3)	4 (0.4)	8 (0.9)
2007	842	9 (1.1)	2 (0.2)	7 (0.8)
2008	1124	24 (2.1)	8 (0.7)	16 (1.4)
2009	1153	26 (2.2)	10 (0.9)	16 (1.4)
Total	**11909**	**131 (1.1)**	**43 (0.4)**	**88 (0.7)**

No in parentheses indicate percentage

Table 2. Clinical features of *V. fluvialis* infected diarrhoeal patients

Clinical features		No of patients (%)	
		Sole	Mixed
Types of Diarrhoea	Watery	36 (84)	72 (81)
	Bloody mucus & loose	7 (16)	16 (19)
Dehydration Status	Severe	12 (28)	14 (16)
	Some & Rare	31(72)	74 (84)
Age	> 5 years	30 (70)	66 (75)
	≤ 5 years	13 (30)	22 (25)
Sex	Male	23 (53)	58 (66)
	Female	20 (47)	30 (34)
Fever	Yes	4 (9)	9 (10)
	No	39 (91)	79 (90)
Abdominal Pain	Yes	5 (12)	11 (12)
	No	38 (88)	77 (88)

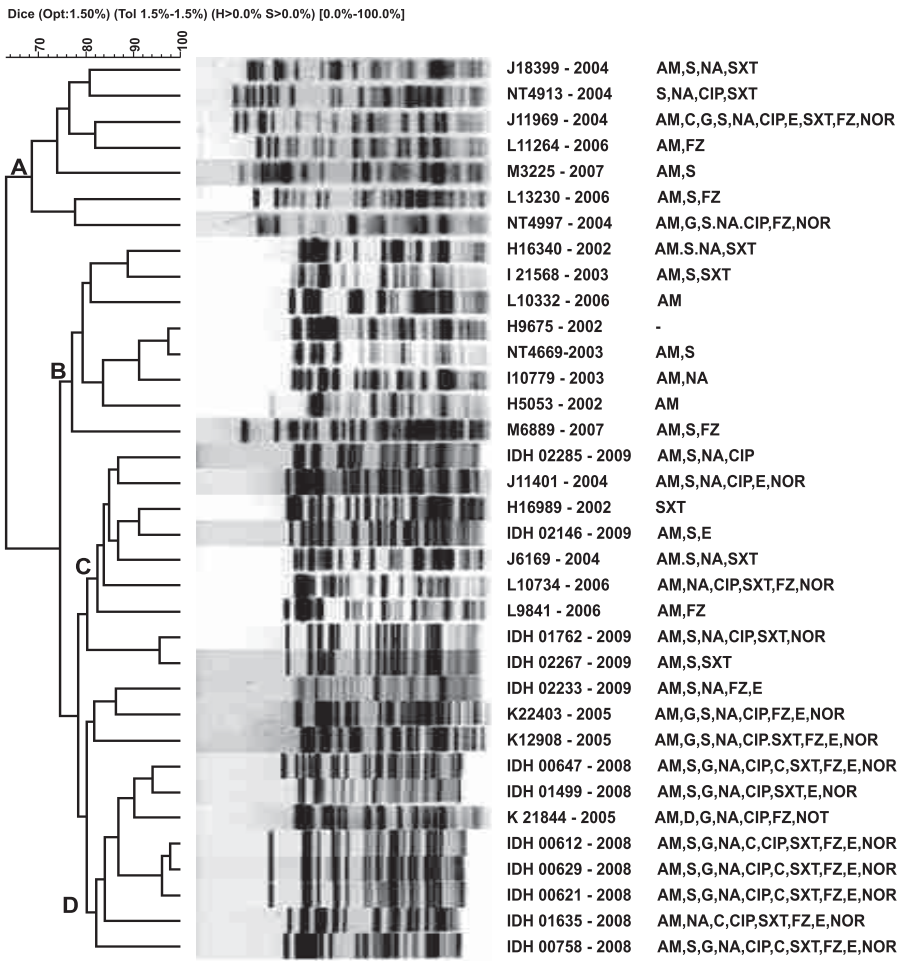

Fig. 5. Dendrogram of NotI-digested PFGE profiles of V. fluvialis strains. Clustering identified 4 clades (A–D). Abbreviations, AM, ampicillin; S, streptomycin; G, gentamicin; NA, nalidixic acid; CIP, ciprofloxacin; C, chloramphenicol; E, erythromycin; SXT, sulfamethoxazole/trimethoprim; FZ, furazolidone; NOR, norfloxacin. Scale bar indicates degree of similarity.

Molecular characterization of *Salmonella enterica* serovar Typhi isolates from Kolkata : antimicrobial resistance pattern and molecular subtypes

Investigators : S. Dutta, S. Das and U. Mitra

Early introduction of antibiotic therapy is the mainstay of typhoid treatment. Recent isolates of *S*. Typhi showed increased resistance to nalidixic acid (Na) (>98% isolates) and reduced susceptibility (≥80% isolates) to ciprofloxacin (Ci), the drug of choice, leading to the treatment failure of typhoid cases. Gradual increase in MDR (multi drug resistant i.e. resistant to chloramphenicol, ampicillin, cotrimoxazole) *S*. Typhi was noticed from 2009-10 (13.6%) to 2013 (25%) (Fig. 6) in contrast to the earlier observation on decline in MDR isolates from 50% in 1998 to 13% in 2005. High MICs of common antimicrobials and fluoroquinolone (FQ) group were obtained in recently isolated *S*. Typhi strains. All these isolates (100%) were susceptible to third-generation cephalosporin and azithromycin resulting in third-generation cephalosporin being used as the best treatment option for typhoid. A total of eight resistance patterns were observed among the Kolkata (n=77) isolates. Mechanisms of antimicrobial resistance (AMR) were investigated. Resistance to ampicillin, chloramphenicol and tetracycline was encoded by the presence of $bla_{TEM-1,}$ *catA* and *tetA* genes respectively. Co-trimoxazole resistance was determined by expression of the following genes like *sulI*, *sulII* and *dhfrI*a. Streptomycin resistant isolates harbored either chromosomal *strA*, *strB* genes or integron mediated *aadAI* gene. Class I integron was found in all *S*. Typhi MDR isolates by PCR with amplified band ranging from 700 bp to 1.3 kb in size.

MIC of Ci for any *S*. Typhi strain depends on the no. of mutation present within the QRDRs of DNA gyrase and topoisomerase IV subunit genes. Plasmid mediated quinolone resistance (PMQR) genes like *qnr* and *aac(6')-Ib-cr* were absent in *S*. Typhi Kolkata isolates. One heavy plasmid (180 kb) was present in all the MDR isolates. None of the plasmids belonged to the following incompatibility groups like incHI, FIA, FIB, FII, FIIS, A/C tested so far.

Molecular subtyping by PFGE (Fig. 7) did not show much variation (>85% similarity) among the Kolkata *S*. Typhi isolates. By PFGE typing the study isolates (n=77) could be discriminated based on their resistance profile.

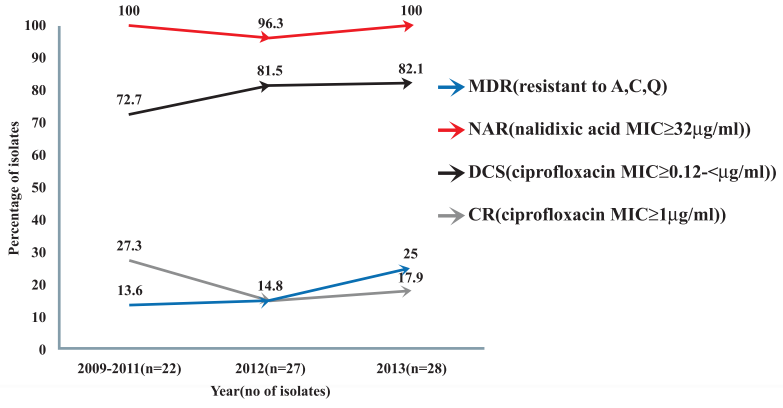

Fig. 6. Year wise percentage distribution of drug resistant S. Typhi Kolkata isolates (n=77) during 2009-2013. MDR, multidrug resistant i,e, resistant to ampicillin (A), chloramphenicol (C), cotrimoxazole (Q); NAR, nalidixic acid resistant; DCS, decreased susceptibility to ciprofloxacin; CR, ciprofloxacin resistant. All data are in %

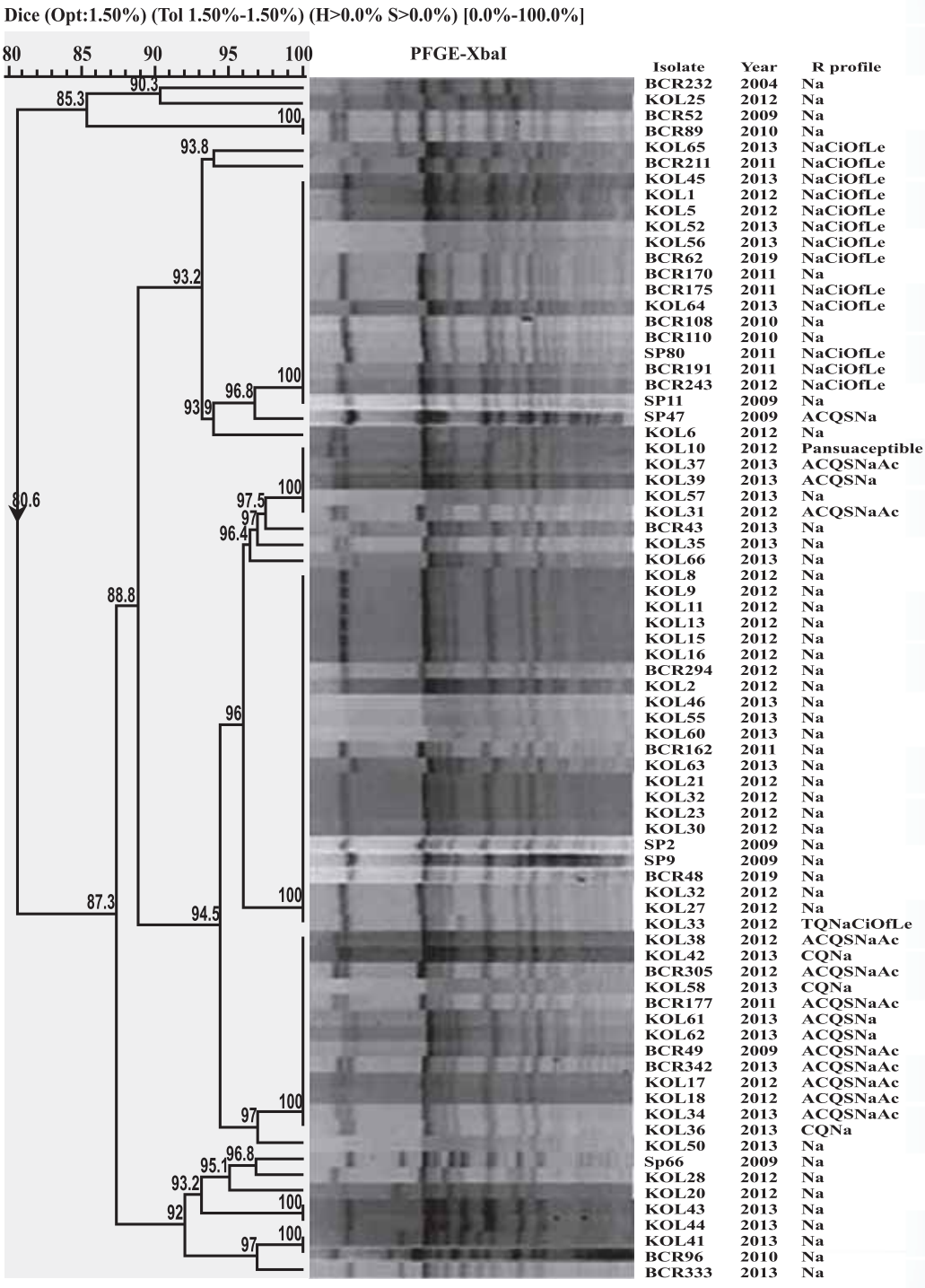

Fig. 7. PFGE profile of Xba 1 digested DNA of Salmonella Typhi isolates from Kolkata; R profile, resistance profile; Na, nalidixic acid; A, ampicillin; C, chloramphenicol; Q, cotrimoxazole; S, streptomycin; Ci, ciprofloxacin; Of, ofloxacin; Le, levofloxacin; Ac, amoxicillin clavulanic acid

Salmonella enterica serovar Weltevreden ST1500 associated foodborne outbreak in Pune India

Investigators : S. Dutta, P. Jain and R. Bharadwaj

Foodborne outbreak due to non-typhoidal *Salmonella* represents an important public health problem globally. Here we report one foodborne outbreak of gastroenteritis by *Salmonella enterica* serovar Weltevreden (*S*. Weltevreden) affecting 150 students in a hostel at Pune, India during January 2010. The students had consumed food comprising milk, rice, sprouted pulses and curd from their hostel canteen for lunch and almost all of them developed acute watery diarrhea within 12 hours of having food, associated with fever, abdominal cramps, nausea and vomiting. They were immediately admitted to the local private hospitals for treatment. Stool samples (n = 25) were collected from admitted patients and processed by standard microbiological method. A total of 9 isolates, provisionally identified as *Salmonella spp*. were sent to NICED, Kolkata for further confirmation, where the isolates were identified as *S. enterica serovar* Weltevreden.

The isolates were characterized w.r.t their antimicrobial susceptibility patterns, molecular types by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), plasmid profiling and plasmid typing. The Pune outbreak isolates were pan-susceptible to all common antimicrobials tested, possessed a single plasmid (63kb) of IncFIIS type, showed identical (100%) pulsotype (Fig 8) and belonged to sequence type (ST) 1500 by MLST. Clustering of the outbreak isolates suggested their spread from common source of origin, although the implicated food samples could not be collected for testing. On comparison, the Pune outbreak isolates and the *S*. Weltevreden outbreak strain 2007-60-3289-1 linked to alfalfa outbreak in Scandinavia, belonged to identical MLST profile (ST1500) and plasmid type indicating their common phylogenetic origin. This study highlighted extension of the *S*. Weltevreden outbreak strain 2007-60-3289-1 to the Indian subcontinent.

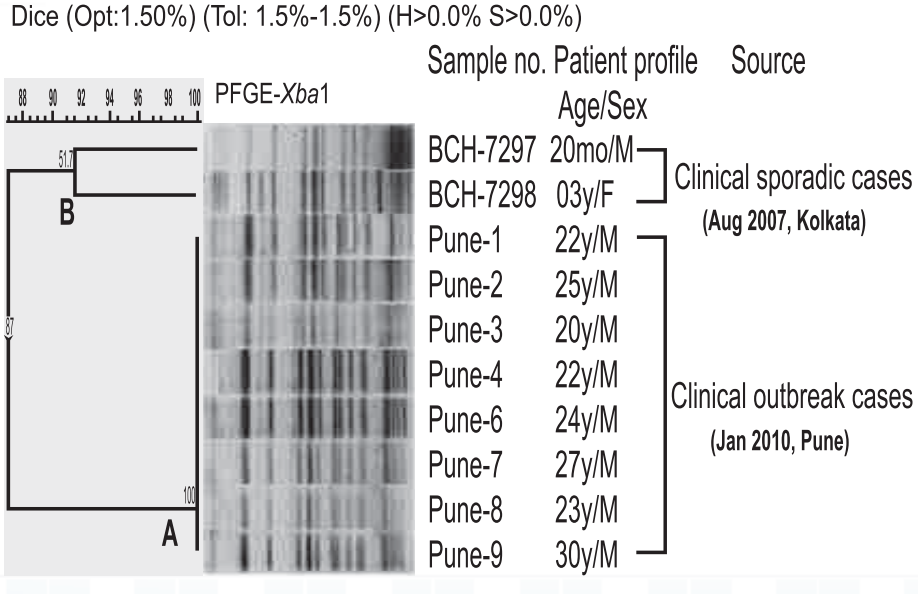

Fig 8: PFGE profiles of XbaI digested DNA of S. Weltevreden Pune outbreak isolates, by cluster analysis and comparison with sporadic S. Weltevreden Kolkata isolates

Validation of a new serology based dipstick test for rapid diagnosis of typhoid fever

Investigators : S. Dutta, S. Das and U. Mitra

Typhoid diagnosis still remains elusive due to lack of simple and suitable diagnostic test. One serology based dipstick test (IC-LFT), developed indigenously for bedside use, has been validated in this study. A total of 336 stored sera samples from fever patients attending hospitals for treatment with known culture result for *S.* Typhi were tested blindly by the Widal test and the new assay system. The analytical sensitivity, specificity of the assay was found 68.8% and 71.1% respectively considering culture positive samples as gold standards. Its analytical efficiency (70.5%) was significantly higher than that of the Widal and culture ($p < 0.001$) (Table 3). To determine the performance ability of the new assay in field situation, prospective sera samples from 102 hospital attending children with clinical diagnosis of typhoid fever (of which 20 confirmed non-typhoidal cases served as negative control) were tested blindly by three methods (Culture, the Widal and new assay) and compared statistically. The diagnostic sensitivity, specificity and efficiency of the new assay were observed 51.2%, 85.0% and 57.8% respectively. Overall performance was not significantly better than the Widal ($p > 0.5$) (Table 4). Studies are in progress to improve the performance of the new assay system before launching it as an efficient test for typhoid diagnosis in field set up.

Table 3: Analytical sensitivity and specificity of the new test (IC-LFT) in comparison with Widal on stored sera samples [a] (n = 336)

Tests	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)	Efficiency (%)
Widal ≥1:80)	50/80, 62.5 (51.6-72.3)	95/256, 37.1 (31.4-43.2)	50/211, 23.7	95/125, 76.0	145/336, 43.2 [b]
IC-LFT	55/80, 68.8 (57.9-77.9)	182/256, 71.1 (65.3-76.3)	55/129, 42.6	182/207, 87.9	237/336, 70.5 [b]

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value
[a], considering 80 blood culture positive cases as gold standard
[b], $p < 0.001$ using McNemer test.

Table 4 : Evaluation and comparison of performance indicators of blood culture, Widal and new test (IC-LFT) on prospective blood samples collected from clinically diagnosed typhoid fever cases [a] (n = 102)

Tests	Sensitivity (95% CI)	Specificity (95% CI)	PPV (%)	NPV (%)	Efficiency (%)
Blood culture	9/82, 11.0 (5.88-19.6)	20/20, 100.0 (NA)	9/9, 100.0	20/93, 21.5	29/102, 28.4 [b]
Widal (≥1:80)	36/82, 43.9 (33.7-54.7)	13/20, 65.0 (43.3-81.9)	36/43, 83.7	13/59, 22.0	49/102, 48.0 [c]
IC-LFT	42/82, 51.2 (40.6-61.7)	17/20, 85.0 (64.0-94.8)	42/45, 93.3	17/57, 29.8	59/102, 57.8 [b, c]

[a], considering 82 clinically diagnosed typhoid cases as true positive and 20 lab confirmed non-typhoid cases as true negative.
[b], $p < 0.001$ using McNemer test.
[c], $p > 0.5$ using McNemer test.

Multi-serotype outer membrane vesicles of Shigellae confer passive protection to the neonatal mice against shigellosis

Investigator : H. Koley

Recently, we have demonstrated, immunization of adult female mice with outer membrane vesicles (OMVs) of Shigella boydii type 4 protected their offspring passively from shigellosis. In our present study, we have advanced our research by formulating multi-serotype outer membrane vesicles (MOMVs), mixing the OMVs of S. dysenteriae 1 stx, S. flexneri 2a, S. flexneri 3a, S. flexneri 6, S. boydii type 4 and S. sonnei to achieve a broad spectrum protection against shigellosis. Adult mice were immunized orally with 50μg of MOMVs, four times at weekly intervals. Immunological parameters were observed at various time points, before, during and after immunization, in adult mice. Western blot analysis of the immunized sera against WCL has detected various proteins, present in the OMVs that were immunogenic in adult mice. Some antigens are more immunogenic than others, as indicated by a massive response directed against proteins between 30 to 45 KDa.

IpaB (62 KDa), IpaC (42 KDa), the major translocator proteins and the effector molecule IpaD (38 KDa) were also found to be immunogenic in adult mice immunized with S. flexneri 2a and S. boydii type 4 outer membrane vesicles (Fig: 9). Passive protection was examined in their offspring by measuring protective efficacy and studying intestinal colonization, after challenging with various Shigella strains. Immunized dams exhibited a consistent broad spectrum antibody response. Three to four day-old offspring of immunized dams showed significant long term passive protection against wild type S. flexneri 2a, 3a, 6, S. boydii type 2 and S. dysenteriae 1 (Fig. 10). Their stomach extracts, essentially containing mother's milk, have also exhibited significant levels of anti-MOMVs immunoglobulins.

In conclusion, MOMVs formulation represents an easy, safe immunization strategy that was found suitable to provide complete passive protection to the neonatal mice against all four serogroups of Shigellae. It could be exploited for the development of a novel non-living vaccine against human shigellosis in near future.

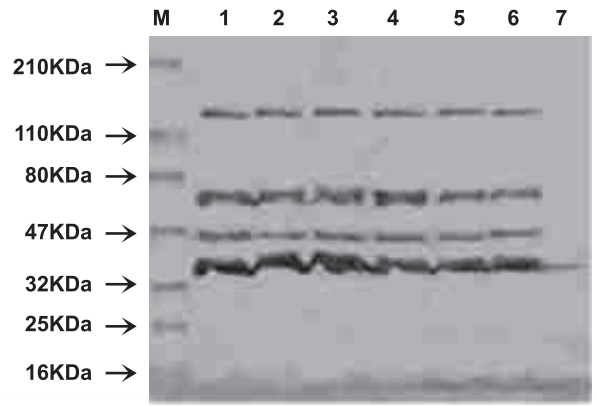

Fig. 9. Representative Immunoblot against WCL of seven Shigella strains probed with 28th day's anti-MOMVs serum from a single mouse. Lane M: prestain molecular weight marker (Pierce, USA) Lane 1: S. dysenteriae 1; Lane 2: S. flexneri 2a; Lane 3: S. flexneri 3a ; Lane 4: S. flexneri 6; Lane 5: S. boydii type4 ; Lane 6: S. sonnei ; Lane 7: non invasive strain S. flexneri 1a.

Fig. 10. Newly developed multi-serotype (hexavalent) outer membrane vesicles formulation an ultimate broad spectrum non-living vaccine candidate that conferred long term passive protection to neonatal mice against shigellosis.

Studies on the transmissible carbapenemase genes and non-transmissible efflux pumps

Investigator : S. Basu

Antibiotic resistance is a global problem that limits theurapeutic options immensely, particularly in neonates. The studies in the laboratory during this period have focused on carbapenem resistance in predominant gram negative bacteria that cause neonatal infections or colonize the neonatal gut.

The emergence of carbapenem resistance in Enterobacteriace and the identification of the potent carbapenemase, NDM-1 (New Delhi metallo-β-lactamse), has necessitated further studies in this area. We retrospectively studied the carbapenem resistance in Enterobacteriacae for a five year period in an attempt to understand the emergence and the diversity of these isolates. The increase in MIC values for carbapenem could be attributed to NDM-1. *Co-resistance to other antibiotics, like aminoglycosides, fluroquinolones were also addressed.* The association of two novel β-lactamases, SHV-type β-lactamases (SHV-167) and AmpC-type-β-lactamase (ACT-16) in two NDM-1 carrying *Enterobacteriaceae* were also noted. *The diversity of the genetic features associated with the* bla_{NDM-1} *gene could be established.*

In a situation with dwindling options of antibiotic therapy, mainly due to the emergence of carbapenem resistance, tigecycline is an alternative. We investigated the trend of tigecycline susceptibility during a period when carbapenem resistance in Enterobacteriaceae due to the presence of NDM-1 emerged in this unit. Resistance to tigecycline remained low in neonatal bloodstream infections during the study period (Fig. 11). Resitance to tigecycline in clonally distinct isolates of *K. pneumoniae* could be attributed to over expression of *ramA* and AcrAB-TolC pump proteins (Fig. 12). Nonsusceptibility to tigecycline was not observed among carbapenem-resistant NDM-1 possessing isolates in this setup.

In summary, horizontal transfer of NDM-1 has important implications in neonatal infections. The ability to harbor other resistance genes and transmit them along with NDM-1 is an important strategy to spread resistance to other groups of antibiotics. Tigecycline however retains its potency against carbapenem-resistant isolates.

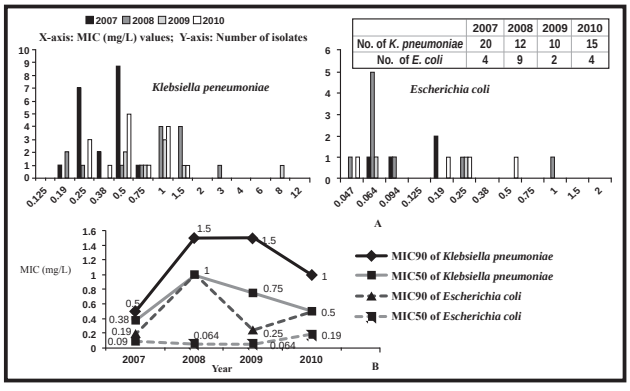

Fig. 11. Distribution of tigecycline MIC (A), MIC_{50} and MIC_{90} values (B) among Klebsiella pneumoniae and Escherichia coli as determined by the E-test method.

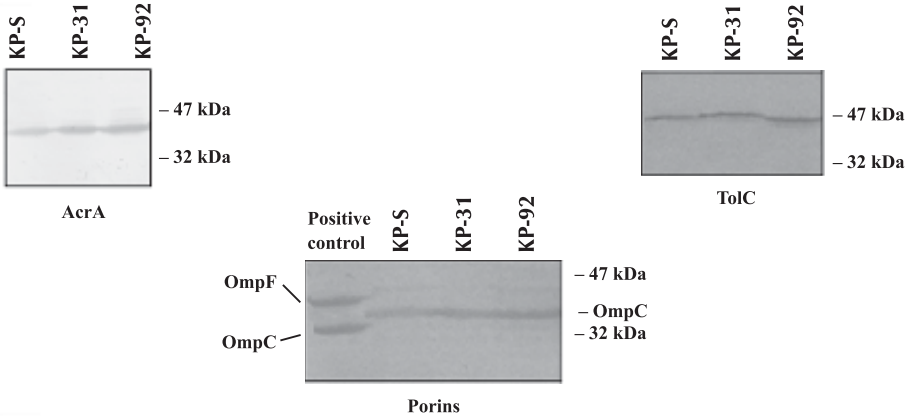

Fig. 12. Western blot analysis to detect the expression levels of AcrA, TolC and porins (OmpC/F) in tigecycline-susceptible (KP-S) and tigecycline-resistant (KP-31 & KP-92) Klebsiella pneumoniae isolates.

Multiple infections of *Helicobacter pylori* in a single host in India

Investigator : A. K. Mukhopadhyay

Helicobacter pylori has a remarkable ability to establish infections in human stomachs lasting for decades and the asymptomatic infection may lead to unwanted outcomes including peptic ulcers and gastric cancer. *H. pylori is* one of the most diverse bacterial species that chronically infects more than 70% of Indian population. Studies from Europe and Western countries showed that almost all strains of *H. pylori* isolated from different sites in the stomach of individual patients show homogeneous DNA profiles. In contrast, Mexican and Chinese populations are infected with genetically heterogeneous strains with high infection rates. In India, the prevalence of *H. pylori* infection is high and the chances of infection and re-

infection of strains in single host is relatively more as compared to the Western populations. But no investigation has been undertaken in India to determine the genetic types of different *H. pylori* strains from a single host. In addition, data showing microdiversity of the *H. pylori* strains within a particular gastric niche remained scarce. The present study was aimed to examine the genetic diversity of *H. pylori* strains from Indian patients. To understand the extent of genetic diversity among *H. pylori* strains within a given host, patients with gastro-duodenal problems were subjected to endoscopy and from each patient 10 single colonies were isolated. Characterization of each of these 10 single colonies by DNA fingerprinting as well as genotyping of several important genetic markers viz. *cagA, vacA, iceA*, *vapD*, *cag* PAI empty site, IS605, RFLP and two other genetic segments within *cag* PAI revealed that all the patients were infected with more than one strain and sometimes strains with 5 to 6 types of genetic variants (Figs. 13, 14). Analyses of certain genetic loci showed the microdiversity among the colonies from single patient, which may be due to the recombination events during long-term carriage of the pathogen (Figs. 15, 16). These results suggest that most of the patients have acquired *H. pylori* due to repeated exposure to this pathogen with different genetic make-up, which may increase the possibility of super infections. Genetic exchanges between these unrelated *H. pylori* strains may support certain *H. pylori* variant to grow better in a given host than the parental strain and thereby increasing the possibility for the severity of the infection. The most probable place for genetic recombination is human gastric mucosa and it is possible that during the long-term colonization the *H. pylori* strains may undergo adaptive changes and eventually become significantly different from the ancestral genotype.

