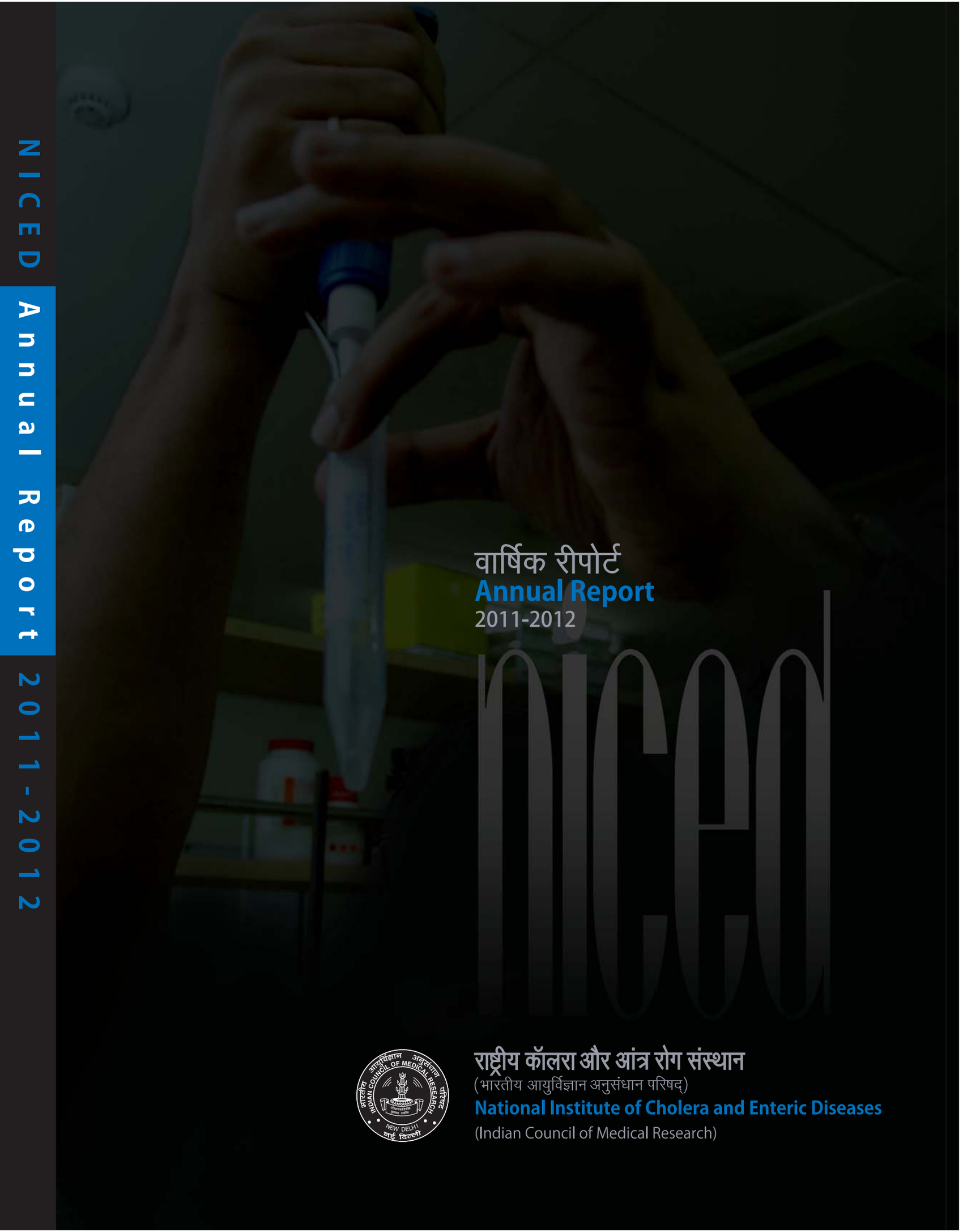


NICED Annual Report 2011-2012

वार्षिक रीपोर्ट
Annual Report
2011-2012

niced

राष्ट्रीय कॉलरा और आंत्र रोग संस्थान
(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)
National Institute of Cholera and Enteric Diseases
(Indian Council of Medical Research)

Annual Report
2011-2012

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

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES
(Indian Council of Medical Research)

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

From the Director's Desk

It is my great pleasure to present the Annual Report of the National Institute of Cholera and Enteric Diseases (NICED) for the year 2011-12. Since beginning of its journey 51 years ago, NICED has travelled a long way, from a four room Centre in a rented house in Central Kolkata to three modern buildings with state-of-art facilities for doing basic as well as operational research in enteric diseases. In spite of tremendous technical advance in biomedical science, cholera and many other infectious diseases of the human gut like rotavirus diarrhea, traveller's diarrhea caused by enterotoxigenic*Escherichia coli*, typhoid and shigellosis still claim thousands of lives. The eradication and control of these diseases is a major challenge to our goal of ensuring health for all by the turn of this decade. It is gratifying for us to record that the NICED has played an important role since its inception in reducing drastically mortality and morbidity due to infectious diarrhea. Since early 1980s, the mandate of the NICED has expanded considerably with the inclusion of Human Immunodeficiency Virus in its ambit.A feature and perhaps, a major advantage of the NICED is that it combines operational and basic research efforts with equal emphasis that is not commonly seen in most other Institutes committed to biomedical science. It is little wonder that operational research like surveillance of enteric pathogens in the community, medical assistance rendered to the local and national health authorities in times of sporadic and epidemic outbreaks of diarrheal diseases, trial of candidate vaccines in community have been combined with in-depth basic studies like unravelling of signal transduction pathways, study of the immunoregulatory mechanism of the human gut and structure-function analysis of proteins. The debate between basic and applied research has been a recurring theme in our National Science Policy. We at the NICED have strived with our humble ability to resolve the apparent contradiction between the two.

The present state of the NICED, whatever it is, has been achieved with the dedication and sincerity of all concerned, viz. the scientists, the research scholars, the technicians, the Administrative Staff and most importantly, the generous support and guidance of the successive Director-Generalsand senior scientists through various mechanisms like deliberations at the Scientific Advisory Committee meetings. The cross-talk between the NICED scientists and the State Health authorities has also contributed enormously to the growth of the Institute. It is my pleasant duty to acknowledge our debt to all of them.

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

निदेशका की मेज से

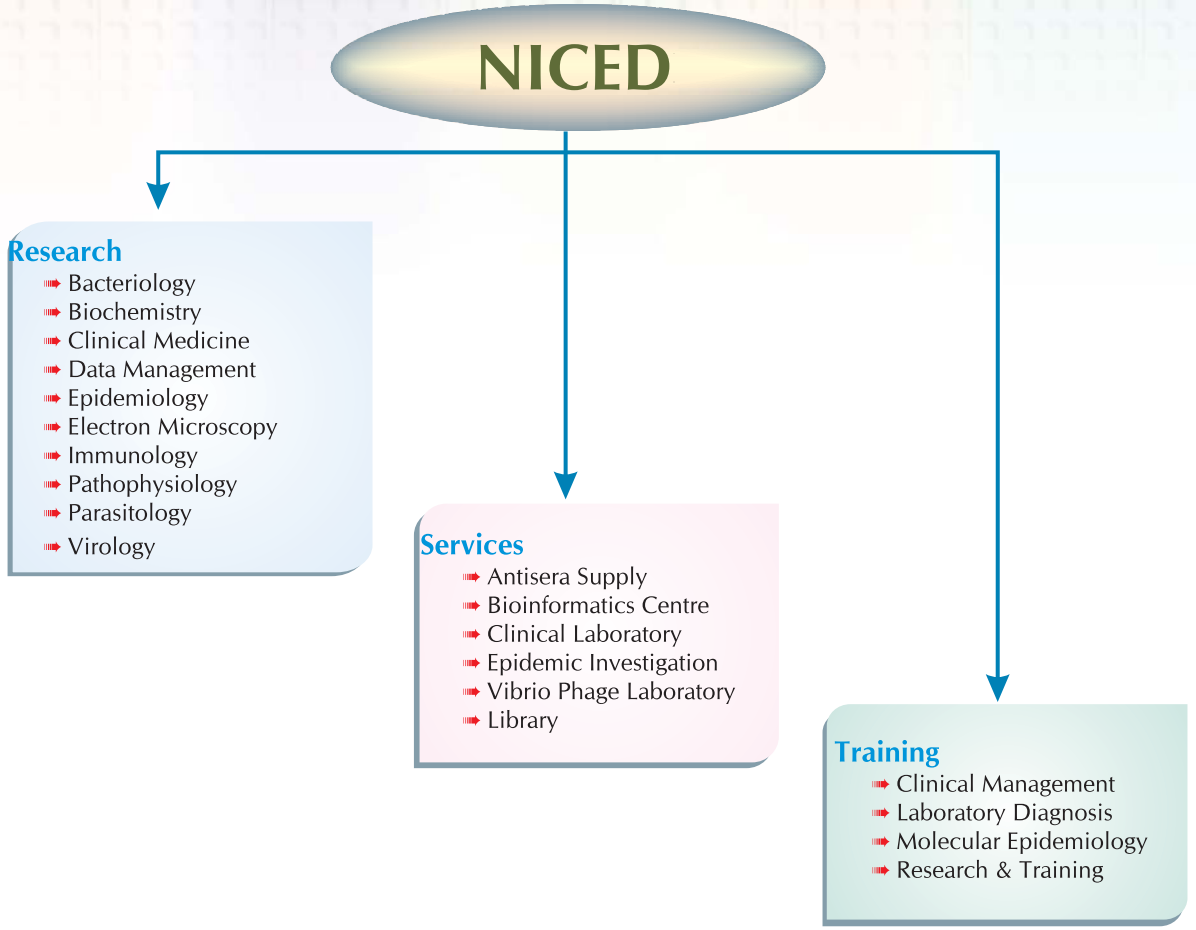
मुझे आप के सामने राष्ट्रीय कॉलरा और आंन्त्र रोग संस्थान (एन आई सी ई डी) वर्ष 2011-2012 की वार्षिक रिपोर्ट पेश करने की अत्यधिक प्रसन्नता हैं। 51 साल पहले अपनी यात्रा की सुरूआत के बाद से (एन आई सी ई डी) ने एक लंबा रास्ता तय किया है, मध्य कोलकाता में किराये के चार कमरों के केन्द्र से आज आंन्त्र रोग पर अनुसंधान के लिए आत्याधुनिक सुविधाओं के साथ तीन इमारतों का सफर। जैव चिकित्सा विज्ञान में जबरदस्त तकनीकी अग्रिम के बावजुद मानव आंन्त्र रोग के कई संक्रामण रोग जैसे हैजा, रोटावायरस, डायरिया, कोलाई, टाइफाइड और शीगेलोसिस की वजह से अभी भी हजारों जीवन जाते हैं। इस दशक के अंत तक इन रोंगो का नियंत्रण उन्मुलन एवं सभी का स्वास्थ्य सुनिश्चित करने का लक्ष्य हमारे लिए एक बड़ी चुनौती हैं।