In India, prevalence of *H. pylori* infection is much higher as compared to the most western countries and almost all infected cases were found to carry multiple *H. pylori* strains. This heterogeneity of *H. pylori* population is clinically very important as the exiting practice of characterization of single isolate from infected individuals may oversight the appropriate target of virulence gene or its alleles. In addition, due importance should be given to the patchy distribution of *H. pylori* throughout the gastric mucosa, as different genotypes may predominate at different sites. Our finding strengthens the view of including multiple colonies from a single host either from single or different sites in the case of multi ulceration.

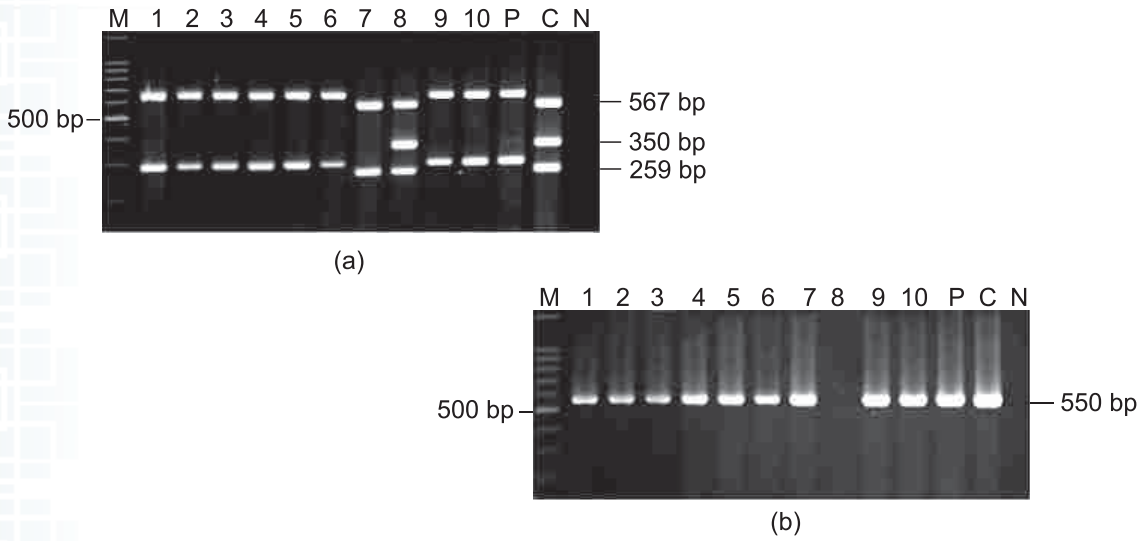

Fig. 13 . Multiplex PCR for vacA subtypes, cagA and cag PAI empty site for the absence of cag PAI. M, 100 bp marker; lanes 1-10, single colonies isolated from PG207; C, 26695 for the first set (a) and AM1 (cag PAI negative strain) for the second set (b); N, Negative control (E. coli DNA). (a) Multiplex PCR showed this particular patient was infected by at least three different strains. Lanes 1-6 and lanes 9-10 showed existence of s2m2cagA$^-$ strains, lane 7 showed existence of s1m1cagA$^-$ strain and lane 8 showed existence of s1m1cagA$^+$ strain. (b) All the single colonies, which failed to produce amplicon for cagA gene, yielded ~550 bp product for cag PAI empty site. The colony (Lane 8) that produced amplicon for cagA did not show any amplicon with primers for cag PAI empty site.

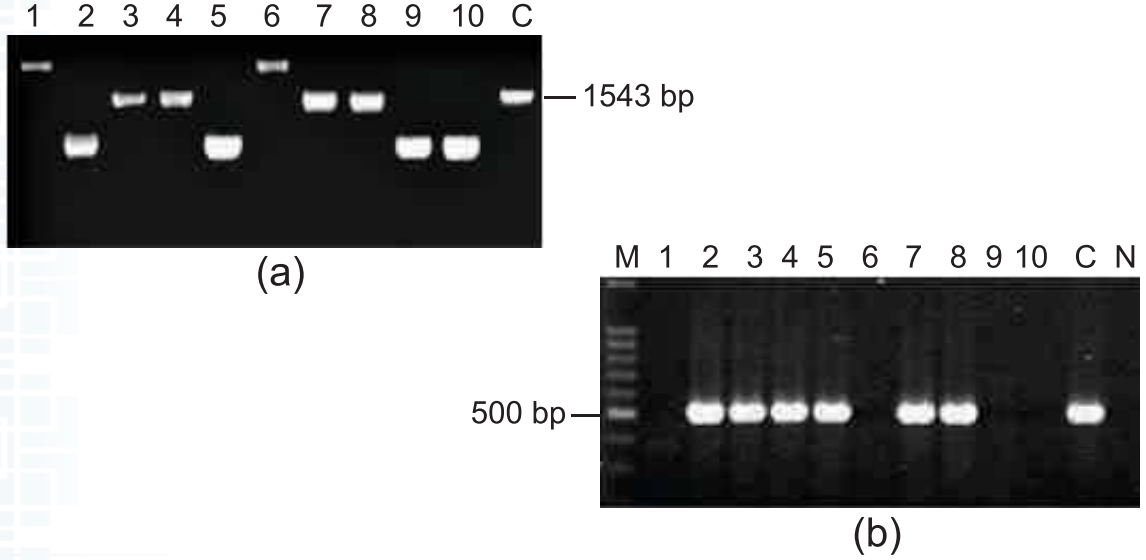

Fig. 14. Multiple strain colonization detected on the basis of HP0527 gene in cag PAI. Lanes 1 to 10, single colonies isolated from the patient; C, Control strain 26695. (a) Three types of colonies were identified in PG93. Lane 1 and 6 gave a higher amplicon than that of 26695; lanes 3, 4, 7 and 8 yielded same amplicon while lanes 2, 5, 9, 10 produced lower amplicon than that of 26695. (b) Mixed infections detected on the basis of vapD genetic locus PCR. M, 100 bp marker; lanes 1-10, single colonies isolated from PG137; 11, positive control (PG225); 12, Negative control (E. coli DNA). All the colonies are positive for vapD except colony numbers 1, 6, 9 and 10.

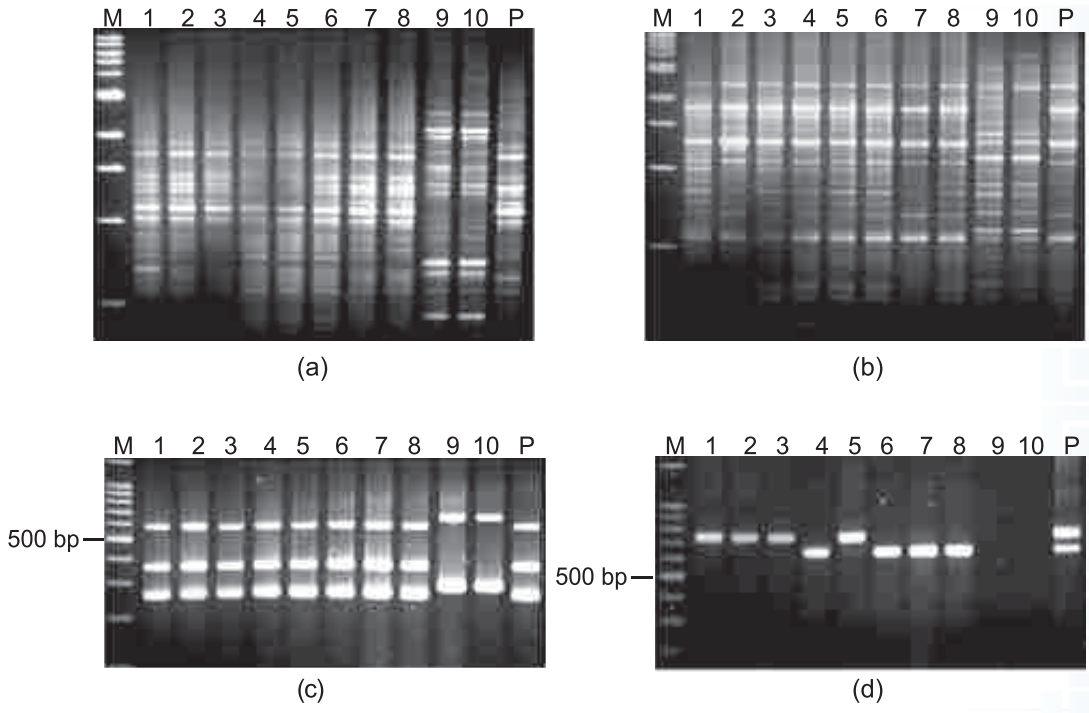

Fig. 15. Combination of genotype and RAPD analysis for PG157 indicated multiple infections and microdiversity in a single host. M, 100 bp marker; lanes 1-10, single colonies isolated from PG157; P, pooled DNA. (a) RAPD patterns using primer 1281 showed two distinct patterns (lanes 1-8 and lanes 9-10) (b) RAPD patterns using primer 1283 also yielded two distinct patterns (lanes 1-8 and lanes 9-10) (c) Multiplex PCR for vacA alleles and cagA showed existence of s1m1cagA$^+$ strains in lanes 1-8 and s2m2cagA$^-$ strains in lanes 9-10. (d) Variant cagA subtypes detected on the basis of PCR for 3' end of cagA using primers CAG1 and CAG2. This PCR assay showed existence of type A strains in lanes 4, 6-8 and existence of type B/D strains in lanes 1-3 and 5. Lanes 9-10, which were detected as cagA$^-$, did not produce any amplicon and the pooled sample yielded amplicons for type A and type B/D strains.

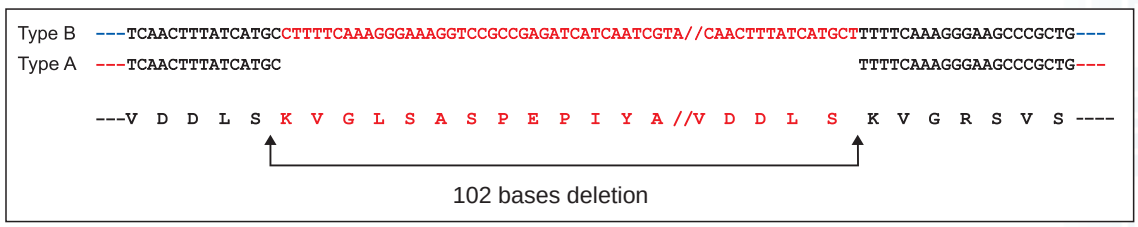

Fig. 16.Sequencing analysis of both Type A and Type B cagA 3'end amplicon of PG157 showed the deletion of 102-bp in type A strains, which contained one "EPIYA" motif.

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

Investigator : B. L. Sarkar.

Phage typing is the ongoing activity of this institute. The strains of *V. cholerae* sent to us from different institutes across the country. A total of 703 strains of *V. cholerae* were received from different parts of the country during the current year for serotyping, biotyping and phage typing. All the 703 (100.0%) strains were 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 681 (96.87%) followed by Inaba 22 (3.23%). A total of 9 (1.28%) 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 (59.03%) followed by type 26 (8.3%), 23 (6.8%), type 13 (5.54%), 12 (0.14%) respectively. It has been shown that type 27 was the predominant phage type circulating in this country. During the current year, we did not receive a single strain of *V. cholerae* O139 for phage typing study from any parts of the country.

Biotype, Serotype and Phage type of *V. cholerae* strains received during the year 2012 - 13

State	No of Strains	Biotype		Serotype		Basu & Mukherjee			New Phage										
		El Tor	Classical	Ogawa	Inaba	T-2	T-4	UT	4	7	12	13	14	16	19	21	23	24	26	27
New Delhi	77	77	-	73	4	70	4	3	-	-	-	5	-	-	3	6	5	4	9	42
Andhra Pradesh	66	66	-	65	1	65	1	-	-	-	-	7	-	-	4	5	6	5	8	31
Gujrat	121	121	-	121	-	118	2	1	4	-	1	4	-	3	6	4	5	7	6	80
Karnataka	13	13	-	13	-	10	3	-	-	-	-	-	-	-	1	-	3	1	2	6
Maharashtra	229	229	-	220	9	215	11	3	-	2	-	13	-	-	9	14	15	11	25	136
Madhya Pradesh	7	7	-	7	-	7	-	-	-	-	-	2	2	-	1	-	-	1	-	1
Tamil Nadu	67	67	-	65	2	60	5	2	-	-	-	3	3	-	7	5	6	7	5	29
Punjab	93	93	-	87	6	86	7	-	-	-	-	5	-	1	5	7	8	4	3	60
West Bengal	30	30	-	30	-	30	-	-	-	-	-	-	-	-	-	-	-	-	-	30
Grand Total	703	703		681	22	661	33	9	4	2	1	39	5	4	36	41	48	40	58	415
Total %		100	-	96.87	3.23	94.02	4.7	1.28	0.56	0.28	0.14	5.54	0.71	0.56	5.12	5.8	6.8	5.68	8.3	59.03

Retrospective analysis of toxigenic traits of *V. cholerae* received for phage typing.

Investigator : B. L. Sarkar, A. Palit and H. Koley

Salient Observations :

1. A total of eighty one strains of *V. cholerae*, biotype El Tor were included from 1990-2005 from seventy different geographic location. We intend to include from 1990 to 2012 in future.

2. All except one of these strains were found to be Ogawa.
3. Majority of the strains were clustered with Type 27 of the new phage typing scheme.
4. 6.25% of strains were found to be sensitive for all the ten number of antibiotics tested.
5. Of these, 86.25% of these strains were found to be resistant against S (Streptomycine) and SXT (Trimethoprim/Sulfamethoxazole).
6. The study is underway.

Awards and Honours

T. Ramamurthy

- Elected as Fellow of Indian National Science Academy, New Delhi

S. Dutta

- Nominated principal member of the Drinks and Drinking water Sectional Committee, FAD 14 of Bureau of Indian Standards, Ministry of Consumer affairs, Food & Public distribution, GOI
- Acted as an invited reviewer for following international/National journals/ WHO Guidelines (WHO Guidelines on the quality, safety and efficacy of Vi polysaccharide conjugate typhoid vaccine, Diagnostic Microbiology and Infectious Diseases, Journal of Antimicrobial Chemotherapy, Journal of Applied Microbiology, BMC infectious diseases-a BMC series journal, PLoS, International Journal of infectious Diseases, and Indian Journal of Medical Research.
- Acted as a reviewer for the following funding agencies to give comments on the submitted research proposals (DST, M/O Science and Technology, Govt. of West Bengal, Central Drugs Standard Control organization, DGHS, M/O Health and FW and STS, ICMR.
- Awarded one ICMR patent on "A Herbal formulation for treatment of typhoid fever and preparation thereof"- The Appl. no. 2775/DEL/2012 dated September 6, 2012

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.
- Invited and chaired as Chairperson plenary lectures at UGC-DST sponsored National Seminar on : Biotechnology for people: Application and awareness"; P.D.Women's College, Jalpaiguri, West Bengal, December 4-5,2012
- Invited and delivered an invitational lecture on "Aquatic environment, biogeoecology and seasonality: Vibrio paradigm" at CSMCRI (CSIR), Bhavnagar, Gujarat, August 6, 2012.

B.L. Sarkar

- Recipient of "Life Time Achievement Award" by Indian Association of Applied Microbiologists at SRM University, Chennai on December 17, 2012.

A.K. Mukhopadhyay

- Editorial Board Members of World Journal of Gastrointestinal Pharmacology and Therapeutics (2009-2013) and Gut Pathogens

S. Basu

- Selected as a Young Investigator at the Symposium on Probiotics in Prevention of Lifestyle Disorders. Bengaluru, India. December 15-16, 2012.

Conferences/ Seminars/ Workshop /Training Attended/ organized

T. Ramamurthy

- Attended the 8th PulseNet Asia Pacific Strategic Planning Meeting from November 5-6, 2012, Shenzhen, China for an oral presentation.
- Attended Consultation workshop for Foodborne Infection from February 11-12, 2013 at National Centre for Disease Control, New Delhi.
- Organized WHO-CDC-ICMR supported GFN Course for Microbiologists and Epidemiologists was held from February 14-16, 2013 in NICED, Kolkata.

S. Dutta

- Participated in the VII th Annual State conference of IAMM (Indian Association of Medical Microbiologists), WB Chapter on 16 Septmber 2012, held at Calcutta National Medical College, Kolkata.
- Delivered a talk on *"Diagnosis of Typhoid fever: An enigma yet to be solved"* in the 100th meeting of Indian Science Congress Association (ISCA 2013) held at Kolkata on January 3-7, 2013.
- Posters Presentation on "*Genetic characterization of Salmonella Typhi isolated from typhoid cases attending Hospitals in Kolkata*" and "*Transferable third generation cephalosporin and fluoroquinolone resistance in non typhoidal Salmonella isolated from neonates in Kolkata"* at the 100th meeting of Indian Science Congress Association (ISCA 2013) held at Kolkata on January 3-7, 2013.
- Participated in the Cochrane workshop held at NICED, Kolkata on 5 July 2012 organized by Wiley Blackwell, India.
- Attended one Indo Russian workshop of "The sub working group on Medical Research" organized by ICMR, New Delhi, DST, Govt. of India and held at New Delhi on November 21, 2012 and delivered a talk on "*Characterization of Salmonella Typhi and non typhoidal isolates from environment and clinical samples in and around Kolkata, India*".

A. Palit

- Organized Training program for Final year BHMS students from national Institute of Homeopathy, Kolkata, orientation training programme, August 31, 2012.
- Organized Training Programs 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 program schedules.
- Organized SAC meeting on August 24-25, 2012 at NICED, Kolkata.
- Invited & participated in Consultation meeting for collaborative project prospective between scientists of NICED (ICMR) and CSMCRI (CSIR), Bhavnagar, Gujarat, August 5-9, 2012.

- Invited and participated in UGC-DST sponsored National Seminar on : Biotechnology for people : Application and awareness"; P.D.Women's College, Jalpaiguri, West Bengal, December 4-5, 2012.
- Delivered Plenary lecture "Aquatic Environment, bio-geo-ecology and seasonality in southern deltaic West Bengal, India : *Vibrio* paradigm" in UGC-DST sponsored National Seminar on : Biotechnology for people : Application and awareness"; P.D.Women's College, Jalpaiguri, West Bengal, December 4-5, 2012.

B. L. Sarkar

- Delivered invited talk on "Cholera and cholera bacteriophage" at CSRI-CSMCRI, Bhavnagar on November 29, 2012.
- Delivered lecture entitled "Bacteriophage : A silent player in the environs" at the seminar Biotechnology for people: application and awareness" organized at P. D. Women's college, Jalpaiguri on December 4, 2012.
- Delivered invited lecture on "Cholera and cholera Bacteriophages : A recent development" at SRM University, Chennai on December 17, 2012.

R. K. Nandy

- Attended the 4th Annual meeting of the Global Network on Malnutrition and Enteric Diseases (MAL-ED) and presented the laboratory aspect of the project proposal on "Exploration of Biological Basis of Underperformance of Oral Polio and Rota Virus vaccine in India" in Baltimore, USA from May 16- 17, 2012.
- Oral presentation at the 47th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections organized by US-Japan Cooperative Medical Sciences; Chiba University, Japan during December 12-14, 2012.
- Attended Centenary Session of Indian Science Congress at Kolkata, India during January 3-7, 2013.

A. K. Mukhopadhyay

- Presentation of the work entitled "Genetic Attributes of Indian *Vibrio cholerae* strains: Evidence for the genesis of Haitian variant".in the "Annual Asia-African Research Forum" organized by the Japan Initiative for Global Research Network on Infectious Diseases held in Tokyo, Japan during January 23-24, 2013.
- Presentation of the work entitled "Genetic traits of Indian *Vibrio cholerae* strains: Clues for the genesis of Haitian variant" in the 47th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections at Chiba, Japan during December 12-14, 2012.
- Presentation of the work entitled "Genesis and route of spread of Haitian variant strains of *Vibrio cholerae*: Evidence from Kolkata and Delhi, India" in the 34th Naito conference organized by the Naito Foundation held in Sapporo, Japan during October 16-19, 2012.
- Attended Consultation workshop for Foodborne Infection from February 11-12, 2013 at National Centre for Disease Control, New Delhi.

- Organized WHO-CDC-ICMR supported GFN Course for Microbiologists and Epidemiologists during February 14-16, 2013 in NICED, Kolkata.

S. Basu

- Poster presentation on "Diverse clones of Carbapenem resistant *Klebsiella pneumoniae* causing neonatal septicemia" in Microcon, 2012, New Delhi, India, November 22-25, 2012.
- Delivered invited lecture on "Neonatal Sepsis – The Gut Connection" at Probiotics in Prevention of Lifestyle Disorders, Bengaluru, India, December 15-16, 2012
- Delivered invited lecture on "Carbapenem Resistance and its Impact on Neonatal Health in a Developing Country" at 100th Indian Science Congress, Kolkata, January 3-7, 2013.

H. Koley

- Poster presentation on "Multi-serotype Outer Membrane Vesicles of *V. cholerae* confer passive protection to the neonatal mice" at United States-Japan Cooperative Medical Science Program, Chiba, Japan during December 12-14, 2012.
- Poster presentation on "Multiserotype outer membrane vesicles of Shigellae confer passive protection against shigellosis in neonatal mice" at 100th Indian Science Congress, Kolkata, India during January 3-7, 2013.
- Poster presentation on "Immunogenicity and protective efficacy of Oral Heat-killed Multi-serotype Shigella (HKMS) vaccine in Rabbit model at 100th Indian Science Congress, Kolkata, India during January 3-7, 2013.
- Poster presentation on "Metallo and Hemagglutinin proteases of *Vibrio cholerae* Regulate Motility and Intestinal Colonization" at 100th Indian Science Congress, Kolkata, India during January 3-7, 2013.
- Delivered invited lecture on "Oral Live Transconjugant Shigella Vaccine" at 100th Indian Science Congress, Kolkata, India during January 3-7, 2013.
- Oral presentation on "Immunogenicity and protective efficacy of Oral Heat-killed Multi-serotype Shigella (HKMS) vaccine in Rabbit model" at annual Asia-African Research Forum" organized by the Japan Initiative for Global Research Network on Infectious Diseases to be held in Tokyo, Japan during January 23-24, 2013

BIOCHEMISTRY

The Division of Biochemistry primarily focuses on in-depth understanding of the molecular mechanisms of pathogenesis of diarrheal diseases using biochemical and biophysical approaches. The molecules of interest are *Vibrio cholerae* cytolysin / hemolysin (VCC), chitinases, a chitin-binding protein and colonization factors of enterotoxigenic *Escherichia* coli. Scientists of this division address characterization of these microbial proteins in relation to structure and pathogenesis of enteric diseases and host immune response. Recent approach in studying host signaling pathways by these proteins and their relevance to diarrhea is a modest beginning of the holistic approach to understand the complexity of bacterial pathogenesis. In addition, the division is working on PCR-based detection of virulence markers in pathogenic strains.

Scientist :

Dr. K. K. Banerjee, *Scientist 'F'*
Dr. N. S. Chatterjee, *Scientist 'E'*

Staff :

R. Naik, *Technical Assistant*

Pre-Doctoral Fellow :

S. Sabui
S. Ganguly
M. Mondal
A. Debnath
A. Mukherjee
S. Mondal
R. Chaurashi

A novel role of the lectin domain of *Vibrio cholerae* hemolysin in toxin assembly to the β-barrel oligomeric channel

Investigators : K. K. Banerjee and N. S. Chatterjee

Vibrio cholerae hemolysin/cytolysin (VCC) is a β-pore-forming toxin (β-PFT) with a jacalin-like β-prism lectin domain specific for β1-galactosyl moiety of asialofetuin and other glycoconjugates. We showed earlier that loss of the lectin domain causes an 800-fold reduction in pore-forming activity along with loss of 7-fold symmetry of the β-barrel transmembrane heptamer. Here, we show that membrane-targeting of VCC was mediated by amphipathicity-driven nonspecific interaction with several erythrocyte integral and peripheral membrane proteins with at most a minor contribution from carbohydrate-dependent interaction with cell surface glycoconjugates. A critical factor regulating efficiency of a PFT is the relative stability of the monomer in comparison to the oligomer. The VCC oligomer was thermodynamically more preferred than the monomer because relocation of

the β-prism lectin domain during toxin assembly was accompanied by a large negative enthalpy ($H = -115\ kJM^{-1}$) and positive entropy ($S = +0.96\ kJK^{-1}M^{-1}$) terms. With the absence of the lectin domain, thermodynamic advantage of the oligomeric structure was lost in the 50 kDa truncated VCC variant. The lower propensity of the 50 kDa toxin to self-assemble was reflected in the lower efficiency of the protein to form an oligomeric pore in the membrane lipid bilayer. The study revealed a novel role of the β-prism lectin domain in toxin assembly that is clearly distinct from its role in targeting the protein to its cell surface carbohydrate receptor, a role that has so far been thought to be universally valid.

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

Investigators : N.S. Chatterjee and K. K. Banerjee

Vibrio cholerae O1, causative agent of epidemic diarrheal diseases, 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, we are presently exploring the importance of ChiA2, a secreted chitinase, in V. cholerae survival in the environment as well as the in the human intestine.

Chitin is known to induce chitinase gene expression. We quantitatively analyzed the expression of *chiA2* gene in varying temperature, pH, salinity and time and found that maximum expression of *chiA2* gene occurred when *V. cholerae* was grown in artificial sea water medium along with chitin for 24 hours. This *chiA2* expression level was 10-fold more compared to laboratory conditions. Similar increase was observed at the protein levels from western blot experiments. Importance of ChiA2 in *V. cholerae* environmental survival was further proved when ChiA2 deleted *V. cholerae* when growth curve was compared to the wild type cultured in artificial sea water medium maintaining optimum environmental conditions. Mice intestinal survival assay showed that *V. cholerae chiA2* survived poorly in mice intestine compared to wild type strain. Fluid accumulation in infant mice model was also 2-fold less after eighteen hours of infection in case of *V. cholerae chiA2* compared to wild type strain. *V. cholerae chiA2* complemented with pChiA2-His was able to survive like the wild type strain. *V. cholerae* survival in presence of different human intestinal cell lines was 4.6-fold higher than the mutant strain. Our observations clearly indicate that *V. cholerae* ChiA2 has an impact on the bacterial survival in the environment as well as in the intestine. Further characterization of ChiA2 is in progress towards understanding its involvement in growth and survival in the intestine.

Molecular characterization of Enterotoxigenic *Escherichia* coli colonization factors

Investigators : N.S. Chatterjee and 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. Our laboratory has been studying different aspects of the colonization factors of ETEC and aims in developing simple methodologies for detection of these factors. In the previous report, we have reported that CS6 is composed of two strongly associated subunits, CssA and CssB,

which due to point mutations in their respective structural genes gave rise to allelic variants designated as AI, AII, AIII, BI and BII. The AIBI and AIIIBI were mostly associated with diarrhea cases whereas AIIBII were associated with asymptomatic cases. The pathogenicity of AIBI was correlated with their stronger adherence to CaCo-2 cells in comparison to that of AIIBII. The affinity of CS6 for mucin was less than that for fibronectin by roughly one order of magnitude and AIIBII had 4-fold less binding affinity for mucin than AIBI. Deletion mutation and complementation of CssAI/BI with CssAII/BII suggested that CssB, rather than CssA subunit, played an important role in mucin binding.

We also found a correlation between surface expression and receptor affinity of CS6 synergistically affects the adherence capability of these two variants. The expression of AIBI was found significantly higher than AIIBII on bacterial surface. During analysis of point mutations by site-directed mutagenesis, we observed that Gly39 in CssAI and Lys97 in CssBI substitution caused a sharp decrease in surface level expression of these proteins. Lys97Asn substitution in CssBI sharply decreases the adherence ability of the respective strain with host cell also. Further, we observed that IL-8 and TNF-α were induced in supernatants of HT-29 cells by 24- and 12-fold, respectively, at 12 h infection of ETEC and then subsequently declined. Mutants are being generated to gain a better understanding of this phenomenon and delineating the role of Cs6.