हमारे लिए यह खुशी की बात है कि अपनी स्थापना के बाद से (एन आई सी ई डी) ने अनुसंधान के प्रयासों से बहुत ही तेजी से मृत्युदर और संक्रामक दस्त के कारण रूग्णता को कम करने में एक महत्वपूर्ण भूमिका निभाई है। 1980 दशक (एन आई सी ई डी) ने अपने अनुसंधान के अधिदेश में हयूमन इम्यूनो डेफ्सिियेंसी वायरस (एच आई वी) को शामिल किया है। जो एन आई सी ई डी की महत्वपूर्ण विशेषता है कि यहाँ बुनियादी एवं प्रचालनात्मक अनुसंधान पर बराबर जोर दिया जाता है। जो कि आमतौर पर अन्य जैव चिकित्सा विज्ञान के लिए प्रतिबद्ध संस्थाओं में नही देखा जाता है। समुदाय में आंन्त्र रोगजनकों की निगरानी की तरह है जो कि इसका परिचालन अनुसंधान में आश्चर्यजनक है।

समुदाय में आंन्त्र रोगजनकों के निगरानी, स्थानीय और राष्ट्रीय स्वास्थ्य अधिकारियो को छिटपट और महामारी फैलने के समय में सहायता प्रदान करना, उम्मीदवाँर टीके का परिक्षण करने के साथ साथ मानव आंत और प्रोटीन की संरचना, इम्युनो रेगुलेटरी तंत्र और सिगनल ट्रान्सडक्शन इत्यादि का गहराई से अध्ययन करना एन आई सी इ डी को आश्चर्यजनक विशेषता है। वुनियादी और अनुप्रयुक्त अनुसंधान के बीच की बहस हमारी राष्ट्रीय विज्ञान निति का एक आवर्ती विषय रहा है। हम दोनों के बीच स्पष्ट विरोधाभास को हल करने के लिए विनम्र क्षमता के साथ प्रयासरत हैं।

राष्ट्रीय कॉलरा ओर आंन्त्र रोग संस्थान ने वर्तमान बुलन्द स्थिति को सभी वैज्ञानिको, शोधकर्ताओं, प्रयोगशाला तकनीशियन एवं प्रशासनिक कर्मचारियों के समर्पण और इमानदारी से हासिल किया है। एन आई सी ई डी का बिकास भारत सरकार स्वास्थ्य अनुसंधार के सचिव भारतीय आर्युविज्ञान अनुसंधान परिषद के महानिदेशक तथा वैज्ञानिक सलाहकार समिति की वैठको में विचार विमर्श और वरिष्ठ वैज्ञानिकों के लगातार समर्थन और मार्गदर्शन से सम्भव हुआ है। एन आई सी ई डी वैज्ञानिकों और राज्य स्वास्थ्य प्राधिकारियों के बीच परस्पर आलोचनाओं से भी संस्थान को विकास में अत्यधिक योगदान मिला है। हम इमानदारी से सभी के आभारी हैं।

डा. शेखर चक्रवर्ती
पी.एच.डी., एफ.एन.ए., एफ.एन.ए.एस.सी., एफ.ए.एस.सी.एण्ड टी,
निदेशक प्रभारी



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

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

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

Staff :
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Mr. S.K. Bhowmik, Technical Officer - A
Mr. A.K. Mondal, Technical Officer - A
Mr. N.K. Sikder, Technical Officer - A
Mr. R.B. Bose, Technical Officer - A
Mr. S.R. Ghosh, Technical Assistant
Mr. Arindam Ganai, Technical Assistant
Mr. Sujit Kumar De, Technical Assistant

Mr. S.C. Saha, Technician - B
Mr. Rajbir Balmiki, Technician - B
Mr. M.L. Gupta, Technician - B
Mr. Khokan Kr. Roy, Technician - B
Mr. Subhadeep De, Technician - B
Ms. Moumita Das, Technician - C
Mr. A.K. Saha, Attendant Services
Mr. P. Samanta, Attendant Services
Mr. Bhaskar Roy, Attendant Services
Mr. K. Ghosal, Attendant Services
Mr. V.K. Singh, Attendant Services
Mr. Satyajit Mondal, Attendant Services

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Dr. Soumik Barman
Dr. Ronita De

Pre-Doctoral Fellows : Abhisek Jaiswal
Anirban Sarkar
Anuradha Sinha
Arindam Naha
Devarati Dutta
Fatema Calcuttawala
Goutam Chowdhury
Jawed Alam
Koel Bhattacharya
Piyali Mukherjee
Prachetash Ghosh
Prasenjit Batabyal
Priyanka Ghosh
Priyanka Jain
Sambit Roy
Sanjucta Dutta
Santanu Ghosh
Saswati Dutta
Shravani Mitra
Soma Mitra

Somdutta Chatterjee
Sourav Sen Gupta
Subham Mookerjee
Subhasree Roy
Sucharita Guin
Suman Nandy
Surojit Das
Taniya Golder
Tapas Patra

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

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

In this study, 212 (6.5 %) Shigella isolates were identified from 3262 diarrhoeal patients. The numbers and proportions of different Shigella serogroups detected were 160 (75.5 %) *S. flexneri*, 33 (15.6 %) *S. sonnei*, 14 (6.6 %) *S. boydii* and 5 (2.3 %) *S. dysenteriae*. Among the *S. flexneri*, serotypes 2a and 3a were frequently identified. Interestingly, 19 (11.8 %) *S. flexneri* isolates were designated as untypable (UT). *S. dysenteriae* types 1 and 3 (one isolate each) and three isolates of type 2 were isolated. Among 14 *S. boydii* isolates, type 12 was common (four isolates) and the others were represented by serotypes 1, 11 and 15 (two isolates each) and serotypes 9, 18 and 10 (one isolate each).

Ninety per cent of the Shigella isolates in this study were resistant to multiple drugs. Majority of the isolates were resistant to trimethoprim–sulfamethoxazole (94.3%), nalidixic acid (93.4%), tetracycline (88.7%), ciprofloxacin, norfloxacin, ofloxacin (85.8% each), furazolidone (79.2%) and chloramphenicol (62.7%). Resistance to ampicillin (53.8%) and azithromycin (34.4%) was moderate, whilst much lower resistance was observed to the third-generation cephalosporin ceftriaxone (1.9%). Among *S. flexneri*, serotype 2a was totally resistant to nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin and streptomycin. Ninety-six per cent of isolates were resistant to co-trimoxazole and tetracycline and 90% of isolates were resistant to ampicillin and chloramphenicol. Resistance to azithromycin was detected among 32% of isolates, whilst 1.3% of isolates were resistant to ceftriaxone.