Conferences/ Seminars/ Workshop /Training Attended/ organized

N. S. Chatterjee

- Delivered a talk titled "Chitin-binding protein GbpA of *Vibrio cholerae* induces interleukin-8 gene expression in intestinal cells through a TLR2/TLR1/CD14 complex" at the Experimental Biology 2012 Meeting organized by Federation of American Societies for Experimental Biology on April 21-25, 2012 at San Diego, California, USA.
- Delivered a talk titled "Intestinal adherence of enterotoxigenic *Escherichia coli* involves both the subunits of colonization factor CS6" at 34th NAITO Conference organized by The NAITO Foundation on October 16-19, 2012 at Sapporo, Japan.
- Delivered a talk titled "Expression and characterization of *Vibrio cholerae* chitinase ChiA2" at the 81st Annual Meeting by Society of Biological Chemists (India) organized by the Society of Biological Chemists (India) on November 8-11, 2012 at Kolkata, India.
- Delivered a talk titled "Colonization factor CS6 of enterotoxigenic *Escherichia coli*: Functional heterogeneity due to sequence variation among CS6 genes" at Asian–African Research Forum on Emerging and Reemerging Infections organized by Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) on January 23-24, 2013 at Tokyo, Japan.

CLINICAL MEDICINE

The Clinical Medicine division is conducting the two major institutional 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. B.C. 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 at the mucosal surfaces and to identify novel virulence factors of Salmonella Typhi and study host-pathogen interactions in human Salmonellosis. Recently, studies have been initiated for induction of protective immune responses by probiotic bacteria isolated from indigenous population.

Scientists have also conducted different facet of clinical research projects funded by external funding agencies.

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 we have evaluated the efficacy of standard dose of Norfloxacin and single dose of Azithromycin in the treatment of cholera in adult and we found that single dose Azithromycin is similar effective as Norfloxacin in treating cholrea. The clinical medicine division is closely associated with the scientist of different division in the research project.

Scientists are involved in investigation of epidemics of diarrheal 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.

Scientists :

Dr. M. K. Bhattacharya, *Scientist 'F'*
Dr. U. Mitra, *Scientist 'E'* (Retired on 31-07-2013)
Dr. S. S. Das, *Scientist 'D'*
Dr. P. Indwar, *Scientist 'B'*

Staff :

A. Pal, *Technical Officer*
K. G. Saha, *Technician 'B'*
S. Turi, *Attendant Services*
S. Routh, *Attendant Services*
S. Dey, *Attendant Services*

Hospital based surveillance system for diarrhoeal diseases

Investigator : M. K. Bhattacharya

This study was initiated to monitor changes in disease pattern, to create a database on diarrhoeal diseases, and to provide regular reports to the Govt. and other agencies and to improvement in better patients care and preventive measures. During January 1, 2012 –December 31, 2012, a total of 20558 diarrhea cases admitted at ID &BG Hospital. Out of these 644 diarrhea cases were enrolled in the surveillance. Majority cases were presented with dehydration 580 (90.8%). Of which 472 (73.4 %) acute watery diarrhea. The number of death cases was 4 (0.6%). In children below 5 years of age rotavirus and *V. cholerae* were main pathogens. *V. cholerae* strains were resistant to tetracycline and susceptible to Azithromycin and Norfloxacin. Shigella isolation was only 5. 7%. Weekly reports sent to Govt. and other agencies for control and improvement for better patients care.

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

Investigator : M. K. Bhattacharya

A total of 120 adult patients aged 12-55 years were enrolled in the study with 60 patients in each treatment group (one group received Azithromycin 1 gm. single dose and another group received Norfloxacin 400 mg twice daily for three days). Of these, 64 patients were positive for *V. cholerae* infection, and 56 patients were negative for *V. cholerae*. Among 64 Cholera positive patients 32 received Azithromycin and 32 received Norfloxacin. On admission characteristics features such as age, body weight, frequency of diarrhoea, and preadmission duration of diarrhoea were comparable between the groups. The data were analysed by using SPSSPC 11.5 statistical package. Primary outcome variables ware total stool output and duration of diarrhoea and secondary outcome variables were total fluid intake (intravenous +ORS). Difference between total stool output (2580.7ml ± 1480.8ml vs. 2863.6ml ± 2617.1ml) and total duration of diarrhoea after starting treatment (26.6h ± 7.5h vs. 29.3h ± 8.9h) between two treatments groups were statistically insignificant ($p \geq 0.05$). Moreover total fluid requirement ORS (4041.9ml ± 1740.5ml vs. 3891.5ml ± 1986.2ml) and IV fluid (4574.2 ml ± 1336.9ml vs. 5098.8ml ± 1635.3ml) is comparable between two treatment groups. In conclusion Azithromycin appeared to be equal in efficacy to Norfloxacin and may be regarded superior to Norfloxacin because of its single dose treatment advantage. This single dose efficacy is quite helpful in cholera epidemics and may be used as a mainline drug in case of emergence of *V. cholerae* resistant to Norfloxacin. The result suggests that single dose of Azithromycin is equally effective as standard dose of Norfloxacin in the treatment of cholera in adults.

Commensal *E. coli* flagellin induces intestinal regulatory responses and protects from experimental colitis

Investigator : S. S. Das

Per-rectal administration of recombinant commensal *E. coli* flagellin (CF, 5µg) in BALB/c mice (~20 gm) for 5 consecutive days resulted in enhanced expression of regulatory cytokines (TGF-β, IL-10) by the ECs and LPDCs and their raised serum levels. In addition, the numbers of CD11c+CD11b+CD103+ tolerogenic DCs and CD4+FoxP3+ cells in the lamina propria (LP) and the mesenteric lymph nodes (MLNs) were significantly increased in CF-treated mice. However, direct stimulation of MLN T cells with CF failed to upregulate cytokine production or FoxP3 expression, suggesting that CF-induced T cell differentiation may be regulated by EC- and DC-derived factors. To prove that CD4+FoxP3+ cells have regulatory functions, an in vitro T cell suppression assay was carried out using Treg (suppressor) cells from CF-treated mice and T effector (Teff) cells from OT-II transgenic mice. Teff proliferation by $OVA_{323-339}$ was increasingly suppressed with rising suppressor to effector ratios, suggesting functional Treg cell generation by CF in vivo. This led us to investigate if commensal flagellin may suppress the manifestations of intestinal inflammatory disorders. We used a model where pre-sensitization of C57BL/6 mice by s.c. injection of TNBS followed after 7 days by per-rectal administration of TNBS results in severe Th1 cell-dependent colitis as evident from macroscopic, histopathological and flowcytometric analysis leading to high Wallace and Ameho scores. Mice that received CF after TNBS showed significantly less inflammation (Fig. 1, upper panel), which correlated with higher serum levels of regulatory and lower levels of pro-inflammatory cytokines. In addition, CF-treated mice showed increased populations of CD103+ LP and MLN DCs as compared with the mice treated with TNBS alone, along with a parallel rise in the number of CD4+FoxP3+ T-regulatory cells in the intestine (Fig. 1, lower panel).

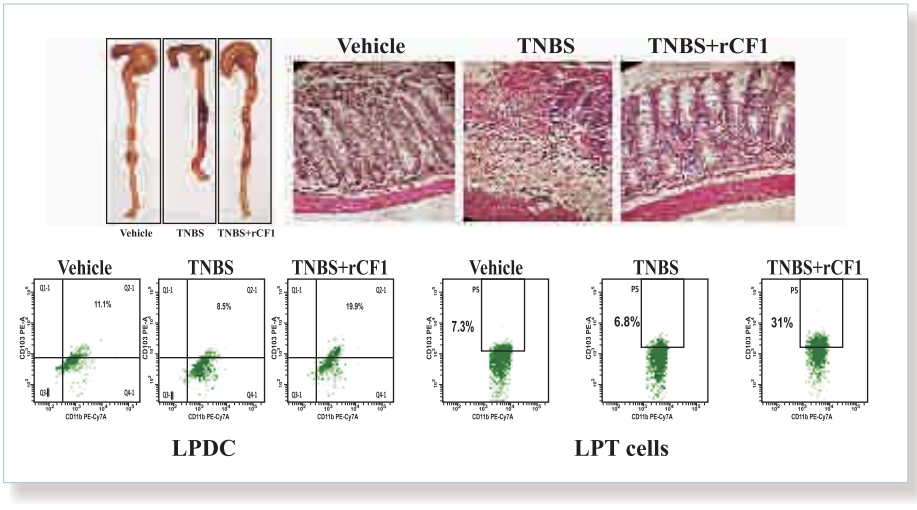

Fig. 1. Upper panel (left). Colon of mice isolated 3 days after intracolonic TNBS administration. Upper panel (right). Histopathology of the colonic tissues isolated as above and stained with hematoxylin and eosin. Lower panel. Cells isolated from the colon of mouse trated as above.

An eukaryotic-like serine/threonine protein kinase (STPK) of *Salmonella enterica* serovar Typhi (S. Typhi) promotes intracellular survival through lysosomal dysfunction.

Investigator : S. S. Das

Intracellular pathogens employ multiple mechanisms to subvert host-induced killing within macrophages. These include suppression of the production and neutralization of reactive nitrogen and oxygen species (RNS and ROS) and AMPs, inhibition of phagososme maturation, phagolysosome formation, lysosomal biogenesis and enzyme activities etc. To address the above issue, we generated isogenic mutants of S. Typhi in the background of a reference strain (Ty2). Wild type (Ty2) and mutant (Ty2 STPK) S. Typhi as well as the recombinant protein (rSTPK) minimally influenced basal production of nitrates and ROS as well as their inducible production by LPS in differentiated human monocytic cells (THP-1) and murine macrophage cell line (RAW264.7). Next, we studied phagosome maturation in Ty2- and Ty2 STPK-infected Thp-1 cells by analyzing the phagososmal acidification marker V-ATPase and a late endosomal marker Rab7, both of which increase with maturation. Phagosomes purified during the early and late intracellular life of the above bacterial strains showed identical expression of the maturation markers, suggesting no effects of STPK. Contradictory reports are available regarding the role of Salmonella in influencing phagosome and lysosome fusion. We investigated by confocal microscopy if S. Typhi interferes with phagolysosome formation through STPK. Lysosomes in the infected Thp-1 cells were stained with the lysotracker(red) dye, while the intracellular bacteria were stained with polyclonal Salmonella antisera. No difference in lysosomal staining and phago-lysosome fusion was noticed after 3h between Ty2 and Ty2 STPK infection, but lysosomal staining was significantly diminished 12 and 24 hours after Ty2 infection or combination of Ty2 STPK infection and rSTPK stimulation (**Fig 2**). In contrast, rSTPK alone induced aggregation of lysosomes and staining of the adjacent cytoplasm. The above difference in the staining patterns after treatment with the purified protein and the whole bacteria may be due to the absence of microbial products in the former that neutralize the acidic pH of the leaked lysososmal contents. These results suggest that STPK induces lysosomal permeabilization and dysfunction, leading to their depletion. Lysosomal dysfunction in our studies was determined by STPK rather than the bacterial numbers, as proved by no alteration in lysosomal staining in Ty2 STPK-infected cells when the multiplicity of infection (MOI) was increased to 10 fold higher (100:1).

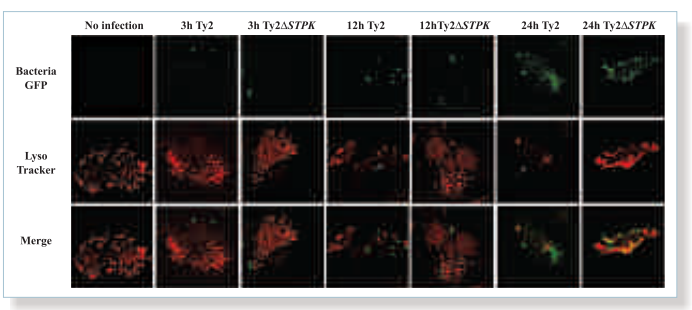

Fig. 2. Confocal microscopic images of THP-1 cells infected with the wild type or mutant S. Typhi for 1 hr, washed and cultured in the presence of gentamicin (50 µg/ml) for the indicated durations. Cells and bacteria were stained with lysotracker(red) and polyclonal Salmonella antisera followed by FITC-conjugated secondary antibody, respectively (The images were taken in the laboratory of Dr. Oishee Chakraborty, Saha Institute of Nuclear Physics, Kolkata).

Awards and Honours

S. S. Das

- Fulbright-Nehru Senior Research Fellowship, 2012 (Dr. S. S. Das, PI).
- Best Poster Award at the 1st Annual Conference and International Symposium organized by Probiotic Association of India held in New Delhi, India during August 27-28, 2012 (Bhupesh Kumar Thakur, SRF (DBT Project)).
- Cytometrist of the Year Award at the 5th Annual Meeting and workshop of the Cytometry Society of India held in Kolkata, India during October 12-15, 2012 (Piu Saha, Postdoctoral Fellow (ICMR)).

Conferences/ Seminars/ Workshop /Training Attended/ organized

M. K. Bhattacharya

- Attended meeting on Oral presentation in the Asian-African Research Forum on Emerging and Reemerging Infections to be held in Tokyo, Japan and Okayama University Forum for Emerging Enteric Diseases in Okayama, Japan during January21 – 27, 2013.
- As a Special Guest lecturer, delivered lecture on "Health & Hygiene Management" on May 7, 2012 at Kolkata Doordarshan, West Bengal.
- As a Guest lecturer, delivered lecture on "INSPIRE SC. CAMP, DST, Govt. India" on November 28, 2012 at National Institute Science and Technology Palur Hills, Berhampur, Orissa, INDIA.
- As a Guest lecturer, delivered lecture on "Health Management during disaster" on February 20, 2013 at ATI, West Bengal.
- As a Guest lecturer, delivered lecture on "Health & Hygiene Management during and Post Flood Disaster" on March 14, 2013 at ATI, West Bengal.

S. S. Das

- Oral presentation at the 34th NAITO Conference on "Infection, Immunity and their Control for Health: Barrier and Vaccine for Infection and Immunity" held at Sapporo, Japan on October 16-19, 2012 (Dr. Santasabuj Das, PI).
- Poster presentation at Kolkata International School cum Conference on Systems Biology (KOLSYSBIO) at Saha Institute of Niclear Physics, Kolkata, India during December 29, 2012-January 3, 2013 (Nirmalya Dasgupta, SRF, UGC).

- Organized Workshop entitled 'Introductory Bioinformatics, Its Applications and Genome Analysis' at the Biomedical Informatics Center of NICED on April 9-10, 2012.
- Organized Workshop entitled 'Molecular Modeling and Drug Designing' at the Biomedical Informatics Center of NICED on April 12-13, 2012.
- Guha Research Conference, 2012 held at North-Eastern Hill University (NEHU), Shillong, Meghalaya on November 28-December 02, 2012.
- 81st Annual Meeting of Society of Biological Chemists (India) held at the Science City Auditorium, Kolkata on November 8-11, 2012.
- 5th Annual Meeting of The Cytometry Society of India held at the Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata on October 12-13, 2012.
- 1st International Meet on Advanced Studies on Cell Signaling Network (CeSiN) 2012 held at Indian Institute of Chemical biology, Kolkata on September 11-13, 2012.
- Training Program on Laboratory Safety held at Indian Institute of Chemical biology, Kolkata on September 6th, 2012.

DATA MANAGEMENT

This division primarily focuses on good data management practices and also compliant with Good Clinical practices (GCP) to produce the reliable, complete and accurate data from the various health research projects of this institute. This division has also crucial role for data management and creation of diarrhoea database from ongoing hospital based diarrhoeal diseases surveillance at Infectious Disease Hospital (IDH), and Dr. B C Roy Children Hospital in Kolkata to identify the pattern of diarroeagenic enteric pathogens. The causative organism of diarrhoea and antimicrobial resistant pattern of cholera and Shigella is communicated on weekly basis to IDH and different department of State Government so as to help the physicians for proper patient management of diarrhoeal diseases.

The division is also working on climate factor and diarrhoeal disease which derives the seasonality pattern and association of diarrhoea in West Bengal. It provides the comprehensible vision of basic research of diarrhoeal diseases empowering the epidemiological, clinical and microbiological data envisaging social, environmental and spatial implication by novel statistical model. It has direct access to the data from all concerned division and to provide data management support including data entry/verification to various studies undertaken in this institute with National like the project on National hospital based Rotavirus surveillance network in Eastern zone of India and Integrated Diseases Surveillance Project (IDSP) and International Collaborators like International Vaccine Institute, Korea, and Centre for Vaccine Development, University of Maryland, Baltimore.

This division always rendered statistical help for epidemiological, clinical and microbiological research as well as to Ph.D. students for their thesis. There are also future plans to conduct local and country level courses on research methodology, biostatistics use in laboratory science, sample size determination for randomized clinical trial for health researchers. Final goal is to publish the research findings using modern and appropriate statistical techniques in peer reviewed journals.

Scientists :

Dr. B. Manna, *Scientist 'F'*
Dr. K. Rajendran, *Scientist 'C'*

Generation of a database on cholera outbreaks in India

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.

The published articles on diarrhoea outbreak /epidemic have been collected through free medical journals, Medexplorer, Medscape, Medhunt and PubMed. Attempt was made 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) web site. About 105 published articles have been collected. Since July, 2009 to May, 2013, a total of 1,926 outbreaks have been reported under Integrated Disease Surveillance Project (IDSP). It has been observed that maximum number of outbreaks occurred during May – July in different year. According to IDSP report, Karnataka state had maximum number of outbreaks followed by Maharastra, Tamil Nadu, Bihar, Andhra Pradesh and West Bengal in 2012 (Fig. 1).

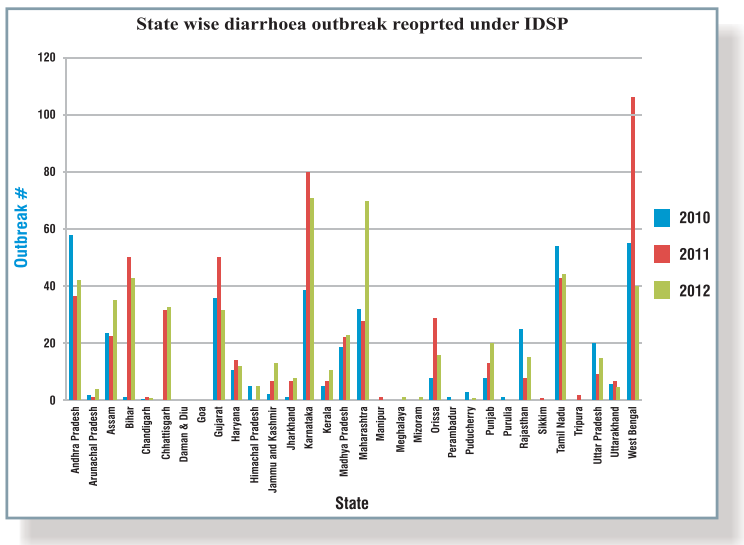

Fig. 1. State wise diarrheal outbreaks reported under IDSP

Time series model study for prediction of cholera and diarrhoea using atmospheric temperature, relative humidity and rainfall in Kolkata, India

Investigator : K. Rajendran

The emerging and re-emerging dynamics of cholera infection is believed to be partly influenced by climatic factors. In the ongoing project, earlier part of analysis has been established that climate was one of the important factors for cholera persistence and spread in Bengal. This part of analysis has been aimed to compare the climatic characteristics with observed infection of diarrhoea and cholera to assess long term changes to develop Time series model.

Active surveillance data collected during 1996-2011 and hospital cholera data recorded from 1980-1995 generated from the Infectious Diseases Hospital (IDH) were used in this study along with the data collected on relative humidity (RH), temperature and rainfall collected from the Meteorological Department Kolkata. During the study period, the cholera infection was assessed using GLM. The climate factors were also considered for Time series analysis of Auto-Regressive Integrated Moving Average (ARIMA) model to investigate relative impact of climatic on cholera. Time series model: ARIMA has created candidate model of year wise *V. cholerae* infection with predictor variables of RH, temperature, and rainfall with ARIM (1, 0, 0) to stabilize model (Fig. 2). Yearly mean RH (73.42 ± 2.56) was consistent. The co-existence of RH and *V. cholerae* infection peaks was observed while 1-5% RH auto regressively (Auto regression: Estimate: 0.80; $|t| = 8.01$; $p < 0.001$) increase of its mean compared to the respective previous years (Fig. 3). The yearly temperature (27.14 ± 0.27) was fluctuating and an increase of 1°C was noticed during the 32 years. The overall yearly mean occurrence of rainfall was observed 13.41 ± 2.41 (Mean $\pm$ Sd) along with the highest peak of cholera. Overall, 32 years data gave convincing information on relation between cholera infection and climatic factors in Kolkata, West Bengal (Fig. 4). It is interesting to note that relative humidity (RH) seems to be the core influential factor to stimulate *V. cholerae* infection. The El Niño and La Niña has definite role in controlling the prevalence of cholera in Kolkata.

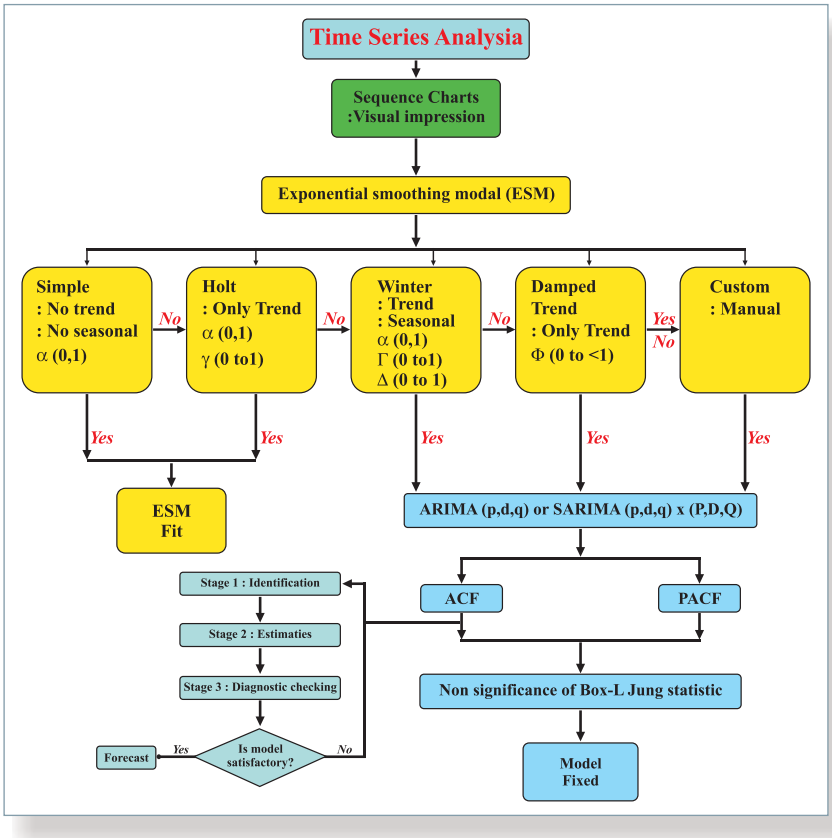

Fig. 2. Flow chart of derived time series model.

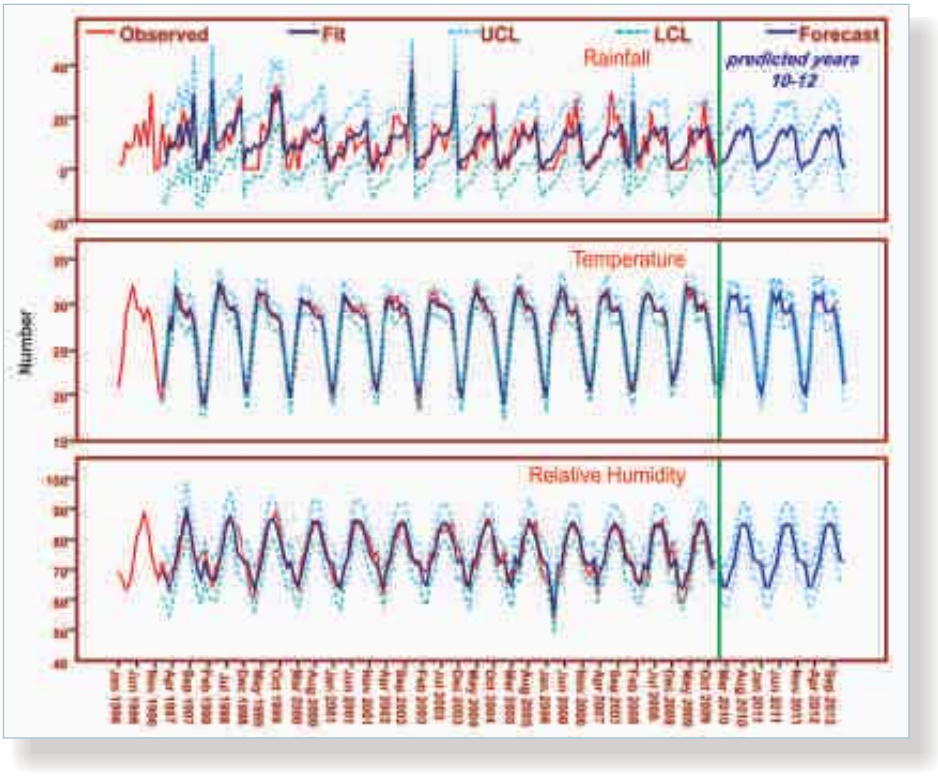

Fig. 3. Candidate model SARIMA (1,0,0) (0,1,1) of climate factors exhibit the fitness of the observed series of cholera infection.

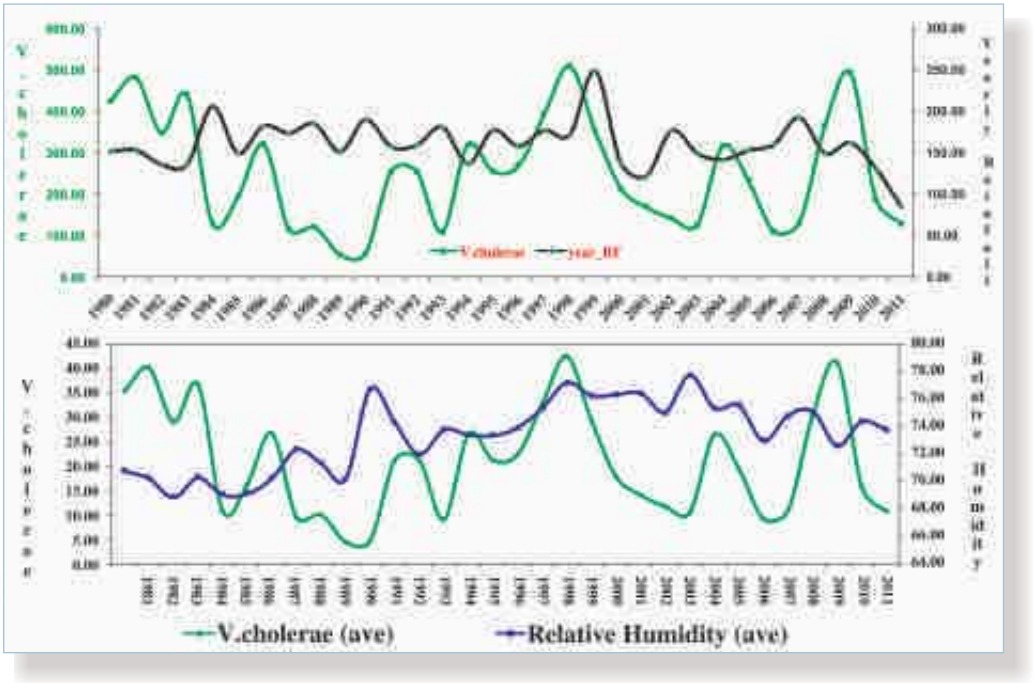

Fig. 4. Analysis of 32 years data showing relationship between relative humidity and rainfall: associated cholera in Kolkata

Conferences/ Seminars/ Workshop /Training Attended/ organized

B. Manna

- Delivered a talk on "Statistics in Scientific Research" in ICMR workshop on "Biomedical Communication" organized by Indian Council of Medical Research on July 28, 2012, at NICED, Kolkata.
- Delivered a talk on "Use of Statistics in Laboratory Science, Hospital & Population based medical research" in "Clinical Research Workshop" Organized by Indian Medical Students Association on June 2, 2012, at NICED, Kolkata.
- Delivered series of lectures for post graduate students in Peerless Hospital & B K Roy Research Centre , Kolkata on September 26, 2012; October 6, 2012 and November 24, 2012.
- Attended workshop on "Clinical Research Informatics and REDCap" organized by NIE, NIRT, NARI and Vanderbilt University, USA at NIE, Chennai from October 3-5, 2012
- Attended symposium on "Probiotics in prevention of Lifestyle Disorders" organized by Yakult India Microbiota and Probiotic Science Foundation at Bengaluru on December 15-16, 2012.
- Attended annual meeting for the PROVIDE Study on May 16-17, 2012 at Baltimore, Maryland, USA.