PFGE analysis of representative serotypes of *S. flexneri* after digestion with NotI revealed three serotype-specific clusters (serotypes 3a, 2a and UT), with approximately 80–100% similarity within each serotype (Fig.1). When XbaI-digested genomic DNAs of current *S. dysenteriae* type 1 isolates and epidemic strains isolated previously in this region were compared, the recent isolates were identified as belonging to a different clone. *S. dysenteriae* type 2 isolates were identified as closely related and the *S. sonnei* isolates were observed to be clonally related (less than three bands difference).

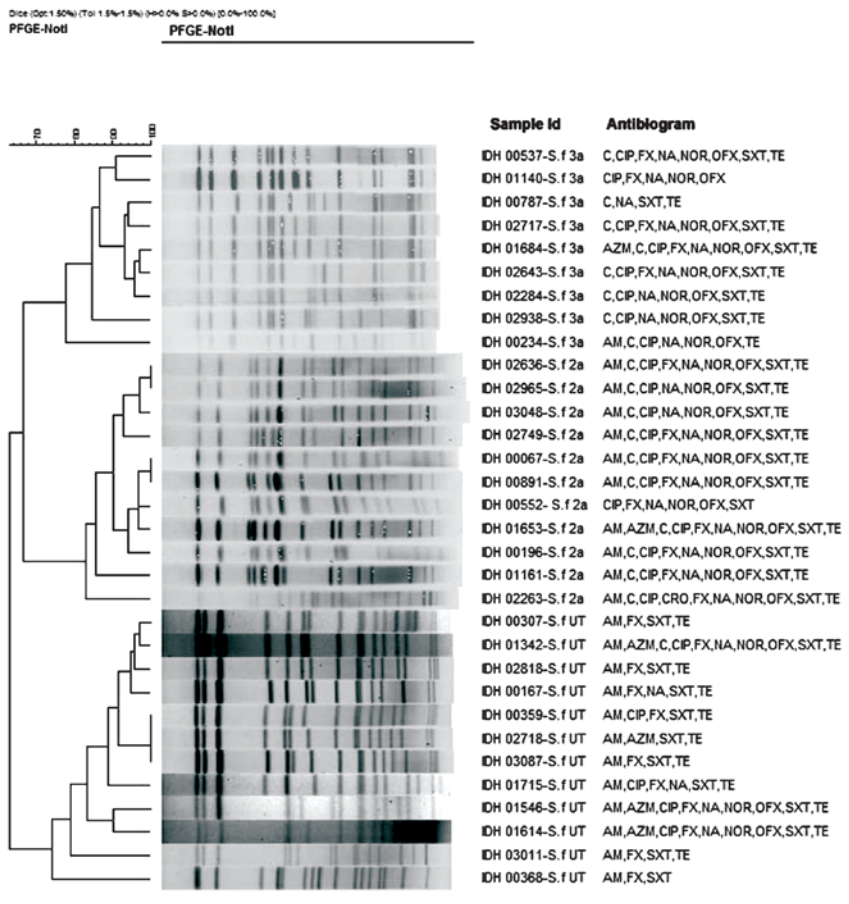


Fig. 2. *NotI* profile of *S. flexneri* strains and dendrogram with percentage similarity.

Foodborne associated *Shigella sonnei* outbreaks in various parts of India

Investigators : S. Dutta, S. Nandy

Serotype switch from *S. flexneri* to *S. sonnei* was observed in Kolkata in recent years (2010-2012). Recent *S. sonnei* isolates developed high level resistance to fluoroquinolones like norfloxacin (MIC 12 µg/ml), ciprofloxacin (MIC 4µg/ml) and ofloxacin (MIC 12µg/ml). Foodborne associated outbreaks by *S. sonnei* although were common in industrialized countries, it was reported for the first time from various parts of India. We reported two foodborne outbreaks of *S. sonnei* in India, one from Thiruvananthapuram, Kerala in February 2009 and the other from Kolhapur, Maharashtra in February 2010, which supported extension of food borne associated *S. sonnei* into India. The outbreak isolates were characterized by antimicrobial resistance, plasmid and pulsed-field gel electrophoresis (PFGE) profiles (Figure).

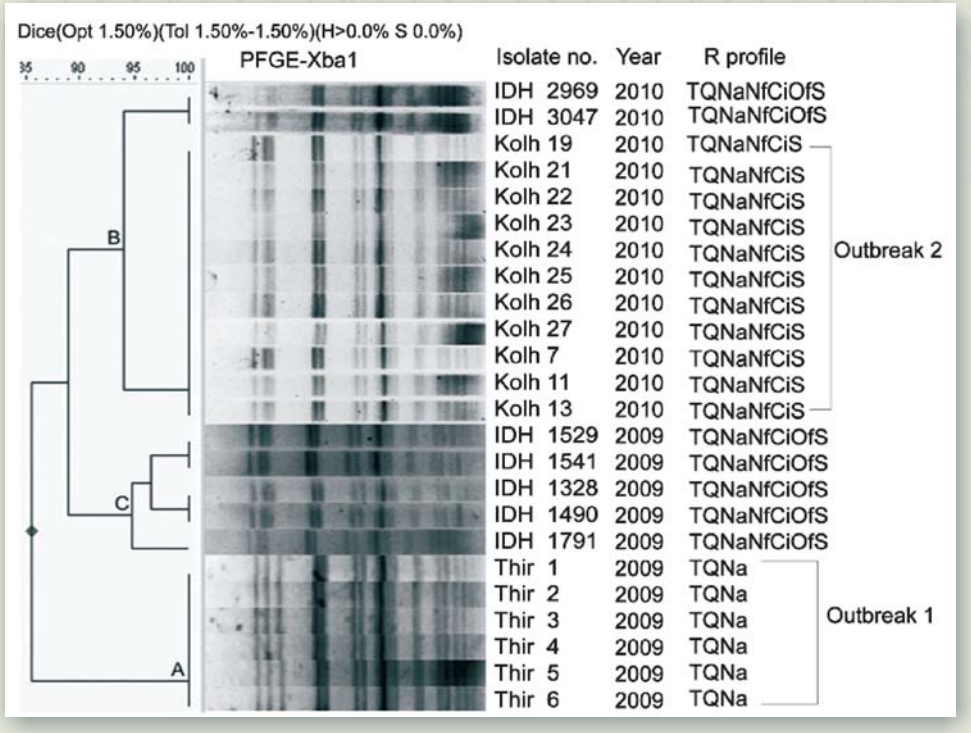


Figure. Pulsed-field gel electrophoresis (PFGE) profiles of Shigella sonnei outbreak isolates, India by cluster analysis and comparison with sporadic isolates (IDH). Thir, isolates from Thiruvananthapuram, Kerala; Kolh, isolates from Ispurli, Shiroli Taluk, Kolhapur district, Maharashtra; IDH, isolates from Infectious Diseases Hospital, Kolkata, West Bengal; R, resistance; T, tetracycline (30 µg); Q, co-trimoxazole (25 µg); Na, nalidixic acid (30 µg); Ci, ciprofl oxacin (5 µg); Nf, norfl oxacin (10 µg); Of, ofl oxacin (5 µg); S, streptomycin (10 µg).

Performance ability of a new serology based rapid, simple and affordable diagnostic test for typhoid fever

Investigators: S. Dutta, S. Das

Actual occurrence of typhoid remains underreported due to absence of proper disease surveillance and lack of suitable diagnostic tests. A no. of serology based new generation commercial kits have been evaluated in the lab but none of these yielded encouraging results. One point of care serological kit, based on the principles of immunochromatography as lateral flow test (IC-LFT) was developed by Span Diagnostics Ltd., Surat, India. It qualitatively detects both IgM and IgG antibodies specific to surface antigens lipopolysaccharide (LPS) and flagellin of *S. typhi* in human serum/plasma. Performance ability of the new kit was validated and evaluated at the Bacteriology Division of National Institute of Cholera and Enteric Diseases, Kolkata. In validation assay, the overall performance of the dipstick typhoid kit for testing stored sera samples with known culture results, was found significantly higher than the Widal ($p < 0.001$) considering blood culture as gold standard. But to determine the performance ability of the kit in real situation, when clinically suspected typhoid fever cases were considered as gold standard in a prospective study, the overall efficiency of the dipstick method decreased from 68.7% to 51.2%. Further improvement is necessary to enhance the

efficiency of the test kit (Table).

Table. Comparison of performance ability of blood culture, Widal and Crystal Typhi based on prospective samples collected from clinically suspected typhoid fever cases (n = 102)

Test	Sensitive (95%CI)	Specificity (95%CI)	PPV	NPV	Efficiency of Test
Blood culture	9/82, 11 (5.88-19.6)	20/20, 100 (NA)	9/9, 100	20/93, 21.5	29/102, 28.4
Widal test (=1:80)	36/82, 43.9 (33.67-54.7)	13/20. 65 (43.29-81.88)	36/43, 83.7	13/59, 22.0	49/102, 48.0
IC-LFT (=1:80)	42/82, **51.2** (33.67-54.7)	17/20, **85** (43.29-81.88)	42/45, **93.3**	17/57, **29.8**	59/102, **57.8**

High-resolution genotyping of the endemic *Salmonella typhi* population during a Vi (typhoid) vaccination trial in Kolkata