K. Rajendran

- Delivered a talk as plenary speaker on "Dynamic focus on diarrhoeal diseases with influence of climate factors in Kolkata: time series and spatial data analysis" in an international conference on epidemiology with "special focus to modeling infectious and chronic diseases data using Time series and Spatial data analysis" held during August 16-18, 2012 at St. Thomas college palai, Mahatma Gandhi University, Kottayam, Kerala-74, India.
- Poster presentation on "Influence of Relative Humidity on Persistence of Cholera in West Bengal: A three decade comparison with Time Series Model" in the 47th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections held during December 12-14, 2012 at Chiba University , Chiba, Japan.
- Delivered a talk on "Bio-statistics in Bio-Medical Science" in the 100th Indian Science congress held at Kolkata, India on January 3-7, 2013.

ELECTRON MICROSCOPY

The Division of Electron Microscopy is engaged in research and diagnosis in the field of diarrhoeal diseases. These include 3-dimensional image reconstruction using cryo-electron microscopy, negative stain analysis, ultrastructural and histopathological studies. The division also organized two workshops on electron microscopy which were attended by a large number of participants from different research institutes, universities and hospitals.

Cryo-negative stain electron microscopy and single particle analysis methods were employed to determine the three-dimensional structures of mature (HlyA; 65 kDa) and truncated (HlyA50; 50 kDa) hemolysin oligomers of *Vibrio cholerae*. Hemolytic activity of the truncated hemolysin is about 1000-fold less than that of the mature one. Although 2-dimensional images of both variants looked similar, there were marked differences in the 3-dimensional structure. Putative atomic structures of the two oligomers were worked out and were used for validation of the 3D models. Interacting amino acid residues, their position in HlyA and HlyA50 and lipid-protein interaction site were also predicted.

During the reported period, two virulent enteroaggregative strains (EAEC) were isolated from our culture collection and were studied histologically in animal model by light microscopy. Enteropathogens like *H. Pylori, V. cholerae* & *A. hydrophilae* related to different projects were studied both by light & electron microscopy. Haemagglutinating activity (HA) & colonization ability of Shigella were studied in suckling mice model. Pathogenic bacteria usually develop various surface structures known as capsule, slime, glycocalyx or fimbriae whose primary function is to interact with receptors on the membranes of target cells. These surface structures of the bacteria have been studied ultrastructurally. Outer membrane vesicles (OMVs) of Shigella as a candidate vaccine in animal model was studied in animal model ultrastructurally. Gut microflora in neonatal sepsis with special reference to gm - ve bacteria were studied in animal model histologically.

Scientist :

Dr. A. N. Ghosh, *Scientist 'F'*
Dr. D. R. Saha, *Scientist 'E'*

Staff :

A. Sarbajna, *Technical Officer 'A'*
S. Kumar, *Technician 'B'*
B. R. Mallick, *Attendant Services*

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

Investigators : A. N. Ghosh and 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. Although 2-dimensional images of both variants looked similar, there were marked differences in their 3-dimensional structures. Three-dimensional structures of mature (HlyA) and truncated (HlyA50) hemolysin oligomers of *Vibrio cholerae* were determined at 16.5 Å and 17.4 Å resolutions respectively using cryo-negative stain electron microscopy and single particle analysis methods. HlyA was observed to have C7 symmetry and a ring-like structure at the top whereas HlyA50 was found to be asymmetric and without any ring. Internal channels were observed in both the oligomers but their structures were also found to be different.

Putative atomic structures of the two oligomers were worked out by homology-modeling method (Fig. 1) and were used for validation of the 3D models. β-prism lectin-like domain was found to be absent in homology-modeled HlyA50 (Figure 1B). Interacting amino acid residues, their position in HlyA and HlyA50 and lipid-protein interaction site were also predicted.

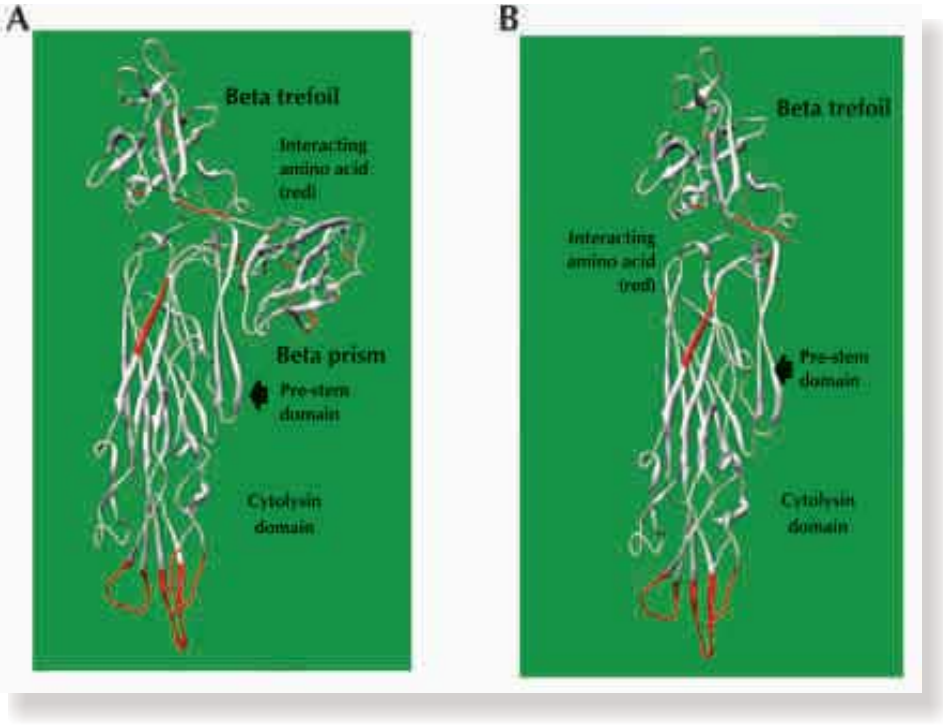

Fig. 1 Protein-protein interaction of V. cholerae hemolysin: (A) The interacting (inter-molecular contacts) amino acids of HlyA represented by red colour and (B) The interacting (inter-molecular contacts) amino acids of HlyA 50 represented by red colour.

Gastric pathology in *Helicobacter pylori* associated diseases in Indians

Investigators : D. R. Saha, A. Chowdhury, A. K. Mukhopadhyay

Helicobacter pylori (H. pylori), a human pathogen that colonizes gastric tissue causing symptoms ranging from mild gastritis to ulcers and confers an increased risk of gastric cancer. Indian subcontinent constitutes about 1/5th of the world population with a very high *H. pylori* infection rate. A variety of adhesions, cytotoxins and enzymes have been identified and implicated in *H. pylori* pathogenesis. It is still not clear how the pathogen chronically colonizes the gastroduodenal epithelium and if attachment alone /or its internalization by epithelial cells is essential for this process. About 100 patients underwent gastroendoscopy based on clinical history and physical examination. Of them 25 were diagnosed as nonulcer dyspepsia (nud), 25 duodenal ulcer (du) and 20gastric carcinoma (gc) cases. *H. pylori* infection was detected in 80% nud, 92% du and 40% gc cases by histology, culture and RUT. Five individual colonies and a pooled bacterial culture were isolated from each patient by RAPD using random primers. For histology other than H&E stain, modified giemsa, immune stain and alcian blue stains were used. *cag A* was present in 89.1% of the tested strains and 69.5% of the strains had vacA s1m1 allele. Other two alleles s1m2 and s2m2 of *vacA* were present in 19.5% and 10.9% respectively. The presence of multiple *H. pylori* strains in a single individual was detected in 38 patients of 46 culture positive cases by RAPD PCR using both primers 1281 and 1283.However,in 9 patients multiple *H. pylori* strains were identified with any of these primers. Vacuolating change was evident histologically in almost all *H. pylori* infected gastric surface mucosa and in several cases even in lamina propria. Bacterial presence was also detected in lamina propria suggesting an evidence of invasive nature of the organism (Fig. 2).

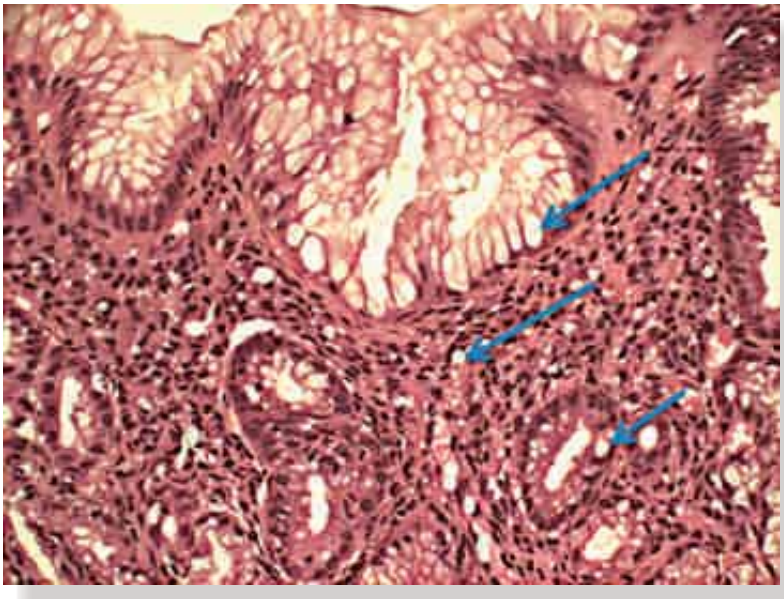

Fig. 2. Vacuolating change in surface mucosa, in lamina propria & inside gland-du-40x (H&E)

Awards and Honours

A. N. Ghosh

- Received Fellowship of Electron Microscope Society of India (FEMSI).

Conferences/ Seminars/ Workshop /Training Attended/ organized

A. N. Ghosh

- Delivered a talk as plenary speaker on "Pore-Forming Toxin of *Vibrio cholerae* : a cryoEM Study" at XXXIII Annual Meeting of the Electron Microscope Society of India, Indian Institute of Science, Bangalore during July 2-4, 2012.
- Convener and resource person of the workshop on "Electron Microscopic Techniques: holey film, carbon film and ultramicrotomy" at NICED organized by National Institute of Cholera and Enteric Diseases and Electron Microscope Society of India, East Zone Chapter during July 30-31, 2012.
- Convener and resource person of the workshop: "Scanning Electron Microscopy in Life Sciences" at NICED organized by National Institute of Cholera and Enteric Diseases and Electron Microscope Society of India, East Zone Chapter during February 7-8, 2013.
- Delivered invited lecture on "Electron Microscopy: Principles and Applications" in the "Workshop on Transmission Electron Microscopy – Theory and Practice" held at Central Electrochemical Research Institute, Karaikudi, Tamil Nadu on September 20, 2012.
- Acted as a Resource Person and delivered invited lecture on "Three-dimensional Image Reconstruction by Cryo-electron Microscopy" in "Workshop in Recent Trends in Biological Electron Microscopy" at North Eastern Hill University, Shillong during March 5-8, 2013.
- Acted as a Resource Person and delivered invited lecture on "Electron Microscopy of native and denatured DNA" in "Workshop in Recent Trends in Biological Electron Microscopy" at North Eastern Hill University, Shillong during March 5-8, 2013.

D. R. Saha

- Delivered a talk in the 100th Indian Science Congress , Kolkata from January 3-7, 2013.
- Participated as organizer for a workshop on 'Electron Microscopic techniques: 'Holey film, Carbon Film and Ultramicrotomy' organized by National Institute of Cholera and Enteric Diseases Kolkata and Electron Microscope Society of India, East Zone Chapter during July 30-31, 2012.

- Participated as organizer for a workshop on 'Scanning Electron microscopy in Life Sciences' organized by National Institute of Cholera and Enteric Diseases Kolkata and Electron Microscope Society of India, East Zone Chapter during February 7-8, 2013.

EPIDEMIOLOGY

Continuing from previous years, the division of epidemiology in NICED thrives to work in the field of public health and translational research. It continued surveillance in urban slums for etiological burden of diarrhoegenic pathogens in under five children, phase II vaccine trials with oral cholera vaccine both live and killed, understanding the biological basis of underperformance of oral vaccines in infants as well as District level health surveys, where NICED targeted population throughout West Bengal for estimation of Hb%, blood sugar and dietary iodine in salt as well anthropometric measurement. Horizon of NICED's work has also expanded from surveillance of diarrhea and HIV to intervention studies. Some of the highlights of the division for the last year are as follows:

On diarrheal disease surveillance and intervention trial :

1. Results for the Phase III study on the efficacy of a bivalent whole cell killed oral cholera vaccine were finalized; it showed a protective efficacy of 65% at 5 years. In 2008, the protective efficacy (PE) of all age group was 67% at two years and at the end of three years post vaccination (2009) it was 65%. As a public health tool, this vaccine will make a dramatic change in reducing the burden of cholera worldwide.

2. A multi centric study of the burden of diarrheal 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. Through this study NICED was able to identify the major pathogens in <5 population in urban slums of pathogens causing moderate to severe diarrhea. The major pathogens were rotavirus, Cryptosporidium, *Shigella* spp, *E. coli*, and adenovirus.

3. Division has also undertaken a phase II study on safety and immunogenicity of the live oral cholera vaccine (VA1.4) developed by Indian scientists and funded by Dept. of Biotechnology, Government of India in collaboration with Society for Applied Studies, Kolkata. The study concluded in 2012 May. Preliminary result showed that the vaccine was safe and immunogenic. Now further plan is age descending study followed by phase III study.

4. Another important work of the Division is to understand diarrhoea-related knowledge and practice of physicians in urban slums of Kolkata, undertaken as intra mural project. Dearth of information regarding physicians' diarrhoea-related knowledge and practice in India necessitated this cross-sectional study of allopathic practitioners in the slums of Kolkata.

5. The division has undertaken a major work on nutritional status of approximately 120, 000 population across whole of West Bengal "District Level Household survey-4 in West Bengal: in collaboration with and funded by National Institute of Health and Family Welfare, Govt. of India. This has household survey and facility survey components. Both these components of DLHS-4 are being implemented in the districts of all states and union

territories other than nine states of Uttar Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh, Bihar, Jharkhand, Orissa, Rajasthan and Assam covered in Annual Health Survey (AHS). International Institute of Population Sciences (IIPS), Mumbai, is primarily responsible for the entire study. They are assisted by National Institute of Health & Family Welfare (NIH&FW), New Delhi, for co-ordinating clinical anthropometry & biochemical (CAB) component of this study. NICED was responsible for training & supervising CAB component of DLHS-4 study at all districts of West Bengal in coordination with NIH&FW (Fig 1). The field work was outsourced and carried out by a Field Agency. Staff of Field Agency were trained and closely monitored by NICED in field situations. NICED had also provided laboratory support to this study and completed a total of 100,000 dried blood spot samples for hemoglobin estimation till August-2013. NICED-DLHS-4 laboratory also maintained their quality through internal and external quality control programme.

6. Division of epidemiology is also undertaking a population based study in Malda district to Assess perceived Health needs and available Health Care Facilities of Malda District (Fig 2). This study is funded by Indian Council of Medical research, with the following objectives:

- Assess the perceived health needs of the population and the pattern of major illnesses by a cross sectional analysis of an Urban and Rural sample.
- Determine the pattern of use of available health care facilities and the morbidity pattern seen in them
- Determine random blood sugar values and the time of the preceding meal to assess the prevalence of Diabetes Mellitus
- Determine Arsenic content of household water to evaluate the potential significance of Arsenicosis in this population.

A total of 10, 000 families will be surveyed along with assessment of health care delivery system and assessment of water quality (for addressing the extent of Arsenicosis) in Malda.

7. A community based study was conducted in the district of South 24 Parganas, West Bengal in which a baseline assessment of health of women and children was carried out at Patharpratima Block of the Sunderbans area. Five panchayats (local village administration) were covered and the study revealed very high prevalence of under-nutrition in children ≤ 5 yr of age and anemia in married women who were ≤ 49 yr. The study findings guided subsequent launch of intervention.

8. Few salient features of studies on HIV :

Care needs assessment of children living with or affected by HIV: A mixed team of people living with HIV and those not infected with the virus has been formed to accomplish the task under this intramural project of public health importance. This team has been trained on various data collection techniques and skill to work in community setting. The tools for this assessment have also been finalized and translated in Bengali. The initial field work has generated a registry in the district of Paschim Medinipur in West Bengal containing details of

362 children in which girls and boys are present in almost equal proportion. Of 362 children, 104 are living with HIV and the rest are not. This latter group of children constitute the 'affected' or 'those who are exposed to HIV' as one or both parents of them lived with the virus. The assessment is now on-going.

Scientist :

Dr. D. Sur, *Scientist 'F'*
Dr. S. Panda, *Scientist 'E'*
Dr. K. Sarkar, *Scientist 'E'*
Dr. A. K. Deb, *Scientist 'D'*
Dr. S. Kanungo, *Scientist 'C'*
Dr. F. Debnath, *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, *Technical Assistant*
A. Chakraborty, *Technician 'B'*

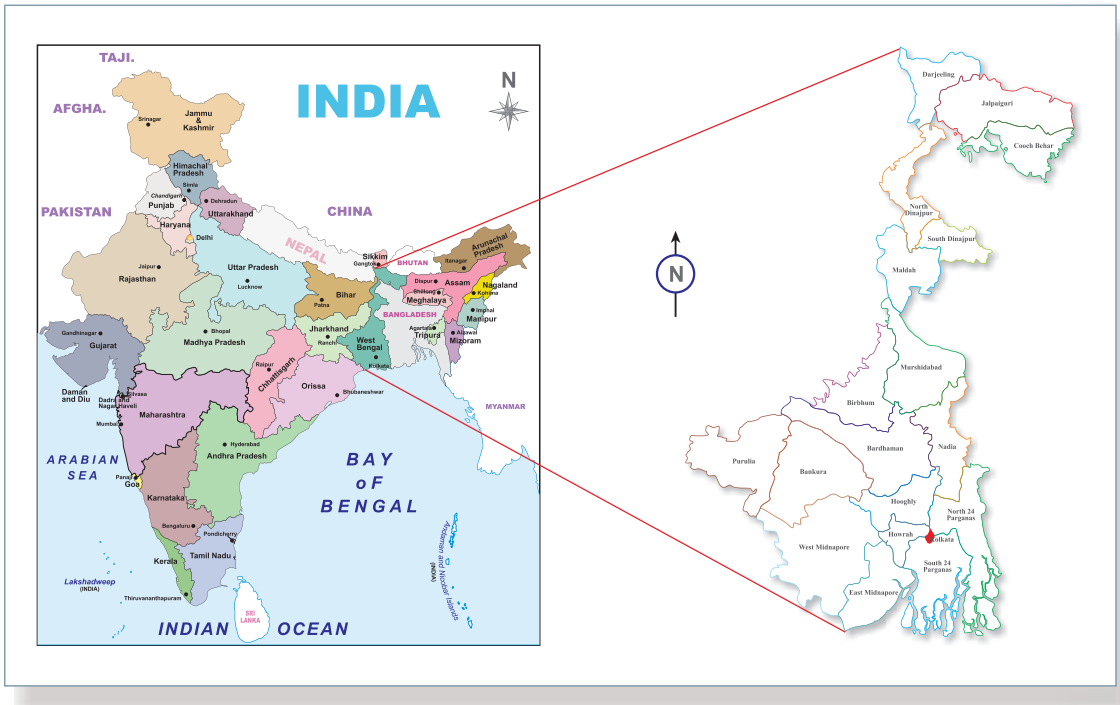

Fig. 1: Area (State) for CAB activities

Fig. 2: Geographical boundary of the state Malda

Situation assessment of health of women and children leading to intervention development

Investigator : S. Panda

A community based study was conducted in the district of South 24 Parganas, West Bengal during the last quarter of 2012 under which selected health parameters of women and children were assessed in the Patharpratima Block. The research was undertaken at the behest of the government of West Bengal and Save the Children. Five panchayats (local village administration) were covered under this assessment and the study revealed 12 to 22% prevalence of under-nutrition in children ≤ 5 yr. Prevalence of anemia in married women ≤49 yr was 64% of which 25% was due to iron deficiency.

The study findings were disseminated to different stakeholders (Fig3) and were used to develop intervention at the local level and Save the Children sent a detailed written communication in this regard to the Director-In-Charge of NICED. A civil society organization named 'Sunderban Social Development Centre' (SSDC) was a partner in this initiative. Capacity building of the field workers of SSDC as well as of the locally recruited field researchers comprising young adult men and women of this underserved and disaster prone district were achieved through this research process.

Fig. 3. Study findings being disseminated at the district magistrate's office in presence of panchayat pradhans, CMOH, Block Development Officer and other stakeholders from the district of South 24 Parganas, West Bengal.

District level household survey-4 in West Bengal: in collaboration with and funded by National Institute of Health and Family Welfare, Govt. of India

Investigators : K. Sarkar, S. Kanungo, and D. Sur

District Level Household and Facility Survey-4 (DLHS-4) has household survey and facility survey components. Both these components of DLHS-4 were implemented in the districts of all states and union territories other than nine states of Uttar Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh, Bihar, Jharkhand, Orissa, Rajasthan and Assam covered in Annual Health Survey (AHS). The facility survey component of DLHS-4 was also implemented in these nine states as facility survey was not a part of the AHS. International Institute of Population Sciences (IIPS), Mumbai, was primarily responsible for the entire study. They were assisted by National Institute of Health & Family Welfare (NIH&FW), New Delhi, for co-coordinating clinical anthropometry & biochemical (CAB) component of this study.

The NIHFW is the nodal agency for the CAB component of DLHS-4 under the overall coordination of IIPS. Similar to other large scale surveys, where information on nutrition and health have been collected through integration of (CAB) tests in the national level household survey, DLHS-4 attempted to undertake a number of CAB tests to produce district level estimates for nutritional status and prevalence of certain life style disorders among not only among women in reproductive ages and their children below age 6 but also among all other members of households. NICED was one of the partner institutes of NIH&FW to carry out the CAB component of the DLHS-4 survey in West Bengal. Major constituents in the proposed

CAB components were measuring height &weight, blood pressure, estimation of hemoglobin, and plasma glucose along with testing of salt used by households for iodine component.

NICED was responsible for training & supervising CAB component of DLHS-4 study at all districts of West Bengal in coordination with NIH&FW. The field work was outsourced and carried out by a Field Agency. Staff of Field Agency were trained and closely monitored by NICED in field situations. NICED carried out dried blood spot samples tests for hemoglobin estimation till March-2013. NICED-DLHS-4 laboratory is also maintaining their quality through internal and external quality control programme. The study was initiated on August 2012. (Table1)

Table 1: Work load and progress

Total No. of districts to be covered within the period	**19**
Total No. of districts completed till March 31, 2013	**2**
Total No. of districts on going till March 31, 2013	**13**
Total No. of PSUs to be covered	**1000**
Total no. of PSUs completed till March 31, 2013	**421**
Total No. of DBSs collected till March 31, 2013	**31676**
Total No. of DBSs processed in NICED Lab. till March 31, 2013	**31676**

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 in collaboration with International Vaccine Institute

Investigators : D. Sur, S. Kanungo, M. K. Bhattacharya, B. Manna, and R. K. Nandy

The primary objective of the study was to compare vibriocidal immune responses to the modified killed WC-OCV when given as two doses, 14-28 days apart, in healthy, non-pregnant adults and children volunteers. And the secondary objective was to confirm the safety of two doses of modified killed WC-OCV in healthy, non-pregnant adults and children volunteers.

The enrolment of a total of 356 subjects has been completed for the main part of the project. Recruitment of 30 subjects for an exploratory study for assessment of other immunologic assays (Elispot, Fecal IgA to LPS and Plasma IgA to LPS) were completed. Laboratory analysis were done. Data entry was completed through Remote Data Capturing system (RDSys). Analysis is ongoing.

Diarrheal disease in infants and young children in developing countries in collaboration with University of Maryland (funded by Bill and Melinda Gates Foundation)

Investigators : D. Sur, B. Manna, and T. Ramamurthy

This project was aimed to find out the etiological burden of enteric pathogens among children less than five years suffering from moderate to severe diarrhoea in an urban slum community. The target was to identify the children with diarrhoea in less than 5 years age group along with selection of controls (Fig. 4). Case and controls were compared for pathogen profile as well as impact of diarrhea on their nutritional status (Fig. 5).

Through this study NICED could able to identify the major pathogens in under 5 population in urban slums of pathogens causing moderate to severe diarrhea. The major pathogens are rotavirus, Cryptosporidium, *Shigella* Spp, *E. coli*, and adenovirus (Table 2).

Fig. 4: Mother feeding the child

Fig. 5: Anthropometric measurements taken for the children

Table 2

Top 5 Attributable Pathogens and Pathogen specific disease burden(attributable cases in DSS /1000 child year)

		0-11 month	Bur den	12-23 month	Bur den	24-59 month	Bur den	Total	Bur den
Top 5 attributed pathogens	1	Rotavirus	283	Rotavirus	155	C. jejuni	54	Rotavirus	99
	2	Cryptosporidium	126	Shigella (any)	39	Rotavirus	44	Cryptosporidium	32
	3	C. jejuni	67	Cryptosporidium	35	Shigella (any)	36	Shigella(any)	20
	4	adenovirus - (non40/41)	39	adenovirus - (non40/41)	30	V. cholera O1	32	ETEC (LT/ST or ST)	18
	5	ETEC (LT/ST or ST)	30	ETEC (LT/ST or ST)	29	ETEC (LT/ST or ST)	26	adenovirus - (non40/41)	12
% of all attributed pathogens			51		42		42		42

Health care utilization and attitude survey regarding diarrhea among parents of children < 5 years: a cross sectional community based study.

Investigators : D. Sur, S. Kanungo, and B. Manna

This study was aimed to provide information on where parents seek care when their children have diarrhea, their attitudes and practices concerning diarrhea as well as public perception of the need for enteric vaccines. Altogether 900 interviews were conducted involving caretakers of under-5 children in the slums of 4 administrative wards (29, 30, 33 and 14) of Kolkata Municipal area and 2 wards (14 & 15) of Bidhannagar Municipal area. Caretakers of under-5 children living in a household having at least one under-5 child in the selected slum area were recruited for interviews after providing informed consents. The study area was divided into two subdivisions: Non-naïve (ward no. 29, 30, 33 and 14 of Kolkata Municipal area) and Naïve (ward no. 14 & 15 of Bidhannagar Municipal area) based on presence/absence of ongoing diarrhea surveillance projects in the area and 450 interviews (150 in each strata of age in months: 0 to 11, 12-23 and 24-59) were conducted in each of the areas. Analysis of the collected data is under process. Dissemination of the gathered knowledge is also being planned in the form of scientific communications.

Rationality of prescription habits by the health care providers for treatment of diarrhea (especially cholera) in urban slums of Kolkata: an observational study.

Investigators : S. Kanungo, D. Sur, and B. Manna

This was an intra-mural project planned to study the knowledge and practice of health care by local health care providers about diarrhoeal disease with special reference to cholera in urban slums of Kolkata, and to study their treatment pattern, especially antibiotic use for

diarrhea as well as cholera. The study showed qualified and Government physicians had better knowledge regarding diarrhoea Better knowledge was associated with a lower likelihood of prescribing antibiotics for diarrhoea (OR 0·72, P<0·001), cholera (OR 0·78, P=0·027) and investigative procedure (OR 0·85, P=0·028). In the slums of Kolkata, diarrhoea-related knowledge and practice were poor with the exception of qualified physicians, hence an improvement in the knowledge of pharmacists and unqualified practitioners is necessary for the overall improvement of diarrhoeal management in these slums.

Awards and Honours

D. Sur

- Elected All India Secretary General of Indian Public Health Association 2013- 2016.