Investigators: S. Dutta, K. Holt, B. Manna, S. K. Bhattacharya, G. Dougan, J. Clemens

We used single nucleotide polymorphism (SNP) typing to investigate the population structure of 372 *S. typhi* isolated during a typhoid disease burden study and Vi vaccine trial in Kolkata, India. Approximately sixty thousand people were enrolled for fever surveillance for 19 months prior to, and 24 months following, Vi vaccination of one third of the study population (May 2003–December 2006, vaccinations given December 2004)(Figure). A diverse *S. typhi* population was detected, including 21 haplotypes. The most common were of the H58 haplogroup (69%), which included all multidrug resistant isolates (defined as resistance to chloramphenicol, ampicillin and co-trimoxazole). Quinolone resistance was particularly high among H58-G isolates (97% Nalidixic acid resistant, 30% with reduced susceptibility to ciprofloxacin) (Table). Multiple typhoid fever episodes were detected in 22 households, however household clustering was not associated with specific *S. typhi* haplotypes. Vi vaccination did not obviously impact on the haplotype population structure of the *S. typhi* circulating during the study period Monthly frequency of *S. typhi* coloured by haplotype (haplotypes defined in Figure 1). Vaccines were administered in December 2004 (indicated by arrows) to approximately two thirds of the study population. (A) *S. typhi* isolated from typhoid fever patients in geographical clusters assigned to Vi vaccine. (B) *S. typhi* isolated from typhoid fever patients in geographical clusters assigned to hepatitis A vaccine Monthly frequency of *S. typhi* coloured by haplotype (haplotypes defined in Figure 1). Vaccines were administered in December 2004 (indicated by arrows) to approximately two thirds of the study population. (A) *S. typhi* isolated from typhoid fever patients in geographical clusters assigned to Vi vaccine. (B) *S. typhi* isolated from typhoid fever patients in geographical clusters assigned to hepatitis A vaccine

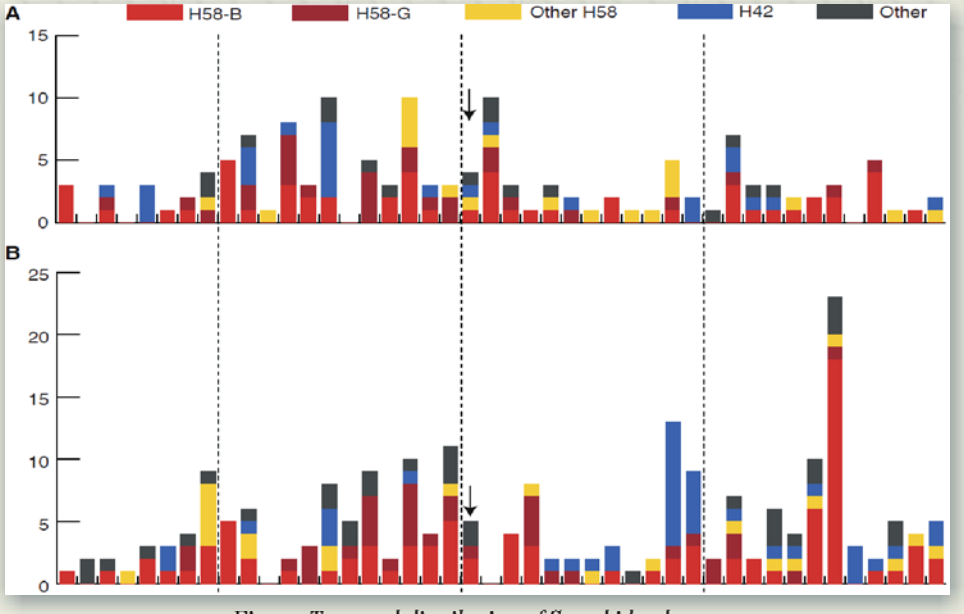


Figure. Temporal distribution of S. typhi haplotypes

Haplotype	NalR				NalS	Total
	CipS	CipI	CipR	All		
H58 subtypes						
- A	14	3	0	17	5	22
- B	23	13	0	36	112	148
- E2	1	0	0	1	0	1
- G	43	20	0	63	2	65
- G0	1	0	0	1	0	1
- H64	12	5	0	17	0	17
- H65	1	0	0	1	0	1
- I1	0	0	2	2	0	2
- I3	1	0	0	1	0	1
- I4	1	0	0	1	0	1
- K1	1	0	0	1	0	1
All H58	98	41	2	141	119	260
H14	14	5	0	19	6	25
H16	1	0	0	1	4	5
H42	24	6	0	30	35	65
H50	4	0	0	4	2	6
H85	5	0	0	5	0	5
Other	1	0	0	1	5	6
Total	**147**	**52**	**2**	**201**	**171**	**372**