S. Panda

- Selected as an expert member of the 'Task Force on Infectious Disease Biology' of Department of Bio-Technology (DBT), Government of India.
- Invited as a technical expert in the 'South to South HIV/AIDS Resource Exchange (SHARE Project) administered by Voluntary Health Association Services (VHS) and supported by USAID.
- Recommendations based on the study findings generated by Dr. Samiran Panda and his team on anemia in women and under-nutrition in children and disseminated to the District Magistrate, Chief Medical Officer of Health (CMOH), Block Development Officers Panchayat Pradhans and other stakeholder of the district of South 24 Parganas were translated by 'Save the Children' and 'Sunderban Social Development Centre' into intervention development.

S. Kanungo

- Elected as editorial board member of Indian Journal of Public Health.

Conferences/ Seminars/ Workshop /Training Attended/ organized

D. Sur

- Speaker at 12th International Advanced Course on Vaccinology in Seoul, Korea on May 1, 2012.
- Annual Meeting for the PROVIDE Study on May 16-17, 2012 at Baltimore, Maryland, USA

- Consultation on vaccines for enteric diseases: an update on latest advancements and a potential WHO research agenda' in Geneva on May 24-25, 2012
- Ethics and policy conference for single dose OCV trial' at Philadelphia on May 30-31, 2012
- Global Enteric Multicentric Study (GEMS) International Strategic Advisory Committee (ISAC) on November 11, 2012 at Atlanta USA
- Speaker at 47th Annual Joint Panel Meeting on Cholera & Other Bacterial Enteric Infections, a United States-Japan Cooperative Medical Science Program during December 12-14, 2012 in Chiba, Japan.
- Attended South East Asian Conference and 56th Annual Conference of Indian Public Health Association, at Science City, Kolkata on February 1-3, 2013 and presented papers on 'Cholera Vaccine trial' and 'GEMS study'

S. Panda

- Speaker at 5th National Conference of 'AIDS Society of India' during November 23-25, 2012.
- Co-ordinated one-day training for medical students on June 2, 2012 at NICED-II building and also at the Laboratories located at JICA building of NICED including the animal house. This was arranged at the formal request of 'Indian Medical Students Association' (IMSA). The training workshop witnessed participation of 72 medical students coming from different parts of the country.
- Guided a post-graduate student (MD in Community Medicine) who was awarded degree under the West Bengal University of Health Sciences, following successful completion of his dissertation work.

K. Sarkar

- Conducted two Training courses (each of 3-day duration) for Field Investigators and Field workers of DLHS-4 project on Clinical Anthropometry & Biochemical component. A total of 60 participants were trained.
- As requested by the Department of School Education, Govt. of West Bengal, an assessment of impact of Cooked Midday Meal Programme was done for the primary & upper primary school students of Uttar Dinajpur District as principal Investigator.
- Supervising the activities of National Monitoring Bureau-West Bengal Unit as Officer-In-Charge in coordination with National Institute of Nutrition, Hyderabad. The unit is currently assessing the prevalence & risk factors of Diabetes. Hypertension & Dyslipidaemia in different urban cities of West Bengal.

- Conducted two training courses & prepared a manual on community-based malaria surveillance for field workers & supervisors at Purulia & Jalpaiguri Districts in collaboration with District Health Authorities and 'GOL'– an international NGO working in West Bengal.

S. Kanungo

- Presentation in the meeting "Oral cholera Vaccine Trial Ethics Meeting organized by Center for Vaccine Ethics and Policy, University of Pennyselvia, USA and International Vaccine Institute, Seoul, Korea in Philadelphia, USA from May 30-31, 2012.
- Nominated and attended the Training workshop on 'Clinical Trials Designs and Statistical Methods' Organized under Clinical Investigator Development Program, by the One World Health-PATH and CDSA at Department of Biostatistics, Christian Medical College, Vellore July 9-14, 2012 and awarded 30 credit units as CME.
- Participated in the Clinical Investigator Development Program, a CDSA-NIH joint Course on "Principals and Practice of Clinical Research" New Delhi, October 29 - November 3, 2012.
- Oral presentation on the topic "Is vibriocidal response a good correlate of protection for a killed whole-cell cholera vaccine: evidence from a phase 3 trial" at the 47th Annual Joint Panel Meeting on "Cholera and Other Bacterial Enteric Infections" held in Chiba, Japan December12-14, 2012.
- Participant in the workshop "Ethics in Clinical Research" as a part of Clinical Investigator Development Program, organized by PATH and CDSA, in Seth GSMC &KEM Hospital, Mumbai from January 7-11, 2013.
- Faculty at training workshop on the "Global Food borne Network" in NICED organized by the National Centre for Disease Control Govt. of India and Centre for Disease Control USA on February 14-16, 2013.
- Attended the workshop on Good Clinical Practice, organized by NICED, Kolkata April 1-2, 2013.
- Expert in the meeting organized by Dept. of Biotechnology, Govt. of India, THSTI, Govt. of India and BIRAC, Govt. of India on " Cholera, Typhoid and Polio Vaccine-From Products to Policy to Practice" on April 4, 2013 at New Delhi, India.

IMMUNOLOGY

The Division of Immunology is engaged in evaluating porin of *Shigella dysenteriae* as an adjuvant. Porins are major outer membrane proteins with pore-forming ability that are strongly immunogenic and can augment humoral response. *Shigella dysenteriae* is a Gram-negative bacterium and significant cause of shigellosis against which a vaccine is being sought. Our study of adjuvanticity of porin of *Shigella dysenteriae* type 1 established the protein primarily as a Toll-like receptor (TLR)2 ligand in association with TLR6. The TLR signaling initiates with MyD88 up-regulation leading ultimately to NF-& κB-dependent chemokine and type 1 cytokine expression. B-1 cell populations have shown porin specific IgA expression, the signature molecule of mucosal immune response. Like B-1 cells, peritoneal cavity B-2 cells expressed IgG2a and IgA. This led us to investigate how splenic B-2 cells, categorized as Follicular (FO) and Marginal Zone (MZ) B cells react to the protein as the ultimate responder to the adjuvant. Expression of proinflammatory cyktoines by FO B cells and anti-inflammatory cytokine by MZ B cells shows porin parallely and differentially dictates maturation of these B cell subsets that includes expression of IgA in particular. Summing up porin can activate systemic B cells and generate antibody response thereby indicating it is a successful adjuvant connecting innate and adaptive immune system.

Scientist :

Dr. T. Biswas, *Scientist 'F'*

Staff :

S. K. Shaw, *Technician 'B'*
N. C. Mondal, *Attendant Staff*

Research Scientist :

Dr. R. Biswas

Pre-Doctoral Fellow :

S. Mukherjee
D. Sinha
A. K. Ghosh

Porin - induced regulation of marginal zone and follicular zone B cells to elicit cytokine and antibody response

Investigator : T. Biswas

Splenic B-2 cells, categorized as Follicular (FO) and Marginal Zone (MZ) B cell respond to porin serving as a good correlate for immune protection. Porin treated FO B cells showed up-regulation of TLR2 and -6, in contrast MZ B cells did not express the TLRs. The early activation markers CD69 and CD25 were up-regulated besides CD40 and MHC II ($I\text{-}A^b$) on FO B cells, indicating potential of the cells to receive cognate or bystanding T cell help. FO B cells showed up-regulation of IL-12, IFN-γ and TNF-α whereas, MZ B cells specifically expressed

IL-10 over untreated control. Expression of proinflammatory cytokines by FO B cells and anti-inflammatory cytokine by MZ B cells indicate porin parallely dictates differential maturation and response of the splenic B cell populations. Blocking experiments have showed TLR2/6 dependent drop in IL-12, TNF-α and IFN-γ expression, indicating the central role played by TLR2/6 together in initiation of signaling in porin mediated response of FO B cells. It is universally accepted that TLR agonists are able to augment antibody response in these B cells, leading us to question whether porin; the key player in mucosal immunity could induce immunoglobulin response in these B cells of systemic origin. Porin is capable of generating IgA, the major immunoglobulin class that participates in the mucosal immune response, in addition to IgG2a and IgG2b in FO B cells. Summing up, this study on one hand highlight the potential of porin as successful adjuvant that has the capability to turn on the adaptive immune system and on the other hand also shows immune counter regulation which is also an important aspect with IL-10 playing an uniquely important role in such mechanisms.

Awards and Honours

T. Biswas

- Acted as invited reviewer for Infection and Immunity and Indian Journal of Medical Research (2013)

PARASITOLOGY

The Department of Parasitology at NICED actively integrates research into the mechanisms of parasitic diarrheal diseases at the molecular and cellular levels with epidemiological studies. While ensuring an increasing understanding of human parasitic diseases, like amoebiasis, giardiasis, cryptosporoidis etc., this provides the foundation for further developments in screening, diagnosis and future therapeutics. One of the major present focus is on the molecular analyses of rRNA biogenesis, mechanism of its formation and its use as a drug target in Giardiasis. Other works also include study the effects of oxidative stress on microaerophilic Giardia at its cellular, genome, proteome and metabolomic level to understand its pathogenic nature during stress response and its regulation, molecular diagnosis and disease pattern recognition of different enteric parasites including opportunistic coccisidians.

During last year enormous genetic diversity of giardiasis among diarreaogenic population of Kolkata has been identified alongwith zoonotic transmission between human and other mammals. It was also reported that this new genetic polymorphism in Giardia is actually a result of a mixed assemblage. A new high resolution genotyping of *Entamoeba histolytica* using its short tandem repeats of tRNA loci showed its efficacy in assessing its virulence and pathogenecity. Based on this genotyping new Indian pathogenic isolates have been identified. It has been observed for the first time that Giardia trophozoites at high oxygen environment produces 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. This is the first demonstration of a possible link in survival of the parasite in high oxygen environment during its pathogenesis.

Scientist :

Dr. S. Ganguly, *Scientist 'D'*

Staff :

Mr. T. N. Boral, *Technical Officer*
Mr. S. L. P. Singh, *Technician 'B'*

Pre Doctoral Fellow :

A. K. Mukherjee
K. Das
D. Raj
S. Karmakar

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

Investigator : S. Ganguly

The study was formulated to assess the extent of genetic diversity among the clinical *Entamoeba histolytica* isolates which have similar diseases outcome. Two tRNA linked loci and two protein coding regions were analyzed and immense genotypic variation were observed between two cases. Some unique short tandem repeat patterns were also found which were never reported before. Moreover the tRNA linked intergenic regions used in our study being the recombination hotspot we have tried to hypothesize the evolutionary pathway of generation of new STR types depending on these locus.

We all know that tRNA linked STR genotyping is one of the most advanced technique for determining the genotype of a wild/clinical *Eh* isolates, but the major problem or drawback of this technique is its high resolution for differentiating isolates. It always produces an immense variety of genotypes that becomes very difficult to be differentiated into groups or taxon. By describing the possible pathway for the generation of new genetic pattern from the existing or vice versa, we have tried to generalize the whole genotyping result and in spite of the genetic variability we could assign few specific genetic groups within the population (Fig. 1). This particular approach could play a very important role in the future genotyping studies.

Studies on burden of parasitic infections among different communities in western part of India to support health impact evaluation of total sanitation campaign

Investigator : S. Ganguly

This study was performed from samples from different parts of semi urban and rural western India (Western Madhya Pradesh, Maharashtra). Recent technological advances of parasites and helminthes have improved our understanding of host specificity, clinical manifestations and transmission mechanisms, although no previous information is available in this part of India in different communities about their incidence, prevalence and disease burden. The overall objective of this study is to gather basic epidemiological data on the prevalence, disease burden and transmission of different helminthes and parasites in children below 12 years of age. Kato katz method was applied for helminth detection while microscopy and PCR was adapted for other diarrheagenic parasites. The principal aim of this impact evaluation is to estimate the impact of the methods/tools (interventions) such as Community Led Total Sanitation (CLTS) implemented under the auspice of *Government of India's Total Sanitation Campaign (TSC)* on the health and welfare of the rural poor. It was suggested that the burden of helminthes were about 30% of which Ascaris was the major helminth followed by hookworm. Among other parasites Giardia and Cryptosporidum were most common followed by Entamoeba, though, rate of Giardia and Cryptosporidium was little lower than found in Eastern and Northern parts of India.

Differential pathogenesis of Giardia : role of Giardia virus

Investigator : S. Ganguly

68 Giardia positive stool samples were randomly taken from the surveillance program of IDBG hospital and were subjected to multi-locus genotyping (Table 1). The DNA was extracted directly from the positive stools using StoolDNAMiniKit (QIAGEN, USA) according to the manufacturer's protocol. A portion of ß-giardin (*ßg*) [511 bp] on 90 kb long contig ctg02-35, Glutamate dehydrogenase (*gdh*) [434 bp] on 231 kb long contig ctg02-15 and *Triose phosphate isomerase* (*tpi*) [530 bp] on 200 kb long contig ctg02-19 (www.Giardiadb.org), were individually amplified nested PCR. The nested PCR products were separated in 1.5% (w/v) agarose gel and purified by gel cut purification process using High Pure PCR purification Kit (Roche, Germany) as per the manufacturer's protocol. Bi-directional sequencing was performed with the respective purified products and nested PCR primers on an ABI 3100 automated sequencer by using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, USA). The sequences obtained from all three loci (*ßg*, *gdh* and *tpi*) were validated using the database BLAST search (i.e. NCBI and *Giardia*db) and were submitted to NCBI GenBank (ACC no. JF918436 – JF918523 & JN647526 – JN647641). The sequences from each locus were separately aligned by using 'MEGA Version 4' software and were manually checked and edited. Previously reported sequences of the respective loci representing different *G. duodenalis* assemblages were included in the analysis to get a better resolution of the assemblage distribution. The extent of sequence diversity among the wild isolates based on the target loci was determined using the 'Maximum Composite Likelihood' method through the MEGA4 software. Based on the cumulative sequence data of all three loci, 41 samples could be assigned as assemblage 'B' (60.2%) and 13 as assemblage 'A' (19.1%), while 14 (20.5%) isolates showed multiple assemblages depending on the marker loci. Viral RNA was isolated by using Viral RNA Minikit, Qiagen. cDNA preparation and PCR amplification was performed by using Superscript-III One Step RT-PCR kit, Invitrogen. Although the primers were designed against the GLV capsid protein but most of the PCR products with variety of PCR conditions were non-specific in nature. Few DNA bands from the desired base pair were purified and sequenced with the specific primers but the results obtained were not desirable. The sequence obtained has no significant identity with reported cds of GLV capsid sequence. (alignment provided as FASTA file named GLV wg_cap_4F). New PCR primers were designed targeting the conserved region of GLV capsid protein.

Table 1. Estimation of average sequence diversity

Target locus					
ß giardin (*ßg*)		Glutamate dehydrogenase (*gdh*)		Triose phosphate isomerase (*tpi*)	
N	*D* ± *SE*	*N*	*D* ± *SE*	*N*	*D* ± *SE*
68	0.001 ±0.004	67	0.053 ±0.011	64	0.106 ±0.019

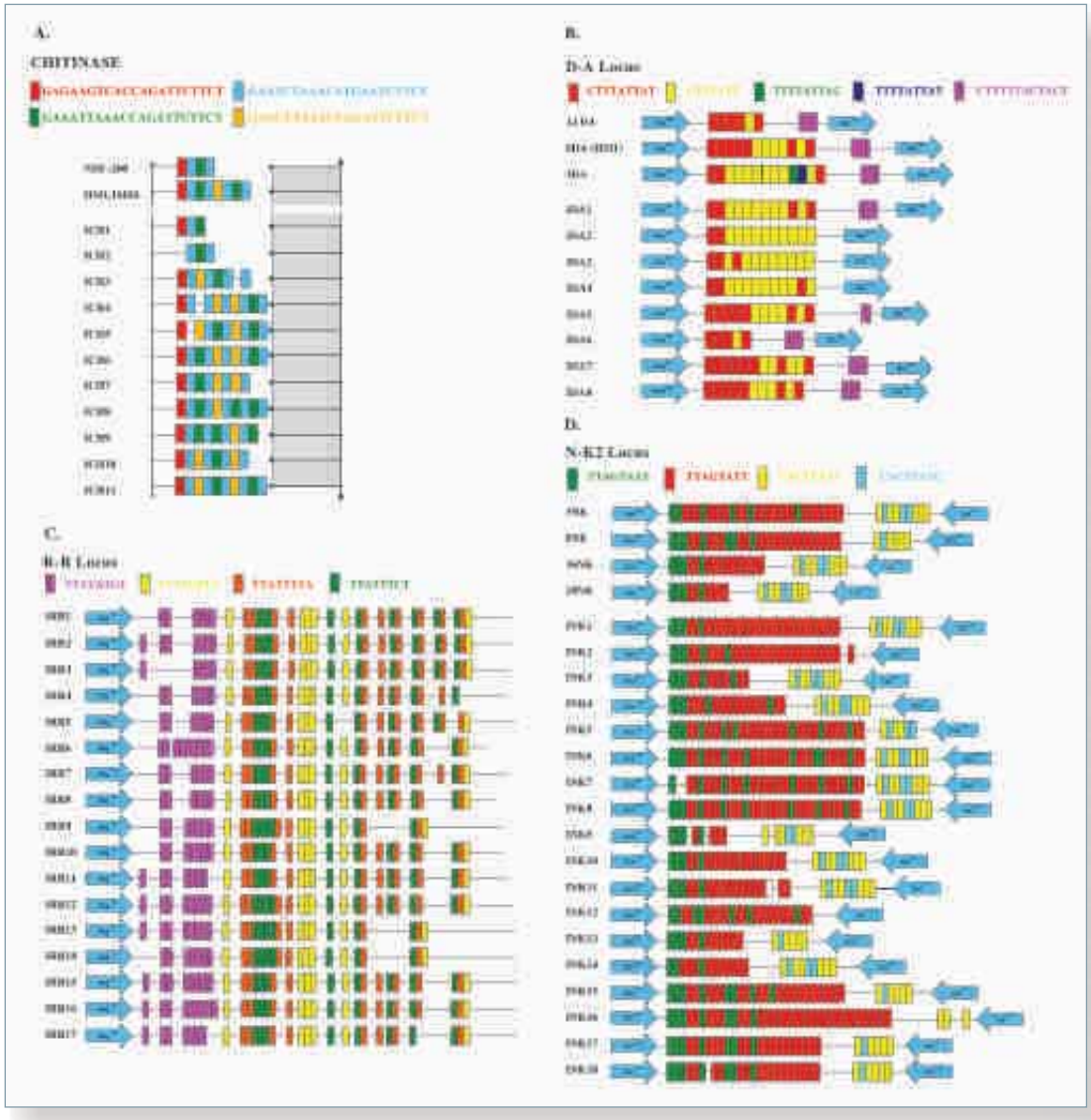

Fig. 1.t-RNA linked short Tandem Repeats (STR) analysis for pathogenic E. histolytica local strains shows diverse variability at different STR loci in Indian samples

Award and Honours

S. Ganguly

- Nominated committee member, board of studies of Department of Microbiology at Saint Xavier's College, Kolkata (Autonomous) .

Conferences/ Seminars/Workshops /Trainings Attended/Organised

S. Ganguly

- Delivered a talk in the 12th International Workshops on Opportunistic Protists held in Tarrytown, New York, USA during August 5-9, 2012.

PATHOPHYSIOLOGY

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

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.

Proteases play a major in pathogenesis in bacterial infection. The major protease secreted by *Vibrio cholerae* is hemagglutinin protease (HAP). The matured 45 kDa and 35 kDa processed forms of HAP were purified from a *ctx* gene negative *Vibrio cholerae* O1 strain. The 35 kDa HAP showed hemorrhagic fluid response in a dose dependent manner in the rabbit ileal loop assay. Almost all results of earlier studies suggest an indirect pathogenic role of HAP; we showed the direct role of HAP in pathogenesis. We also showed the presence of a novel 59-kDa serine protease in a *hapA prtV V. cholerae* O1 strain and its role in hemorrhagic response in RIL model.

The other research interests include to understand how intestinal ion transport is being regulated both normally as part of normal digestive physiology and how this becomes abnormal in diarrhoeal diseases. In future we will explore a better understanding of new targets involved in restitution of transport process and barrier function and the development of agents that specifically modify these targets in alleviating patients suffering from diarrhoeal diseases.

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 :

Dr. M. K. Chakrabarti, *Scientist 'F'*
Dr. A. Pal, *Scientist 'E'*

Staff :

B. Roy, *Technician 'B'*

DBT Ramalingaswami Fellow :

Dr. M. K. Hoque

Pre-Doctoral Fellow :

P. Karmakar
R. Tapader
Sk. Irshad Ali
R. Bhowmick
A. Mondal
P. Sarkar

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

Investigator : M. K. Chakrabarti

In our earlier studies we have shown 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, established itself as highly immunogenic. Moreover, 34 kDa protein has been found to up regulate the expression of adaptor protein MyD88, p38 MAP kinase, NF-kB, production of type-1 cytokines and chemokines as well as other molecules (MHC II, CD40 and CD80) known to modulate the adaptive response towards Th1 type in macrophages. As the yield is very low in traditional purification procedures, we have cloned and over expressed the 34 kDa protein from *S. flexneri* 2a (N.Y-962/92) genomic DNA. MALDI-TOF MS analysis of the purified 34 kDa OMP of S. flexneri 2a shows considerable sequence homology (Identity 65%) with the OmpA of *S. flexneri* 2a. Immunogenicity and protective efficacy of the recombinant OmpA has been evaluated in an intranasally immunized murine pulmonary model. Results demonstrate that the expressed OmpA of S. flexneri 2a induces strong immunogenicity and protective efficacy in a murine model of intranasal challenge (Fig. 1). Further study revealed that OmpA induced release of proinflamatory cytokines and NF-kB activation in TLR2 transfected HEK 293 cells and RAW macrophages is TLR2 dependent (Fig. 2). Transfection of RAW 264.7 macrophage with TLR2 SiRNA functionally knockdown the cellular response to OmpA by significant reduction in the activation of NF-kB and other factors known to modulate the adaptive immune response such as macrophage presentation MHCII & CD80 molecules as well as production of NO and IFN-γ release in macrophage:$CD4^+$T cells co-culture (Fig. 3). Furthermore in vivo studies demonstrate that intranasal immunization of mice with OmpA selectively enhances the release of IFN-γ IL-2 by $CD4^+$ T cells. Addition of inhibitory anti-IL-12p70 mAb efficiently inhibits IFN-γ production by the Ag-primed splenocytes. Moreover, coincubation with OmpA-pretreated macrophages enhances the production of IFN-γ by OmpA-primed $CD4^+$ T cells. Taken together these results suggest that reduced $CD4^+$T cell activation might be associated with reduced IL-12 signalling to the $CD4^+$ T cells from IL-12 inhibited and TLR2 knockdown macrophages as well as lessened presentation of OmpA by the MHCII pathway because of reduced secretion of NO and decreased expression of MHCII and CD80. In conclusion TLR2 is the critical regulatory molecule in initiating the host innate response

required for optimal CD4^{+} T cell responses to OmpA. Moreover, OmpA of S. flexneri has been identified as a novel molecule coordinating the innate and adaptive immune responses, hence proving itself as an optimal vaccine candidate.

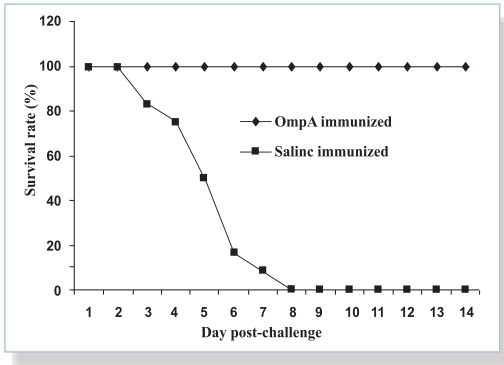

Fig. 1. Mice survival rate after immunization with recombinant his-tag OmpA. Two groups of 20 mice were immunized with normal saline or OmpA. Mice were immunized intranasally at 2-week intervals with 3 μg of OmpA in each immunization. Three weeks after the final immunization, all animals were intranasally challenged with 1×107 CFU of S. flexneri 2a. Figure representing the result for 16 mice from each group, as 4 mice from each group were sacrificed within 24 h after challenge for histopathology and cytokine assay. Percent survivors are plotted for each of 14 days post challenge. Pvalue, calculated by the Fisher exact test and is < 0.001.

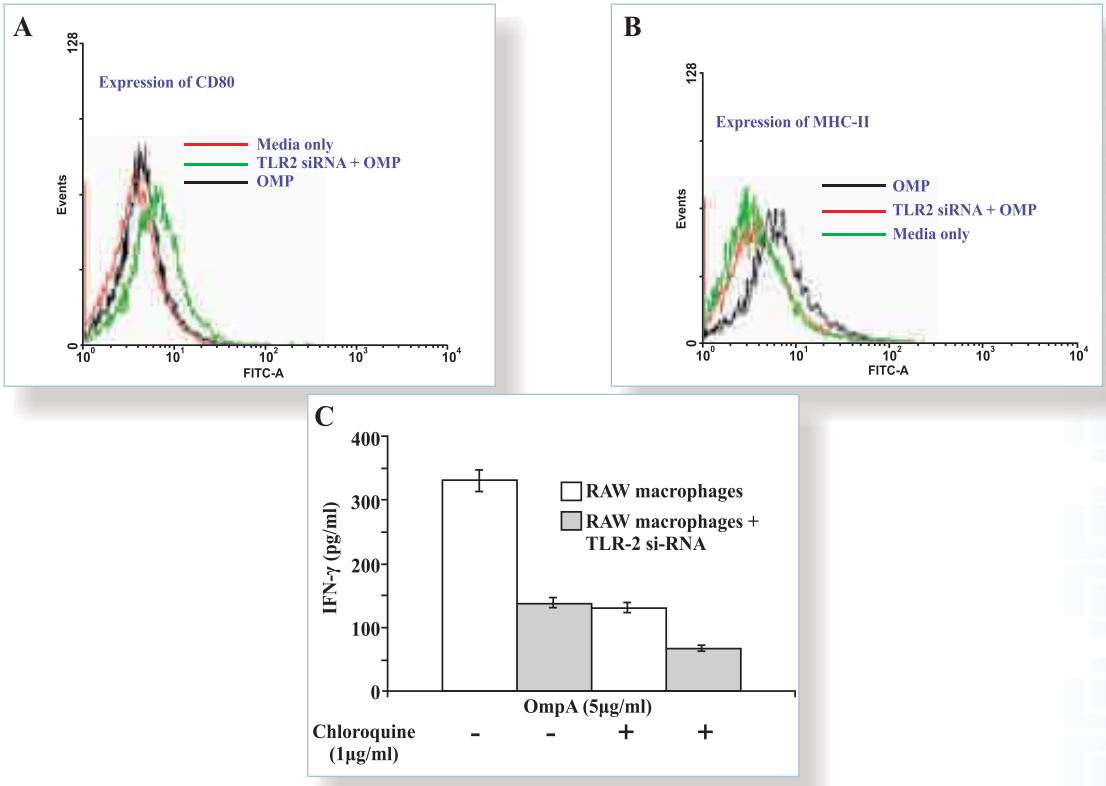

Fig. 2. ***A TLR2 is critical for OmpA triggered expression of MHCII (A) and CD80 (B) on RAW264.7. RAW264.7.*** *Macrophages were incubated with and without OmpA or transiently transfected with TLR2 siRNA for 48 h prior to treatment with OmpA for 6 h. Cells were harvested and assayed for cell surface expression of MHCII and CD80. Representative data from three independent experiments are shown. C, involvement of both innate and adaptive immune responses in the OmpA-induced release of IFN-_ by CD4_ T cells. Wild type and TLR2 knockdown RAW264.7 macrophages were co-cultured with CD4_ T cells for 24 h and incubated without or with OmpA in the presence or absence of chloroquine. The cell-free supernatants were assayed for IFN-_ after 24 h of incubation. Data represent mean S.E. of three independent experiments, p 0.005.*

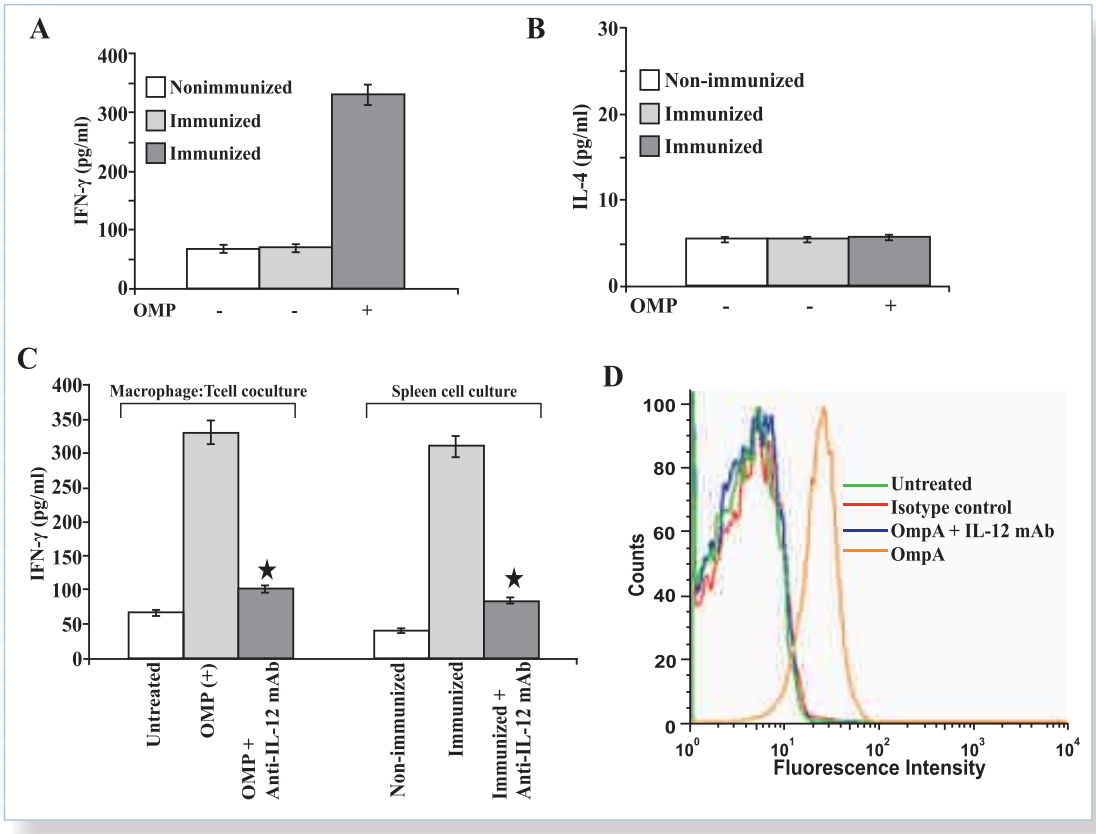

Fig. 3. Macrophages pretreatedwithOmpAenhance IFN-γ production by OmpA-primed CD4⁺ T cells. Peritoneal macrophages of BALB/c mice were preincubated in the presence or absence of OmpA. After 6 h, the cells were washed and co-cultured with either saline-immunized or OmpA-immunized CD4⁺ T cells (10^6 cells/well) for 4 days. The culture supernatants were harvested and then the released IFN-γ (A) and IL-4 (B) were determined by ELISA. C, IL-12p70 is the critical factor involved in the OmpA-induced IFN-γ production in both the peritoneal macrophages:CD4⁺ T cell co-culture and OmpA-primed spleen cell cultures. Macrophages were stimulated in the absence and presence of OmpA or preincubated with neutralizing IL-12p70 mAb for 1 h separately prior to addition of OmpA followed by co-culture with OmpA-primed CD4⁺T cells. In addition, OmpA-immunized and -nonimmunized spleen cells were restimulated with OmpA in the presence of neutralizing IL-12p70 mAb. Cell-free supernatants were collected 4 days later and assayed for IFN-γ production by ELISA. D, multicolor flow cytometric analysis of the intracellular IFN-γ expression after gating on CD4⁺ T cells from the macrophage:CD4 T cell co-culture. Macrophages were cultured with and without OmpA or preincubated with neutralizing IL-12p70 mAb for 1 h separately before stimulation with OmpA followed by co-culture with OmpA-primed CD4 T cells. After 3 days cells were recovered and stained for IFN-γ expression.

Studies on proteases of *Vibrio cholerae*

Investigator : A. Pal

The major protease secreted by Vibrio cholerae is hemagglutinin protease (HAP). The matured 45 kDa and 35 kDa processed forms of HAP were purified from a ctx gene negative *Vibrio cholerae* O1 strain. Almost all results of earlier studies suggest an indirect pathogenic role of HAP; this is the first study to show the direct role of HAP in pathogenesis. PrtV a

metalloprotease other that HAP has a role in the protection from predator grazing in natural aquatic environments and has also has a role in human pathogenicity. The *hapA* and *prtV* knock out mutant, *V. cholerae* O1 strain CHA6.8 prtV still retains residual protease activity. The residual protease secreted by CHA6.8 *prt*V was partially purified 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. Our results show the presence of a novel 59-kDa serine protease in a *hapA prtV V. cholerae* O1 strain and its role in hemorrhagic response in RIL model. Many Gram-negative bacterial species can extend their pathogenicity by releasing outer membrane vesicles (OMVs) which can expose host cells to relatively high concentrations of toxins and additional virulence factors without the requirement of a close contact between the bacterial and target human cells.Our results show that hemagglutinin protease is also transported through the outer membrane vesicles of *Vibrio cholerae*. Using protease knock out mutants in mice ileal loop assay we have shown that the 59 kDa serine protease is also secreted through the OMVs. Further work is in progress to show the transport and role in pathogenesis of proteases through the OMVs.

Awards and Honours

M. K. Chakrabarti

- Served as the General Secretary (HQ) of Indian Science Congress Association 2010-2013.
- Convener of the Section of medical and veterinary sciences, 2008-2010, 2010-2012, West Bengal Academy of Science and Technology and conducted memorial lectures and other scientific activities in different institute including NICED.
- As Vice-President of The Physiological Society of India, 2010-2014 was deeply involved in the scientific activities of the Society.
- 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.

P. Karmakar

- Smt. P. Karmakar, SRF, was awarded ISCA young Scientist award,2013 at the Section of Medical Sciences(including Physiology), ISCA

Conferences/ Seminars/ Workshop /Training Attended/ organized

M. K. Chakrabarti

- Delivered invited lecture on 'Vaccine Against Enteric Pathogens' at UGC 94th Orientation course for College and University Teachers, Academic Staff College, Calcutta University, Kolkata on July 13, 2012 as a resource person.
- Delivered invited lecture on 'Environmental factors and Infectious Diseases' at UGC 95th Orientation course for College and University Teachers, Academic Staff College, Calcutta University, Kolkata on July 27, 2012 as a resource person.
- Delivered keynote address on 'Present status of vaccines against shigellosis' at a seminar of ISCA Bangalore Chapter on August 30, 2012.
- Delivered keynote address on 'Pathophysiology of Diarrhea' at a seminar of ISCA Kanpur Chapter on October 03, 2012.
- Delivered keynote address on 'Impact of climate on the Spread of Infectious Diseases' at a conference on 'Science for Shaping the Future of India' organized by ISCA Jaipur Chapter on October 06, 2012.
- Delivered invited lecture on 'Probiotics: A New Approach to Gastrointestinal Health and Disease' at Victoria Institution (College), Kolkata on October 16, 2012.
- Delivered invited popular lecture on 'Water borne microbial Diseases' at Purba Barasat Adarsha Vidyapith, North 24 Pgns. on November 07, 2012.
- Delivered invited lecture on 'Diarrheal diseases: An unending journey' at Patharpratima College, South 24 Pgns.,on March 22, 2013.

A. Pal

- Delivered a talk on "Studies on role of proteases in pathogenicity in *Vibrio cholerae*" in the 100th Indian Science Congress held on January 3-7, 2013 at Kolkata.

VIROLOGY

The researchers and staff of Division of Virology are involved actively in the surveillance studies undertaken by the National Institute of Cholera and Enteric Diseases to understand the etiological role and disease burden of different diarrhoeagenic viruses in and around Kolkata. Molecular phylogenetic analysis of the circulating enteric viruses is being carried out with focus on Rotaviruses, Caliciviruses viz. Norovirus and Sapovirus, Astroviruses, Picobirnaviruses and Adenoviruses to a study their genetic diversity and monitor the emergence of new strains and variants in a stringent manner. The basic research activities cater towards understanding functional aspects of host Rotavirus interaction through analysis of the signaling mechanisms activated during infection. In addition functional role of rotaviral non structural proteins (NSPs) in modulating cellular innate immune responses and host proteins which play a positive role during rotavirus infection is being studied.

The Division has also extended its activites 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 Influenza virus strains. The Division also maintains necessary laboratory facilities to carry out diagnosis during sudden Influenza outbreaks for effective patient management. The Virology Division has also made notable contribution to understand the transmission of HIV from injecting drug user's to their spouses in North eastern states and conducted invaluable research to unravel the molecular aspects of HIV strains among infected individuals and their partners.

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 diseases of national importance across the country. The research programs include intramural projects and extramural projects with national and international funding and collaborating scientists. The current programs are associated with DBT, ICMR, WHO, CDC Atlanta and Okayama University, Japan.

Scientist :

Dr. S. Chakrabarti, *Scientist 'G' and Director-in-Charge*
Dr. T. Krishnan, *Scientist 'E'*
Dr. M. Chawla-Sarkar, *Scientist 'D'*
Dr. B. Ganesh, *Scientist 'C'*

Staff :

S. Omesh, *Technical Officer 'A'*
M. Mullick, *Technical Assistant*
K. Sen, *Technician 'C'*
P. De, *Technician 'B'*
MD Musharraf Hossain, *Technician 'B'*
B. K. Bera, *Technician 'B'*

Post-doctoral Fellow :

Dr. A. Mukherjee
Dr. R. Sarkar

Pre-Doctoral Fellow :

M. Pativada
S. Chattopadhyay
S. Nandi
U. C Halder
R. Bhowmick
S. Chanda
P. Mandal

Characterization of accessory genes *vpr, vpu* of HIV-1 among female injecting drug users from north-eastern states of India

Investigator : S. Chakrabarti

North-Eastern states of India, Manipur and Mizoram are reported to harbor a large number of HIV positive cases. The rising rate of HIV infection in these states might be due to close proximity of neighboring countries like Myanmar and Thailand from where drugs like heroin are smuggled. The major cause of spreading infection among these IDU's is due to sharing of needles among each other. India is reported to have subtype C as the predominant strain of HIV-1. Information on the genetic subtypes of HIV-1 is important for understanding of the global evolution of HIV-1 and for vaccine development. The genetic diversity of HIV-1 has mainly been characterized by analysis of the env and gag genes. To date, there are limited studies on genetic diversity of accessory genes (*vpr* and *vpu*) from north-eastern part of India. Among other studies the different rate of progression to AIDS have been linked to HIV-1 accessory genes (*vif*, *vpr* and *vpu*) since they have been shown to impact on viral replication and pathogenesis of HIV-1. Thus, it is important to study the accessory genes and assess sequence diversity and evolution of these genes as HIV-1 accessory genes are potential vaccine candidates. Identification of the different subtypes of HIV-1 accessory genes is an important aspect of HIV research in the context of vaccine design. Further molecular characterization of the HIV-1 accessory genes of those different subtypes at the structural and functional level is necessary in order to compare the phylogenecity of the virus, its replication potency, host relationship & viral fitness. In this context, study of the HIV-1 accessory genes which controls the regulation of the gene expression and the efficient viral propagation of those different subtypes is of utmost relevance.

A set of 27 HIV positive plasma samples were selected for phylogeny analysis of HIV-1 structural (env and gag) and accessory (*vpr* and *vpu*) genes to compare the genetic diversity of HIV-1 subtype C between accessory (*vpr* and *vpu*) and structural (gag and env) genes. The

samples were drawn from the female injecting drug users from the North-eastern states of India; Manipur & Mizoram. Amplicons of the gag, *env*, *vpr*, & *vpu* genes segment were purified by a QIA quick PCR purification kit (QIAGEN, Germany, and Hilden) and were subjected to cycle sequencing reactions using fluorescent dye-labeled di-deoxy nucleotides in an ABI PRISM 3100 automated sequencer following the manufacturer's protocol. The sequences were edited manually using BIOEDIT sequence alignment editor program (version 5.0.6; Department of Microbiology, North Carolina State University). The edited sequences were Blast searched and further aligned with the reference sequences from different geographic regions available in the HIV database for phylogenetic analysis using the Molecular evolutionary genetics analysis software version 4 (MEGA 4) and evolutionary distances were measured by a Kimura two-parameter distance matrix method. Phylogenetic analysis of the *vpr* gene of Manipur & Mizoram female injecting drug users with the reference subtype C and subtype B sequences clearly showed that all samples except FDU-17 clustered with Indian subtype C (Fig. 1). For FDU-17, *vpr* gene clustered with subtype B strains from Thailand. Same thing happened in case of another accessory gene *vpu* (Data not shown).Previous studies from Manipur detected the presence of Thai-B as the second major subtype after subtype-C, circulating among IDU's. Recent studies have also revealed the same in case of female injecting drug users but this time the analysis has clearly shown that the accessory genes *vpr* & *vpu* are more conserved than the structural genes *gag* & *env*.

Next the inter-sample sequence homology was evaluated with ClustalW software. The genetic diversity of HIV-1 subtype C was compared between accessory (*vpr* and *vpu*) and structural (*gag* and *env*) genes. The *gag* gene sequences were highly conserved (89% to 96%), as compared to *vpr* gene (84% to 94%), the *env* gene (83% to 93%) and finally the *vpu* gene (73% to 92%). The high variability of the *vpu* gene was seen towards the end of the gene, most probably due to primer binding region. We believe that the PCR amplicons for accessory genes could have contributed to the high variability of the *vpu* gene because the binding region of the reverse primer was very close to the end of the *vpu* gene, creating difficulties with interpretation of the sequence chromatograms of the *vpu*. In addition, a number of conserved regions were observed in all the four genes (*vpr*, *vpu*, *gag* and *env*). Generally *gag* and *vpr* showed to be more conserved. In addition, a number of conserved regions were observed in all the four genes (*vpr*, *vpu*, *gag*, and *env*). The genetic diversity between the genes was clearly seen on the phylogenetic analysis, with the *vpu* phylogeny more branched as compared to the env phylogeny, the *vpr* phylogeny, and lastly the gag phylogeny.

The comparison of accessory genes and structural genes revealed that generally accessory genes are more conserved than structural genes, with exception to the *gag* gene. *vpu* appears to be more diverse compared to *vpr* genes. *Gag* gene are conserved compared to *env* gene. Finally, the comparison of accessory genes and structural genes showed that gag, and *vpr* are more conserved as compared to *env* and *vpu* genes. The inter-sample sequence homology ranged from 89% to 96% for *gag*, 84% to 94% for *vpr*, 83% to 93% for *env* and 73% to 92% for *vpu* (Fig. 2).

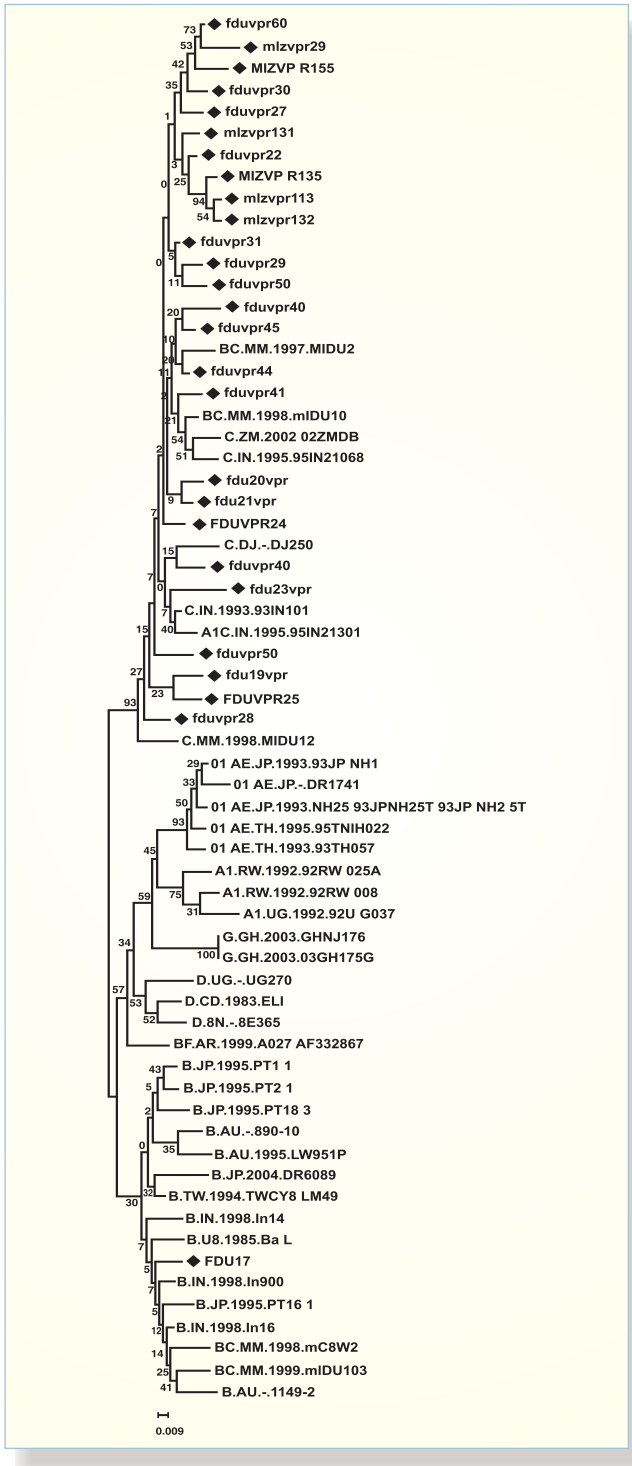

Fig. 1. Phylogenetic analysis of vpr gene of the HIV-1 strains isolated from the Manipur & Mizoram Female IDU samples

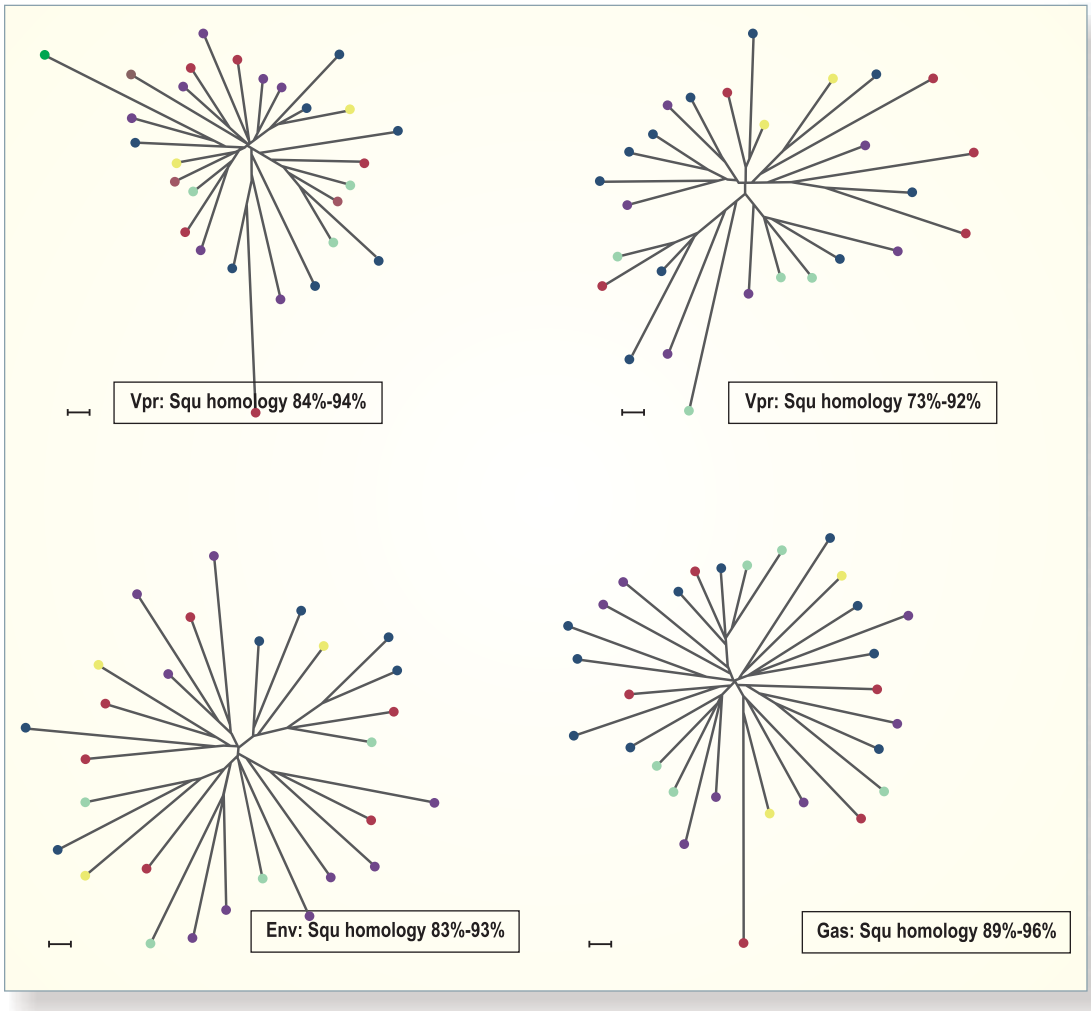

Fig. 2. Phylogenetic comparison between accessory and structural genes. The studied samples are shown in round shapes and references in squares

Studies on detection and molecular characterization of astroviruses among viral gastroenteritis cases

Investigator : T. Krishnan

Human astroviruses (HAstVs) were associated with acute gastroenteritis (AGE) among infants, younger children (up to 6 years), older children and adolescents (>6-17 years) and adults (18 years and above). Molecular characterization enabled monitoring of human astrovirus strains circulating among hospitalized diarrhea cases in Kolkata, India. Sole or mixed infections were detected among 60 cases (2.4%), among all age groups; mixed infections included other enteric viral, bacterial and parasitic pathogens such as Rotavirus, *Vibrio cholerae, Cryptosporidium spp* and *Giardia lamblia*. Eleven HAstV positives were analyzed for their sequences of overlap region between ORF1b (RdRp) and ORF2 (capsid).

Among these, ten strains were found to have close genetic relatedness to the Japanese strain HAstV_G1 [AB009985] and another Kolkata strain showed close genetic match with the Thai HAstV_G3 strain [EU363889] as shown in Fig 3.

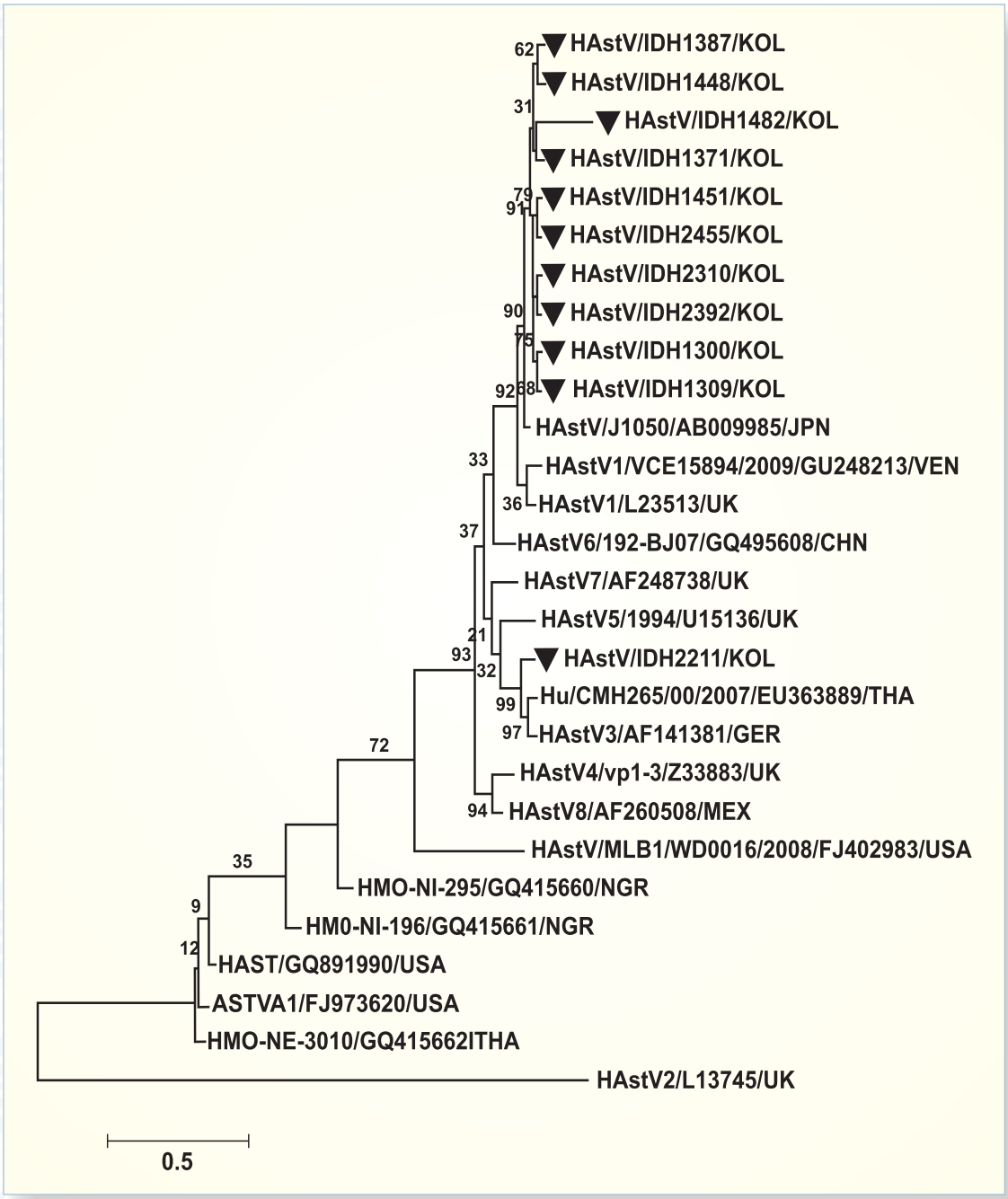

Fig. 3. Phylogenetic analysis of nucleotide sequences of overlapping region between ORF1b and ORF2 (capsid) of human Astrovirus strains detected in Kolkata, India

Surveillance and molecular characterization of Influenza Virus strains circulating in Eastern India

Investigator : M. Chawla Sarkar

Nasal or throat swabs were collected from symptomatic patients (fever >37.5, running nose, cough/sore throat, body ache etc) from three hospitals in Kolkata (BC Roy Children Hospital, Nil Ratan Sircar (NRS) Hospital and National Medical College) after obtaining informed consent form from the guardian/parent. A total of 1878 samples were screened during this period by real time PCR for Influenza A and B. Of 1878 samples, 306 (16.28%) were positive for Inf A/B and one was positive for both A + B. Of 306 samples, 07 (2.3%) were typed as Inf A and 299 (98%) as Inf B. Of 306 Real time PCR samples, 179 (approx 60%) were inoculated in MDCK cells for virus culture. Of 179, 99 isolates were obtained. Of 99 isolates 01 was H3N2, 6 were pH1N1 and 93 were Inf B subtypes. One sample had both Inf A and B. Five samples were positive for Influenza C. Full genomic characterization of Influenza C strain was done which revealed that the HE, matrix, NS, PB1 and PB2 gene of the studied strain (C/Eastern-India/1202/2011) possessed a close relatedness to C/Yamagata/26/81 like strains. The P3 gene shows proximity with C/Mississippi/80 like strains whereas NP gene revealed similarity with C/Miyagi/1/93 like strains (Fig. 4). Majority of samples were from pediatric population (0-5 yrs) and no correlation with gender was observed. The virus positivity correlated positively with rainfall as shown in previous years. Kolkata does not have cold winter and no secondary peak is observed.

Exploring role of the rotavirus encoded non structural protein (s) in evasion of cellular immune responses

Investigator : M. Chawla Sarkar

Rotavirus is known to express six nonstructural proteins. Previously we have reported functions of NSP1, NSP3 and NSP4. NSP3 interacted with cellular protein Hsp90. Hsp90, a cellular chaperone is essential for dimerization and stability of NSP3. Hsp90 inhibitors resulted in degradation of NSP3 and lower viral titers. NSP4 is the enterotoxin, known to disrupt cellular Ca^{+2} homeostasis by translocating to endoplasmic reticulum. NSP4 translocated to mitochondria by confocal imaging resulting in dissipation of mitochondrial membrane potential both during viral infection, NSP4 overexpression as well as in in vitro condition. NSP1 was shown to activate the anti apoptotic signaling via activation of PI3Kinase/Akt pathway delaying virus induced apoptosis. Phosphorylated AKT was found to up regulate IAPs and prevent caspase activation. Both NSP1 and NSP4 have counteracting function to maintain cellular homeostasis. In current year we analyzed the molecular mechanism by which NSP1 activates PI3 Kinase. Mammalian two hybrid assay using CheckMate Mammalian Two-Hybrid system (Promega, WI, USA) and GST pull down assays were done to study direct interaction between rotavirus NSP1 and α and β isomers of PI3K regulatory subunit p85. Study confirmed direct interaction of NSP1 with both α and β isomers of PI3K regulatory subunit p85 and revealed the importance of full length RV NSP1 and both α

and β isomers of PI3K p85 for interaction between NSP1 and PI3K in cellular condition which results efficient PI3K activation. Our results indicate a probable conformational change of cellular PI3K after direct interaction with full length rotavirus NSP1 that may lead to efficient activation of PI3K/Akt pathway (Fig. 5).

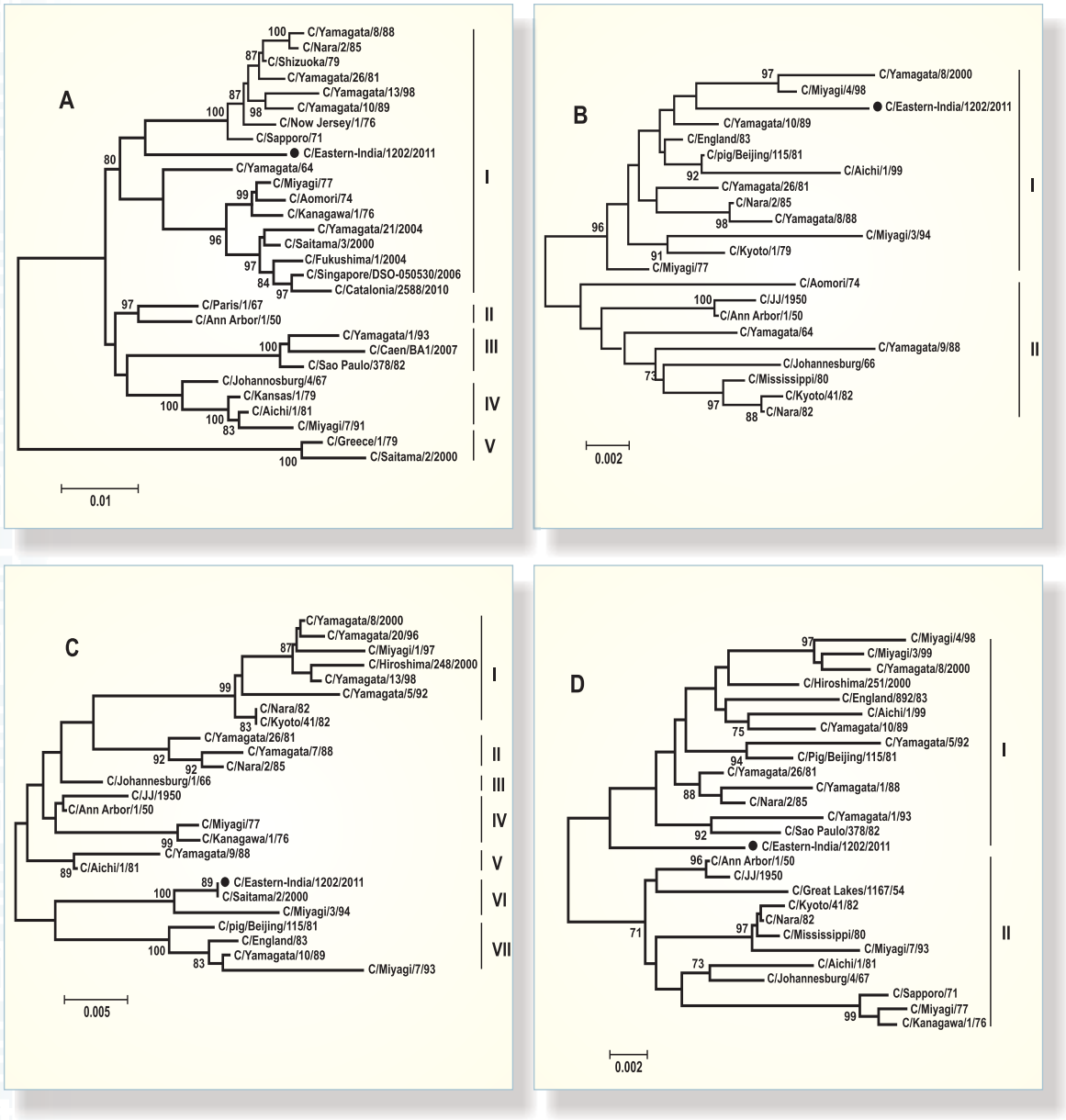

Fig.4. Phylogenetic comparison based on nucleotide sequences of HE (A), Matrix (B), NP (C) and non-structural genes (D) of C/Eastern-India/1202/2011 from Kolkata, eastern India. The Kolkata strain was marked as '●'. The tree was created by using neighbor-joining method with 1000 bootstrap replicates.

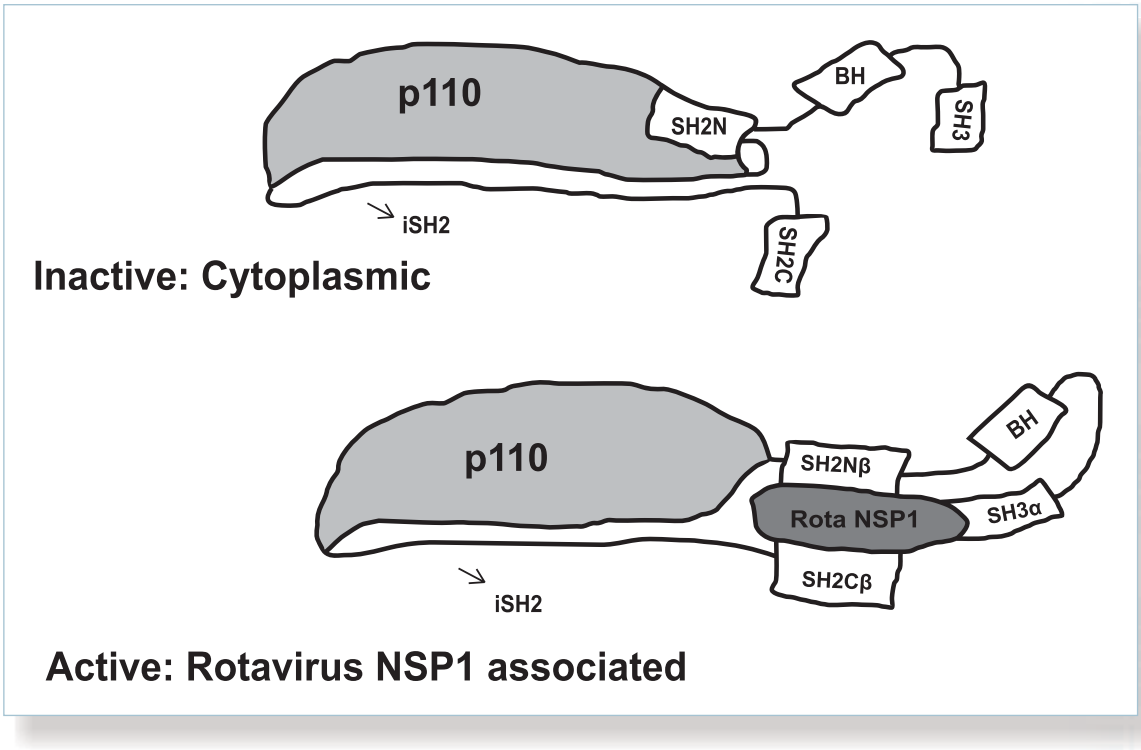

Fig. 5. Probable conformational change of PI3K after direct interaction with full length rotavirus NSP1 which may lead to efficient activation of PI3K/Akt pathway

Detection and molecular characterization of complete nucleotide sequence of human picobirnaviruses causing acute watery diarrhea among children in Kolkata

Investigator : B. Ganesh

The genus, Picobirnavirus (PBV), Spanish *'pico'* = 'small', *'birna'* for *'bipartite RNA'* genome, belongs to the family *Picobirnaviridae* under the proposed order *Diplornavirales*. The virion is non-enveloped, small spherical virus, 35-41 nm in diameter without any apparent surface morphology. They have a characteristic double-stranded, bisegmented RNA genome of two types viz. large genome profile and small genome profile. PBV infections have been reported from different animal species and humans with or without diarrhea. The detection of porcine-like picobirnaviruses in diarrheic children aged <5 years, was suggestive of zoonotic transmission. Similarly, the human isolates of PBVs obtained during our study genetically related to European porcine PBV strains, we have initiated analyzing the porcine fecal samples of Kolkata. Surprisingly, we have found that the sequence data of Kolkata (Indian) porcine PBV strains genetically related to European PBV strains and environmental PBV strains of North America. These results insists that a zoonotic origin of PBV infections as well as a chance of water-borne route of transmission, which presents a wide spread of PBV across globe in various hosts ranging from humans, domestic animals, wild animals, birds, poults, reptilian and environmental samples too. The full genome of segment 2 of the picobirnavirus which encodes viral RNA-dependent RNA polymerase were sequenced for 5 strains for further analysis.

Awards and Honours

S. Chakrabarti

- Selected as member of Guha Research Conference, India
- Delivered Prof J K Sarkar memorial oration from Calcutta School of Tropical Medicine

T. Krishnan

- Invited as the external examiner for evaluation of Ph.D. thesis submitted to AIIMS, New Delhi in June 2012.
- Invited as the external examiner during Ph.D. Viva voce exam held in AIIMS, New Delhi on October 31, 2012
- Invited as editorial board member of OA Publishing London for OA Infectious Diseases from November 2012.
- Editorial Board Member of Research Journal of Infectious Diseases from March 2013.
- Member of Academic Council for Ramkrishna Mission Vidyamandira, Belur Math during Fifth meeting of the Board of Studies for UG Course in Microbiology held on the March 22, 2013.
- Invited to ResPub as Editor-in-Chief in February 2013.
- Selected as member for Asia Pacific Journal of Tropical Disease expert database
- Invited as editorial Board member of Scirene in September 2012 to contribute a chapter in the volume on Infectious Diseases in 21st Century.

Conferences/ Seminars/ Workshop /Training Attended/ organized

S. Chakrabarti

- Delivered a keynote lecture on "Knowing a virus and to make a vaccine against challenges posed by HIV" at the Kolkata Annual Research and Medical International Congress (KARMIC-2012) organized by the Indian Medical Students Association (IMSA) on June 4, 2012 at the Upohar-Conclave, Kolkata.
- Delivered a lecture on "Diarrhoeal disease research activities at NICED" at the Workshop on "Current Trends in Researches on Biomedical Sciences" for the South Asian Association of Physiologists (SAAP) young scientists from Bangladesh, Pakistan, Sri Lanka, India and Nepal at NICED, Kolkata on June 23, 2012.

- Delivered a lecture on "The Prevention of HIV/AIDS: Dreams or Reality" to observe Doctor's Day at Dept. of Physiology, University College of Science, Technology and Agriculture (UCSTA) organized by ISCA Kolkata Chapter on July 2, 2012

- Delivered a lecture on "Challenges posed by HIV/AIDS: Where we stand" at the UGC-Academic Staff College Orientation Program on July 3, 2012

- Delivered a lecture on "Diarrhoeal Disease : A Solveable Enigma" at the UGC-Academic Staff College Orientation Program on June 25, 2012

- Delivered a lecture on "Challenges posed by HIV/AIDS: Virus to Vaccine" at the Indian Association for the Cultivation of Science, Kolkata on July 21, 2012.

- Delivered a lecture on HIV subtypes and recombinants in India at the 5th National Conference of AIDS Society of India 2012 (ASICON) and Co-Chaired a Session: HIV Drug Resistance held during November 23-25, 2012 at Yeshwantpur, Bangalore

- Delivered a talk on "HBV Diversity in Eastern India: Clinical Implications" at the 3rd Molecular Virology meeting held at National Institute of Virology, Pune from December 10-11, 2012.

- Delivered a Keynote address on "Rational use of drugs in the context of treatment for HIV/AIDS" during the session on "Public Health Research Initiative by ICMR" at the South East Asia Regional Public Health Conf. and 57th Annual Conf. of IPHA on February 1, 2013 at Science City, Kolkata

- Delivered a guest lecture for Prof. J. K. Sarkar Memorial Oration on "Molecular Characterization of HIV-1 in North-eastern States of India" on March 8, 2013 at the Lecture Theatre Hall, School of Tropical Medicine, Kolkata

- Delivered a talk on "HIV-1 Subtypes and Recombinants in India" at the Heritage Institute of Technology , Kolkata on January 30, 2013.

- Attended 7th Meeting of the Technical Advisory Committee (TAC) of the "Centre of Excellence and innovation in Biotechnology (CEIB) in Conf. Room No.816, 8th floor, DBT, New Delhi during April 9-10, 2012.

- Attended Selection Committee meeting as Subject Expert for the post of Prof. of the Presidency University, Dept. of Botany & Zoology in Vice Chancellor's office, Presidency University, Kolkata on May 7, 2012.

- Attended as a Member of the 1st meeting of the Scientific Advisory Committee of Biomedical Genomics Centre, Kolkata at Seminar Room, School of Digestive & Liver Disease, IPGME&R on June 28, 2012.

- Attended National Task Force meeting on Laboratory Containment to review the protocol for preparation of the inventory of laboratory storing polio virus developed by Enterovirus Research Centre, Mumbai at ICMR Hqrs. on July 5, 2012.
- Attended Planning Board meeting of the West Bengal University of Health Sciences, Kolkata as Expert Member on August 21, 2012.
- Attended Project Review Committee (PRC) meeting on HIV/AIDS and Sexually Transmitted Diseases (STDs) as a Member on September 4, 2012 at ICMR HQ, New Delhi.
- Attended Scientific Advisory Committee (SAC) meeting of Regional Medical Research Centre, Bhubaneswar held during October 16-17, 2012.
- Attended TRG meeting of the Indo-US Jt. Call for research proposals under the Indo-US joint statement on Prevention of Sexually transmitted infections and HIV/AIDS at ICMR Hqrs., New Delhi on October 18, 2012.
- Participation in the 8th PulseNet Asia Pacific Strategic Planning meeting held during November 5-6, 2012 in Shenzhen, China
- Attended Scientific Advisory Committee (SAC) meeting of National Institute of Immunohematology, Mumbai held during December 5-6, 2012
- Attended the 8th meeting of the Technical Advisory Committee (TAC) of the Centre of Excellence and Innovation in Biotechnology (CEIB) held during January 17-18, 2013 at DBT, New Delhi
- Attended Joint Working Group (JWG) meeting of the activities under the Indo-US Statement on Prevention of Sexually transmitted Infections and HIV/AIDS held on February 8-9, 2013 at Indian International Centre, New Delhi
- Attended the CDC-WHO-NICED workshop on Global Foodborne Infections Network (GFN) workshop on "Laboratory and Epidemiology Training Course for Foodborne Infections" held during February 14-16, 2013 at NICED, Kolkata

T. Krishnan

- Delivered a talk titled Human Ethics for NICED Ph.D. students as part of their Course work on September 12, 2012.
- Delivered a talk titled Laboratory techniques for detection and molecular characterisation of viruses to explore genetic diversity at the Heritage Institute of Technology, Kolkata on January 30, 2013.

M. Chawla Sarkar

- Poster presentation on "Modulation of both cell survival and apoptotic pathways during virus infection by rotavirus encoded non structural-4 (NSP4) and non structural-1

(NSP1) proteins" in the 34th Naito Conference on Infection, Immunity and their Control for Health: Mucosal Barrier, Pathogen and Vaccine, Sapporo, Japan, October 16-18, 2012.

- Delivered a talk on "Modulation of cellular apoptosis by Influenza viruses during infection: role of Matrix 1 (M1) protein." in Virocon 2012; XX1 National Conference on Immunobiology and Management of Viral Diseases in 21st Century, Indian Veterinary Research Institute, Mukteshwar, India, November 8-10, 2012.
- Delivered a talk as invited speaker on "Case-Control community based rotavirus surveillance study in Kolkata revealed increased prevalence of G9, G12 and unusual animal like strains". in Virocon 2012; XX1 National Conference on Immunobiology and Management of Viral Diseases in 21st Century, Indian Veterinary Research Institute, Mukteshwar, India, November 8-10, 2012.
- Delivered a talk on "Modulation of cellular apoptosis by Rotavirus nonstructural proteins (NSP4 and NSP1) for efficient viral infection" in 100th Indian Science Congress, Medical Sciences Section, Kolkata, India, January 3-7, 2013.
- Poster presentation on "Identification and Full genomic analysis of a reassortant Influenza A (H1N2) Virus in Eastern India: Evidence of reassortment between co-circulating A(H1N1) pdm09 and A/Brisbane/10/2007-like H3N2 strains" in 100th Indian Science Congress, Medical Sciences Section, Kolkata, India, January 3-7, 2013.
- Attended training programme "WB Health Department Training Programs for Health Personnel" at Swasthya Bhavan, November 24 and 30, 2012.

B. Ganesh

- Poster presentation on "Epidemiology of picobirnavirus diarrhea in hospitalized adults in Kolkata" in 100th Indian Science Congress, Kolkata, India, January 3-7, 2013.
- Attended one-day workshop on "Electron Microscopic Techniques: holey film, carbon film and ultramicrography" jointly organized by NICED, Kolkata and Electron Microscope Society of India, East Zone Chapter on July 30, 2012.
- Attended two-days workshop on "Scanning Electron Microscopy in Life Sciences" jointly organized by NICED, Kolkata and Electron Microscope Society of India, East Zone Chapter on February 7-8, 2013.

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Services

INVESTIGATIONS

During the epidemic outbreaks (2012-13) of diarrhea 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. Water samples had been received from different PHCs of N. 24 Pargana, S. 24 Pargana, Murshidabad and Hooghly as well as from endemic and epidemic affected Municipal wards under the Kolkata Municipal Corporation and its adjoining areas. Results have been conveyed to the respective agencies with a copy of the same to State Health secretariat, Govt. of West Bengal. During the period under report, 45 samples had been received from various sources of which 20 had been found to be positive for faecal coliforms and 12 for presence of *V. cholerae* O1 (Table 1).

TABLE 1: Analysis of water/ environmental samples

Sl No.	District	No. of samples received	Source					Culture Positive	PCR positive
			Tap	Tube well	Pond	Unknown	Stored		
1.	South 24Pgs	2	1	1	-	-	-	-	-
2.	North 24Pgs	28	9	15	-	-	4	15	10
3.	Murshidabad	4	4	-	-	-	-	-	-
4.	Hooghly	11	4	2	2	-	3	4	2
	Total	45	18	18	2	-	7	19	12

Vibrio Phage Reference Laboratory

NICED operates as a WHO Collaborating Center for Diarrheal Diseases Research and Training. Strains of *V. cholerae* are sent to Vibrio Phage Reference Laboratory for biotyping, serotyping and phage typing since 1968 from all parts of India and abroad. During the current year, a total of 703 strains from 9 states from different parts of the country were received. All 703 (100%) strains were confirmed as *V. cholerae* O1 biotype El Tor. All these strains were included in phage typing study and reports have been sent to respective counterpart.

Gastrointestinal Tract Pathogen Repository (GTPR)

GTPR is an extramural project funded by ICMR. The aim of the GTPR is to archive enteric pathogens to ensure their preservation for periods ranging from several years to decades and to provide authentic bacterial strains for research with retrospective analysis on the nature of strains. So far, a total of 2,000 strains including *V. cholerae* O1, O139, Non O1 non O139, *V. parahaemolyticus, Shigella sp, Salmonella sp, E. coli, H. pylori* and environmental *V. cholerae* have been archived in nutrient agar stab and also in glycerol stock (15%) based on the standard operating manual (SOM) according to WHO guidelines. Clinical isolates of all *V. cholerae* O1El Tor strains are subjected to simplex PCR assay to detect the harbors classical

ctxB and hybrid strains that possess both the *ctxB* classical and El Tor alleles. A Multiplex PCR-based assay is employed to determine the presence of the *wb* genes specific for *V. cholerae* O1 *(wbe)* and O139 *(wbf)*, and the *ctxA* gene, encoding subunit A of cholera toxin. All strains are screened by antibiotic sensitivity test according to CLSI Guidelines. We have adopted Material Transfer Agreement (MTA) and Authorization Format (AF) for authorizing and confidentiality during dispatching of strains. A dedicated webpage (www.gtpr.org.in) has been developed and to be linked with ICMR website shortly.

Identification and characterization of enteric pathogens for medical colleges, hospitals etc.

Identification and determination of the serotypes of the *Salmonella and Shigella* isolates sent to NICED from various Medical Colleges of India.

NICED assisted West Bengal State Govt in identification of cholera cases. During the current year NICED has screened 96 samples of which 28 were positive for *V. cholerae* O1.

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

NICED provided Quality control (QC) and Quality Assurance(QA) support facility in eastern India for parasitic detection under Indo-US joint program.

The virology lab provides laboratory diagnosis for Influenza A viruses (H1N1/ H3N2/ H5N1) for effective patient management.

Highlights of other services

National HIV Reference Laboratory, National Institute of Cholera and Enteric Diseases (ICMR) has been assessed and accredited in accordance with the International Standard ISO-15189:2007, Medical Laboratories - Particular Requirements for Quality and Competence in the field of medical testing (Microbiology and Serology) for the duration from September 8, 2011 to September 7, 2013 with the scope of HIV testing employing Rapid Immunoassay, ELISA and Western Blot.

NICED Lab as a member of "Consortium of National Reference Laboratories for Kit Quality" evaluated HIV, HBV and HCV kits for quality assurance for all the National procurements for AIDS Control Program.

NICED Lab implemented HIV testing Quality Assurance program for the State Reference Laboratories of A&N, Assam, Jharkhand, Meghalaya and Odisha.

Regional Institute (East), NICED, implemented HIV Sentinel Surveillance (HSS) for the states of A&N, Meghalaya, Nagaland, Sikkim and West Bengal with the objectives to monitor the (i) trends in prevalence of HIV infection, (ii) distribution and spread of HIV

prevalence in different population subgroups and in different geographical areas and (iii) to identify emerging pockets of HIV epidemic in the country.

NICED has the credit to identify, for the first time, that HIV prevalence among MSM population in Chattisgarh as highest and in Nagaland as 2nd highest in the country indicating the emerging pockets (in both the states) as well as changing dynamics in Nagaland where the HIV epidemic is considered driven primarily by IDUs.

Provided inputs for "Technical Report-India-HIV Estimates-2012"as Special Invitee in the Technical Resource Group on Surveillance and Estimation.

Molecular diagnosis of HIV among babies (upto 18 months) born to HIV infected mothers employing Dry Blood Spot (DBS) samples, for the first time in the country in a massive scale covering all the 14 eastern and north-eastern states of India is being done at NICED Lab to address the monumental challenge of implementing this Nationwide program.

The NICED lab received Certificate of Excellence from Molecular Monitoring Team, Division of Global AIDS, Centre for Global Health, CDC&P (Centre of Diseases Control and Prevention), USA, for excellence in lab performance in Molecular Diagnosis employing DBS sample.

Manpower development for all the eastern and northern states of India through numerous hands on training conducted at NICED as well as at different remote places in the respective states to ensure quality in HIV testing, HIV Sentinel Surveillance and Molecular Diagnosis of HIV employing DBS.

Quality Assurance for HIV Testing

Quality Assurance for HIV testing for the states of Andaman & Nicobar Islands, Assam, Jharkhand, Meghalaya and Orissa under the External Quality Assurance Scheme of National Reference Laboratory funded by Department of AIDS Control (DAC), Government of India (Table 2).

EQAS and Panel Sera preparation for State Reference Labs.

Quality Assurance for HSS Lab result (Retesting of all positive and 5% negative) (Table 3)

Referral for confirmation of HIV testing results of the samples received from different SRLs.

Training for Medical Officers, Lab/Program Supervisors and Medical Lab Technologists for HIV testing as and when requested by different organizations.

Testing of Dry Blood Spot and serum samples for HIV Sentinel Surveillance.

TABLE 2 : External Quality Assurance and referral service for SRLs under NRL, NICED, Kolkata

Name of States	Name of SRLs	Samples received from SRLs	No. of Concordant Result at NRL	No. of Discordant Result at NRL
Andaman & Nicobar Islands	G.B Pant Hospital *Andaman & Nicobar Islands*	02	02	**00**
Jharkhand	Rajendra Institute of Medical Science, *Ranchi, Jharkhand*	00	00	**00**
	MGM Medical College *Jamshedpur, Jharkhand*	02	02	**00**
	Patuliputra Medical College *Dhanbad, Jharkhand*	00	00	**00**
Odisha	SCB Medical College *Cuttack, Orissa*	02	00	**02**
	VSS Medical College *Burla, Orissa*	03	00	**03**
	MKCG Medical College *Beharampur, Orissa*	05	01	**04**
Assam	Guwahati Medical College *Guwahati, Assam*	02	00	**02**
	Assam Medical College *Dibrugarh, Assam*	01	00	**01**
	Silchar Medical College *Silchar, Assam*	00	00	**00**
Meghalaya	NEIGRIHMS, *Meghalaya*	01	00	**01**

TABLE 3 : HIV Sentinel Surveillance 2012-13 (ANC): Quality Assurance for SRLs under NACO NRL, NICED, Kolkata (sample received upto March'2013).

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.	**Rajendra Institute of Medical Science** *Ranchi, Jharkhand*	219	09	00	219	09	Nil
2.	**MGM Medical College** *Jamshedpur, Jharkhand*	81	05	00	81	05	Nil
3.	**Patuliputra Medical** *College, Dhanbad, Jharkhand*	77	00	03	74	00	Nil
4.	**SCB Medical College** *Cuttack, Orissa*	162	13	00	-	-	-
5.	**VSS Medical College** *Burla, Orissa*	129	03	00	-	-	-
6.	**MKCG Medical College** *Beharampur, Orissa*	101	08	01	-	-	-

Referral Services

National Reference Lab, NICED has been entrusted with the responsibility of verifying results for all samples sent by several Hospitals (Table 4). The samples are tested, result communicated within the turnaround time (TAT) of 5 working days. NICED analyzed the root cause of discordance and trained the referring lab for improvement and technical capacity building. Most of the samples are positive for HIV antibody indicating great improvement of quality of the referring labs.

TABLE 4 : Referral Service done for the institutions at NACO NRL, NICED, Kolkata.

Sl. No.	Source of Samples	No. of sample Tested	No. of sample Positive
1.	**Command Hospital** Kolkata	44	42
2.	**Woodlands Multispecialty Hospital Ltd.** Kolkata	03	03
	Sub Total -	47	45

Kit Evaluation done by NICED Consortium Lab during April 2012 to March 2013.

Request for evaluation is routed through the consortium secretariat and all the labs are assigned the task for evaluation in a predefined rotational basis to avoid any bias (Table 5).

TABLE 5 : Diagnostic Kits Evaluated by NICED Lab

Type of Kit	No of Batch/ Lot evaluated
HIV ELISA	06
HIV Rapid	28
HBsAg ELISA	04
HBsAg Rapid	02
HCV ELISA	04
HCV Rapid	03
Total	**47**

Counseling and Testing for HIV

Integrated counseling & testing center

Service for HIV counselling and testing started with a designated ICTC having financial support from WBSAP&CS at NICED in 2008. It has grown gradually not only with large client load (Table 6), but also with various other activities. The main functions of the ICTC are :

Conducting HIV diagnostic tests.

Providing basic information on the modes of HIV transmission, and promoting behavioural change to reduce vulnerability.

Providing psycho-social support

Link people with other HIV prevention, care and treatment services.

TABLE 6 :

ICTC Data from April 2012 - March 2013					
Total Tested	**Positive**	**Positivity**	**Referred from ID Hospital**	**Referred from RNTCP**	**HRG tested (from TI/Non TI)**
982	24	2.50%	417	144	107

The ICTC unit of NICED is very much open to all kind of people irrespective of their sexual orientation. Transgender people are free to access the counseling & testing service from this unit. The sex ratio of the clients attending this ICTC made this picture very clear (Figure 1).

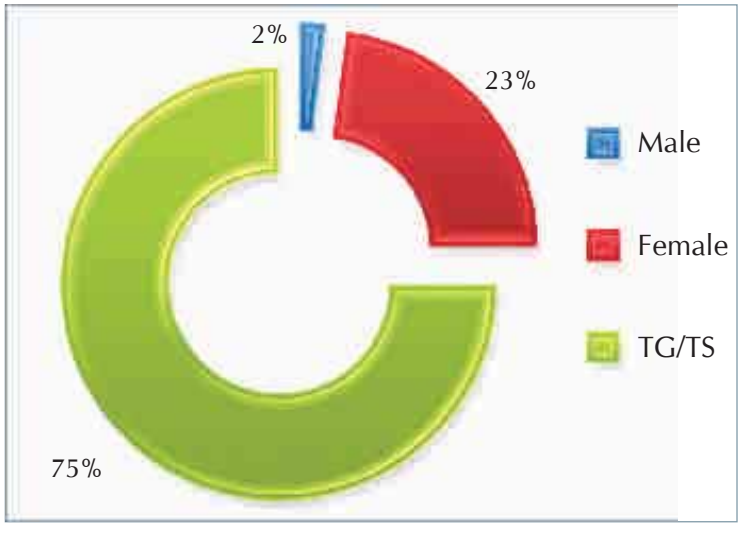

Figure 1 : Sex Ratio of Clients Tested at ICTC (Apr'12 - Mar'13.)

Early Infant Diagnosis

NACO conducted EID Program which 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 trans-placental 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, 2010 initially with three states West Bengal, Orissa and Chhattisgarh. With gradual success of the program, the north eastern states (Jharkhand, Bihar, Assam, Manipur, Mizoram, Nagaland, Meghalaya, Arunachal Pradesh, Sikkim, Tripura, and Andaman & Nicobar Islands) were also included under NICED-RRL.

Presently, 116 ICTCs are involved in collection of DBS samples in 14 states under NICED-RRL and 30 linked ART centres are collecting Whole Blood Samples from infants reactive for DBS-HIV-1 DNA PCR. Different testing algorithms (Algorithm A: for infants < 6 months and Algorithm B: for child 6-18 months) have been followed for two different age groups of HIV exposed infants in this EID program for detection of HIV-1 DNA.

A total of 1695 DBS and 110 Whole Blood Samples received at NICED-RRL for the period of April1, 2012 to March 31, 2013 and their status is depicted in Table 7 and Figure 2.

TABLE 7 : Status of DBS and Whole Blood Samples received at NICED from April' 2012 to March' 2013.

Name of States	No. of DBS samples received	No. of DBS samples tested	HIV-1 DNA detected in DBS	No. of Whole Blood samples received	No. of Whole Blood samples tested	HIV-1 DNA detected in whole blood
West Bengal	671	665	46	42	41	32
Orissa	174	173	25	13	08	07
Chhattisgarh	131	131	19	12	12	11
Bihar	238	238	24	12	12	10
Jharkhand	99	98	15	08	08	06
Mizoram	88	86	16	08	06	05
Assam	80	80	09	08	08	07
Manipur	103	103	08	06	06	04
Nagaland	100	100	13	01	01	01
Meghalaya	10	10	01	-	-	-
Arunachal Pradesh	-	-	-	-	-	-
Sikkim	-	-	-	-	-	-
Tripura	-	-	-	-	-	-
A & N Islands	01	01	-	-	-	-
TOTAL		**1695**	**1685**	**176**	**110**	**10283**

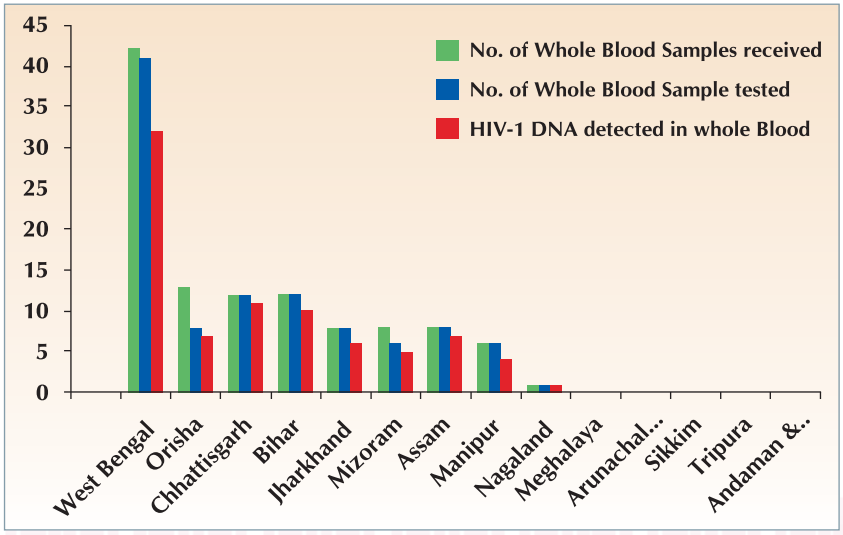

Figure 2 : Status of WBS samples received at NICED from Apr'12 to Mar'13.

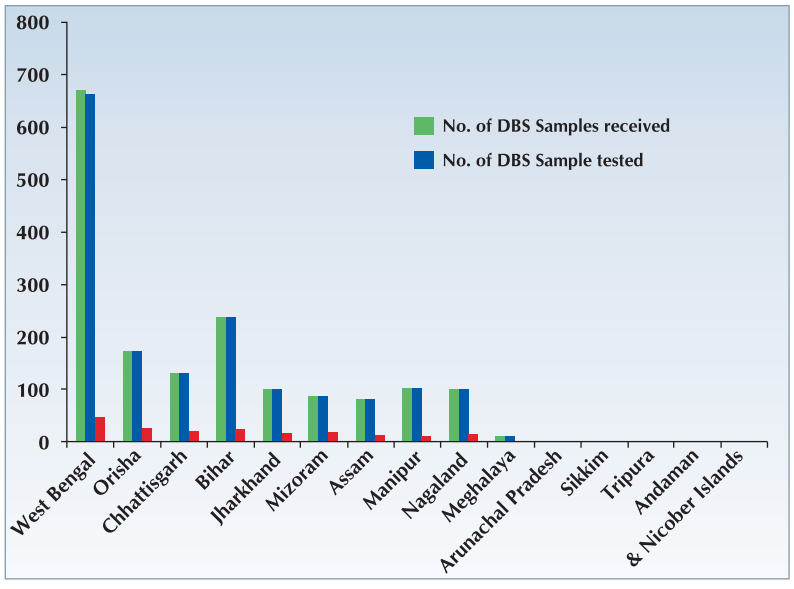

Figure 3 : Status of DBS samples received at NICED from Apr'12 to Mar'13.

All DBS DNA PCR reactive specimens are further confirmed by 2nd HIV-1 DNA PCR test performed with Whole Blood samples. Status of Whole Blood HIV-1 DNA PCR reactive specimens are presented in Figure 3.

Estimation of HIV as Regional Institute for HIV Sentinel Surveillance

The Regional Institute (East) for HSS has been functioning at NICED since 2008 to ensure quality for the purpose of HIV Sentinel Surveillance (HSS) for eastern region. Initially four states (A&N, CG, SK and WB) were attached to RI (E) and later on two more states (MG and NL) were added (Table 8).

Spectrum of Activities (Table 9)

Technical support & guidance to SACS in overall planning & implementation of HSS activities in Andaman & Nicobar Islands, Chhattisgarh, 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, modifying, freezing & cleaning through SIMS.

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 8 : HIV Sentinel Surveillance Sites

	ANC	STD	TOTAL
A&N	4	1	5
CG	18	0	18
ML	8	0	8
NL	13	0	13
SK	4	0	4
WB	22	0	22
Site Type Totals	69	1	70

TABLE 9 : RI (East) Activities

Purpose	Venue/Place	Date
Workshop on National HIV estimation and projection	Delhi	1st May 2012–5th May 2012 28th May 2012–30th May 2012 28th Sept 2012–29th Sept 2012
New site validation for Sikkim	Sikkim	24th Sept 2012–26th Sept 2012
National Pre-Surveillance Meeting	NIHFW, Delhi	5th Oct 2012–6th Oct 2012
New site and testing lab validation for Chhattisgarh	Chhattisgarh	19th Nov 2012–22nd Nov 2012
Regional ToT and Pre-surveillance Meeting	NICED, Kolkata	6th Dec 2012–7th Dec 2012
West Bengal Site Personnel Training for HSS 2012-13 (ANC Round) in three batches	NBMCH, Darjeeling & Kolkata	20th Dec 2012–21st Dec 2012 27th Dec 2012–28th Dec 2012 3rd Jan 2013–4th Jan 2013
Chhattisgarh Site Personnel Training for HSS 2012-13 (ANC Round) in two batches	Raipur & Bilaspur Chhattisgarh	26th Dec 2012–29th Dec 2012
A&N Islands Site Personnel Training for HSS 2012-13 (ANC Round)	Portblair A&N Islands	31st Dec 2012–1st Jan 2013
Sikkim Site Personnel Training for HSS 2012-13 (ANC Round)	Gangtok, Sikkim	4th Jan 2013–5th Jan 2013
New site validation and Meghalaya Site Personnel Training for HSS 2012-13 (ANC Round) in two batches	Shillong, Meghalaya	7th Jan 2013–11th Jan 2013
Training of Lab in-charges and Lab-technicians of ANC/STD testing labs	Guwahati, Assam	10th January, 2013
Nagaland Site Personnel Training for HSS 2012-13 (ANC Round) in two batches	Kohima, Nagaland	15th Jan 2013–18th Jan 2013

Staff of Public Health Laboratory Division :

Dr. M. K. Saha, *Scientist 'D'*
Mr. C. R. Pal, *Technical Officer 'A'*
Dr. S. C. Bhunia, *Technical Officer 'A'*
Dr. S. K. Sadhukhan, *Technical Officer 'A'*
Mr. P. Bhaumik, *Tech. Asstt.*
Mr. C. Das, *Attendant Services*

Training

TRAINING ACTIVITIES

The Division of Training and Extension (a WHO collaborating centre for research and training on diarrhoeal diseases) is actively engaged in the following salient activities in the reported (2012-13) period:

IA. Organized the following international/national meeting/ workshops/ seminars/ trainings :

- Training of medical students at NICED was coordinated at the formal request of 'Indian Medical Students Association' (IMSA) on June 2, 2012. This training comprised of theoretical and laboratory exposures on various aspects of medical research; witnessed participation of 72 medical students coming from different parts of India.
- Training cum review meeting on Early Infant Diagnosis during July 20, 2012; number of participants: 19. Participants were SACS Program Officers / Medical Officers, Counselors and Lab Technicians from WB.
- Training on "Electron microscopic techniques: holey film, carbon film and ultramicrotomy" during July 30-31, 2012; number of participants: 31; participants were researchers of research institutes and universities, junior faculties of colleges and research institutes, and technicians.
- Training programme of final year B.H.M.S students from National Institute of Homeopathy, Kolkata during August 31, 2012; number of participants 30; participants were final year B.H.M.S students (19th batch), National Institute of Homeopathy, Kolkata, India.
- EQAS workshop cum Sera Panel Distribution during September 5, 2012; number of participants: 7; participants were Technical Officers / Lab Technicians from A&N, Assam, Jharkhand, Meghalaya and Odisha.
- Training cum review meeting on Early Infant Diagnosis during September 26-29, 2012; number of participants: 48; participants were SACS Program Officers, Medical Officers, Counselors and Lab Technicians from Manipur.
- Training cum review meeting on Early Infant Diagnosis during November 3, 2012; number of participants: 20; participants were SACS Program Officers, Medical Officers, Counsellors and Lab Technicians from Assam.
- EQAS workshop cum Sera Panel Distribution during December 14, 2012; number of participants: 8; participants were Technical Officers / Lab Technicians from A&N, Assam, Jharkhand, Meghalaya and Odisha.

- Training on "Scanning Electron Microscopy in Life Sciences" during February 7-8, 2013; number of participants: 63; participants were researchers of research institutes and universities, doctors from hospitals, junior faculties of colleges and research institutes, and technicians.
- Training on "Global Foodborne Infections Network Course for Microbiologists and Epidemiologists" during February 14-16, 2013; number of participants 14; participants were 7 medical doctors and 7 technicians.
- A Scientific seminar was organized by Scholars Association NICED on February 22, 2013.
- Induction Training for Technical Officers during February 26-28, 2013; number of participants: 12; participants were from State Reference Labs of Punjab, Haryana, Maharastra, Mizoram, Nagaland, Manipur, Tripura, Assam, Meghalaya, Jharkhand, Odisha and West Bengal.
- Regional Institute (East), NICED, has implemented HIV Sentinel Surveillance for the states of A&N, Chattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal with the objectives to monitor the a) trends in prevalence of HIV infection, b) distribution and spread of HIV prevalence in different population subgroups and in different geographical areas and c) to identify emerging pockets of HIV epidemic in the country.
- Summer trainee (M.Sc. M.V.Sc.) trained 35 in different laboratories of NICED during the period April 2012 to March 31, 2013

IB. Organized the following meetings of the Institute:

- SAC meeting on 24-25 August, 2012 at NICED Kolkata
- Ethical committee meeting
- Biosafety committee meeting
- IVI training meeting
- Meeting of NACO from time to time through the year viz. induction training programmes for medical technologists
- Observance of National Science Day
- Seminars and oration lectures organized by ISCA, Kolkata Chapter & NICED Kolkata
- Oration lecture of Indian Science Congress Association, Kolkata Chapter
- NACO Regional Institute meeting for HIV surveillance

II. Prepared the following documents for the Institute:

- Compilation and submission of Annual report of WHO Collaborating Centre for research and training on diarrhoeal diseases (2012-13)
- Training modules of different workshops
- Document for the Institutional Scientific Audit
- Report of the Training Programme to WHO (SEARO)

III. Prepared the following documents for ICMR Head Quarter:

- Highlights of the diarrhoeal disease research carried out by the Institute
- Highlights of the Institutional activities for ICMR Annual Report
- WHO training details with prospective budget estimate conducted by NICED
- Report for Department of Health Research

IV. Submission of WHO-collaborating centre Annual Report, administrative and account related documents for WHO and Institutional profile

V. Organized workshops/ meetings for professional bodies at this Institute:

- Dr. B. C. Deb Auditorium was the venue of an outreach programme organized by Indian Science Congress Association on January 5, 2013. The programme was organized to make general people aware of the different health activities conducted by different institutes of the Indian Council of Medical Research.
- NICED II auditorium was the venue of "Dr. Sunil Chandra Bose Memorial oration 2013" organized on January 17, 2013 by West Bengal Academy of Science & Technology – CSIR – Indian Institute of Chemical Biology. The oration was delivered by the eminent pulmonologist Dr. Partha Sarathi Bhattacharya, M.D., D.N.B.E, D.M.(Institute of Pulmocare & Research on 'COPD' & left heart function).

Staff :

Mr. R. J. Mukherjee, *Technical Officer 'A'*
Mr. A. Jana, *Technician 'B'*
Mr. A. Roy, *Technician 'B'*
Mr. S. Adhikary, *Attendant Services*

Events

100th Indian Science Congress, January 3-7, 2013, Kolkata public outreach session

As a part of Indian Science Congress centenary celebration, a public outreach program was organised for school going children. About 100 students of local schools joined this programme. On January 5, 2013 Public outreach session for the activities of the ICMR was organised at NICED, Kolkata.

Dr Shekhar Chakrabarti, Director-in-Charge, NICED, Kolkata in his opening address welcomed the guests and the participants and highlighted the usefulness of this program. Dr P. K. Nag, Director, National Institute of Occupational Health (NIOH), Ahmedabad, spoke on the various occupational and environmental hazards. He also informed about the new user friendly model of cycle rickshaw developed by ROHC (East), Kolkata. Dr V.K. Srivastava, Scientist F & Head, Publication & Information Division of the ICMR, Hqrs, New Delhi presented a brief overview of the Council's activities and achievements and also explained about the various outreach activities of the ICMR and its Institutes. Dr D. Raghunath Rao, Scientist E of the NIN, Hyderabad explained the benefits of the fruits and green leafy vegetables and the importance of breakfast particularly for the school going children. A short audio-video film on the activities and achievements of ICMR with the title 'Raising the Bar' was also shown to the participants. Dr Rajni Kant, Scientist D of the ICMR Hqrs, New Delhi extended vote of thanks to the organizer of the Science congress for entrusting the responsibility of hosting the outreach session.

Scientific Advisory Committee (SAC) Meeting

41st SAC meeting was held at NICED on August 19-20, 2013, Prof N.K. Ganguly was the Chairperson of the meeting.

EXTRAMURAL PROJECTS

BLANK

ONGOING EXTRAMURAL PROJECTS

1. **Title** : Exploration of the biological basis of underperformance of oral polio and rota virus vaccines in India.
   PI : **Dr. D. Sur**
   Funding Agency : International Vaccine Institute, Seoul, Korea.
   Duration : 2012-2014

2. **Title** : Diarrheal disease in infants and young children in developing countries.
   PI : **Dr. D. Sur**
   Funding Agency : Bill and Melinda Gates Foundation
   Duration : 2010- 2013

3. **Title** : Presentation of *Shigella*-Porin by dendritic cell to $CD3^+$ $CD4^+$ cell for the T helper activation, differentiation and generation of the adjuvant-specific memory cells.
   PI : **Dr. T. Biswas**
   Funding Agency : DST, New Delhi
   Duration : 2009-2012

4. **Title** : Effect of probiotics on carriage of extended spectrum- β-Lactamase producing Enterobacteriacae in neonatal gut: a randomized controlled trial at a tertiary care Neonatal Intensive Care Unit.
   PI : **Dr. S. Basu**
   Funding Agency : DBT, India
   Duration : 2011-2014

5. **Title** : Cefotaximases in *E.coli* isolated from hospitalized neonates.
   PI : **Dr. S. Basu**
   Funding Agency : DST, West Bengal
   Duration : 2010-2013

6. **Title** : Host intestinal response induced by *Vibrio Cholerae* chitin-binding protein GbpA and the subsequent effect on the pathogen.
   PI : **Dr. N. S. Chatterjee**
   Funding Agency : CSIR
   Duration : 2010-2013

7. **Title** : Vibrio dynamics in aquatic-riverine-estuarine ecosystem in West Bengal: cholera paradigm
   PI : **Dr. A. Palit**
   Funding Agency : Ministry of Environment. Govt. of West Bengal.
   Duration : 2012-2015

8. **Title** : Role of Toll-like and NOD receptors in probiotics-induced mucosal tolerogenicity.
   PI : **Dr. S. S. Das**
   Funding Agency : DBT
   Duration : 2011 - 2014

9. **Title** : Development and pre-clinical studies on safety and immunogenicity of novel candidate vaccines against *Salmonella enterica* serovar Typhi and Paratyphi.
   PI : **Dr. S. S. Das**
   Funding Agency : DBT
   Duration : 2012 - 2015

10. **Title** : A study on the role of eukaryotic-like protein kinases in the pathogenesis of *Salmonella* Typhi.
    PI : **Dr. S. S. Das**
    Funding Agency : DBT
    Duration : 2012 - 2015

11. **Title** : 2nd Phase of the Task Force Project Biomedical Informatics Center of ICMR.
    PI : **Dr. S. S. Das; Co-PI : A. K. Mukhopadhyay**
    Funding Agency : ICMR
    Duration : 2013 – 2018

12. **Title** : Studies of the emerging El Tor variant *Vibrio cholerae* in Asia and Africa
    PI : **Dr. A. K. Mukhopadhyay**
    Funding Agency : Okayama University
    Duration : 2010 – 2015

13. **Title** : Development and evaluation of a heat killed multi-serotype oral Shigella vaccine.
    PI : **Dr. H. Koley**
    Funding Agency : Okayama University
    Duration : 2010-2015

14. **Title** : Multisite monitoring of influenza virus strains in India- Phase II.
PI : **Dr. M. Chawla-Sarkar**
Funding Agency : ICMR, India DHHS, USA
Duration : 2010 – 2015

15. **Title** : Analysis of rotavirus and their interactions with the host: a viral proteomics approach
PI : **Dr. M. Chawla-Sarkar**
Funding Agency : Okayama University
Duration : 2010 – 2014

16. **Title** : Studies on the regulation of antimicrobial peptide expression and their role in mixed and opportunistic infections of the gut.
PI : **Dr. S. S. Das**
Funding Agency : Okayama University
Duration : 2010 – 2015

17. **Title** : Comparative analysis of the *Helicobacter Pylori* strains isolated from North East India with other part of India in causing gastroduodenal diseases
PI : **A. K. Mukhopadhyay**
Funding Agency : DBT
Duration : 2012 – 2014

18. **Title** : National Rotavirus Surveillance Network.
PI : **Dr. M. Chawla Sarkar**
Funding Agency : ICMR, India
Duration : 2013 – 2016

Publications

PUBLICATIONS

1. **Alam, J., S. Maiti, P. Ghosh, R. De, A. Chowdhury, S. Das, R. Macaden, H. Devarbhavi, T. Ramamurthy, and A. K. Mukhopadhyay**. 2012. Significant association of the dupA gene of *Helicobacter pylori* with duodenal ulcer development in a South-east Indian population. J. Med. Microbiol. **61**:1295-1302.
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3. **Badhai, J., P. Kumari, P. Krishnan, T. Ramamurthy, and S. K. Das**. 2013. Presence of SXT integrating conjugative element in marine bacteria isolated from the mucus of the coral *Fungia echinata* from Andaman Sea. FEMS Microbiol. Lett. **338**:118-123.
4. **Bagchi, P., S. Nandi, M. K. Nayak, and M. Chawla-Sarkar**. 2013. Molecular mechanism behind rotavirus NSP1-mediated PI3 kinase activation: interaction between NSP1 and the p85 subunit of PI3 kinase. J . Virol. **87**:2358-2362.
5. **Bagchi, P., S. Nandi, S. Chattopadhyay, R. Bhowmick, U. C. Halder, M. K. Nayak, N. Kobayashi, and M. Chawla-Sarkar.** 2012. Identification of common human host genes involved in pathogenesis of different rotavirus strains: an attempt to recognize probable antiviral targets. Virus Res. **169**:144-153.
6. **Batabyal, P., S. Mookerjee, and A. Palit**. 2012. Occurrence of toxigenic *Vibrio cholerae* in accessible water sources of cholera endemic foci in India. Jpn. J. Infect. Dis. **65**:358-360.
7. **Bhattacharya, S. D., S. K. Niyogi, S. Bhattacharyya, B. K. Arya, N. Chauhan, and S. Mandal**. 2012. Associations between potential bacterial pathogens in the nasopharynx of HIV infected children. Indian J. Pediatr. **79**:1447-1453.
8. **Bhattacharya, S. K., D. Sur, and D. Mahalanabis**. 2012. Public health significance of shigellosis. Indian Pediatr. **49**:269-270.
9. **Bhowmick, R., U. C. Halder, S. Chattopadhyay, S. Chanda, S. Nandi, P. Bagchi, M. K. Nayak, O. Chakrabarti, N. Kobayashi, and M. Chawla-Sarkar**. 2012. Rotaviral enterotoxin nonstructural protein 4 targets mitochondria for activation of apoptosis during infection. J. Biol. Chem. **287**:35004-35020.
10. **Chadha, M. S., S. Broor, P. Gunasekaran, V. A. Potdar, A. Krishnan, M. Chawla-Sarkar, D. Biswas, A. M. Abraham, S. V. Jalgaonkar, H. Kaur, A. Klimov, R. B. Lal, A. Moen, L. Kant, and A. C. Mishra**. 2012. Multisite virological influenza surveillance in India: 2004-2008. Influenza. Other Respir.Viruses **6**:196-203.

11. **Chattopadhyay, D., H. Mukherjee, P. Bag, D. Ojha, K. A. Kumar, S. Dutta, P. K. Haldar, T. Chatterjee, A. Sharon, and S. Chakraborti**. 2012. Inhibition of NO(2), PGE(2), TNF-α, and iNOS eXpression by *Shorea robusta* L.: an ethnomedicine used for anti-inflammatory and analgesic activity. Evid. Based Complement. Alternat. Med. **2012**:254849.

12. **Chattopadhyay, S., R. Patra, R. Chatterjee, R. De, J. Alam, T. Ramamurthy, A. Chowdhury, G. B. Nair, D. E. Berg, and A. K. Mukhopadhyay**. **2012**. Distinct repeat motifs at the C-terminal region of CagA of *Helicobacter pylori* strains isolated from diseased patients and asymptomatic individuals in West Bengal, India. Gut Pathog. **4**:4.

13. **Chattopadhyay, S., T. Basak, M. K. Nayak, G. Bhardwaj, A. Mukherjee, R. Bhowmick, S. Sengupta, O. Chakrabarti, N. S. Chatterjee, and M. Chawla-Sarkar**. 2013. Identification of cellular calcium binding protein calmodulin as a regulator of rotavirus A infection during comparative proteomic study. PLoS.One **8**:e56655.

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15. **Choudhury, S. R., S. Roy, A. Goswami, and S. Basu**. 2012. Polyethylene glycol-stabilized sulphur nanoparticles: an effective antimicrobial agent against multidrug-resistant bacteria. J. Antimicrob. Chemother. **67**:1134-1137.

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17. **Dasgupta, A., R. Banerjee, S. Das, and S. Basak**. 2012. Evolutionary perspective on the origin of Haitian cholera outbreak strain. J. Biomol. Struct. Dyn. **30**:338-346.

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21. **Dutta, S., S. Guin, S. Ghosh, G. P. Pazhani, K. Rajendran, M. K. Bhattacharya, Y. Takeda, G. B. Nair, and T. Ramamurthy**. 2013. Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. PLoS One **8**:e56068.

22. **Ganesh, B., K. Bányai, S. Kanungo, D. Sur, Y. S. Malik, and N. Kobayashi**. 2012. Detection and molecular characterization of porcine picobirnavirus in feces of domestic pigs from Kolkata, India. Indian J. Virol. **23**:387-391.

23. **Ganesh, B., K. Bányai, V. Martella, F. Jakab, G. Masachessi, and N. Kobayashi**. 2012. Picobirnavirus infections: viral persistence and zoonotic potential. Rev. Med. Virol. **22**:245-256.

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31. **Khatib, A. M., M. Ali, L. von Seidlein, D. R. Kim, R. Hashim, R. Reyburn, B. Ley, K. Thriemer, G. Enwere, R. Hutubessy, M. T. Aguado, M. P. Kieny, A. L. Lopez, T. F. Wierzba, S. M. Ali, A. A. Saleh, A. K. Mukhopadhyay, J. Clemens, M. S. Jiddawi, and J. Deen**. 2012. Effectiveness of an oral cholera vaccine in Zanzibar: findings from a mass vaccination campaign and observational cohort study. Lancet Infect. Dis. **12**:837-844.

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33. **Kotloff, K. L., W. C. Blackwelder, D. Nasrin, J. P. Nataro, T. H. Farag, A. van Eijk, R. A. Adegbola, P. L. Alonso, R. F. Breiman, A. S. Faruque, D. Saha, S. O. Sow, D. Sur, A. K. Zaidi, K. Biswas, S. Panchalingam, J. D. Clemens, D. Cohen, R. I. Glass, E. D. Mintz, H. Sommerfelt, and M. M. Levine**. 2012. The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case/control study. Clin. Infect. Dis.**55** Suppl **4**:S232-S245.

34. **Kumar, N., A. K. Mukhopadhyay, R. Patra, R. De, R. Baddam, S. Shaik, J. Alam, S. Tiruvayipati, and N. Ahmed**. 2012. Next-generation sequencing and de novo assembly, genome organization, and comparative genomic analyses of the genomes of two Helicobacter pylori isolates from duodenal ulcer patients in India. J. Bacteriol. **194**:5963-5964.

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38. **Li, W., V. Cama, F. O. Akinbo, S. Ganguly, N. M. Kiulia, X. Zhang, and L. Xiao**. 2013. Multilocus sequence typing of *Enterocytozoon bieneusi*: Lack of geographic segregation and existence of genetically isolated sub-populations. Infect. Genet. Evol. **14**:111-119.

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ADMINISTRATION

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- Supervision and coordinate of staff activities
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- Conduct orientation programs for new employees
- Disbursement of salaries and maintenance of leave records
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- Staff training and development, preparation of job descriptions, staff assessments and promotions
- Maintain management information systems (manual or computerised)
- Review and answer correspondence
- To provide secretarial or executive services for committees.
- Parliamentary report/reply
- Disbursement of Pension
- To control Institutional and Project Accounts
- To maintain RTI records
- To maintain all records for the interest of SC/ST/OBC/PH
- To maintain records of Group Insurance Scheme
- To maintain APAR
- To promote under MACP scheme
- To maintain TA/LTC
- To make purchase of all consumable/nonconsumable items
- To maintain stores
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- Online administration

Office Administration is a set of day-to-day activities related to financial planning, billing and record keeping, personnel, and physical distribution and logistics within the Institution.

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