Table. Distribution of quinolone resistance phenotypes among S. typhi haplotypes

Scientific validation of Anti typhoid activity of some ethnomedicinal plants

Investigators: S. Dutta., D. P. Chattopadhyay, D. Ojha, T. Nag

Escalating antibiotic resistance of the microbes is the major threat in infectious diseases in recent times. For addressing the issue, investigation on anti bacterial activity of ethnomedicinal plant extracts was undertaken, as practiced by tribal healers, the Kaatabhai tribes of Maharashtra for treatment of typhoid fever, Practice No.: 12:42MH15A0028 and tribes of Jharkhand.

In-vitro study showed considerable anti typhoid activity (MIC50≤ 400µg/mL) with aqueous extracts of some ethnomedicinal plants (especially in one). In-vivo (animal model) tests have confirmed the anti typhoid activity. As human is the only known host for *S. typhi*, standard animal could not be used for in-vivo testing. One reference strain of *Salm typhimurium* NCTC 74 was used in swiss albino mice to develop typhoid like symptoms. The in vivo toxicity study with aqueous extract upto 1200 mg/kg oral dose did not produce any visible toxic effect in the extract treated animals. All tested hematological and biochemical parameters were within normal range and abnormalities were not observed in histopathological results. In the in vivo protective efficacy study with *S. typhimurium* NCTC 74, 100% protection of the animals were observed when treated with 92.5 mg/kg of extract i.p. or 300 mg/kg of extract per orally and significant decrease of viable count of bacteria was obtained in liver and spleen homogenates 72 h after i.p. dose and 7 days after oral (p.o.) dose of the extract following i.p. administration of 1.26 x 108 cfu/ml (50 MLD) of *S. typhimurium* NCTC 74 (Table). Search is in progress for isolation of active compounds. ICMR patent has been applied on the antityphoid activity of the plant extract.

Time of sampling	Group	No of Mice	Extract Dose/Mice (mg/kg)	Route	Initial inoculums (50 MLD)	CFU/organ		CFU/ml Blood
						Liver	Spleen	
24h	I	6	92.50	ip	$1.26x10^8$	$2.68x10^3$	$4.14x10^4$	-
24h	II	6	Control*	ip	$1.26x10^8$	$6.92x10^5$	$8.64x10^6$	2.0×10^2
72h	III	6	92.50	ip	$1.26x10^8$	$1.8x10^2$	$1.4x10^2$	-
72h	IV	6	Control*	ip	$1.26x10^8$	$7.48x10^6$	$2.92x10^7$	-
72h	V	6	300	po	$1.26x10^8$	$2.64x10^4$	$1.32x10^5$	-
72h	VI	6	Control*	po	$1.26x10^8$	$7.14x10^6$	$2.56x10^7$	-
7 days	**VII**	**6**	**300**	**po**	**$1.26x10^8$**	**1.6x10**	**2.8x10**	**-**
7 days	VIII	6	Control*	po	$1.26x10^8$	$6.34x10^6$	$1.22x10^7$	-

**, Normal saline was used as vehicle control*

Table. Viable bacterial count (cfu/ml) in organ homogenates of experimental mice treated with extract(s).

This study may give rise to the development of a non-toxic, cost effective natural lead against typhoid fever, particularly in cases where infection is caused by multidrug resistant *S. typhi*.

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

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

SALIENT OBSERVATIONS:

- Water temperature influences bacterial growth and abundance in that environment. Therefore an increasing trend in bacterial population is observed in summer months (~ 109CFU/L) compared to winter season (~105 CFU/mL).Evidence of implication of salinity gradient on Vibrio abundance is being established.
- Coliform contamination at all study sites (TCC) ranges between 200cfu/mL to 6500cfu/mL, highest being at Howrah reflecting the anthropogenic influence as well as indiscriminate sewage disposal in the flowing riverine aquatic system. Though the higher prevalence of TEC along with TCC has been observed at Kolkata and Basonti, prevalence of TFCC could only be noticed in Kolkata (Hooghly) site.In Diamond Harbour field station tidal variance influences physico-chemical parameters and prevalence of bacterial community as well.
- During high tide, the intrusion of saline brackish water triggers the salinity level as well as the halophillic bacterial community and at low tide lowest prevalence of all type of bacterial community except coliform bacteria has been detected (Fig-I).

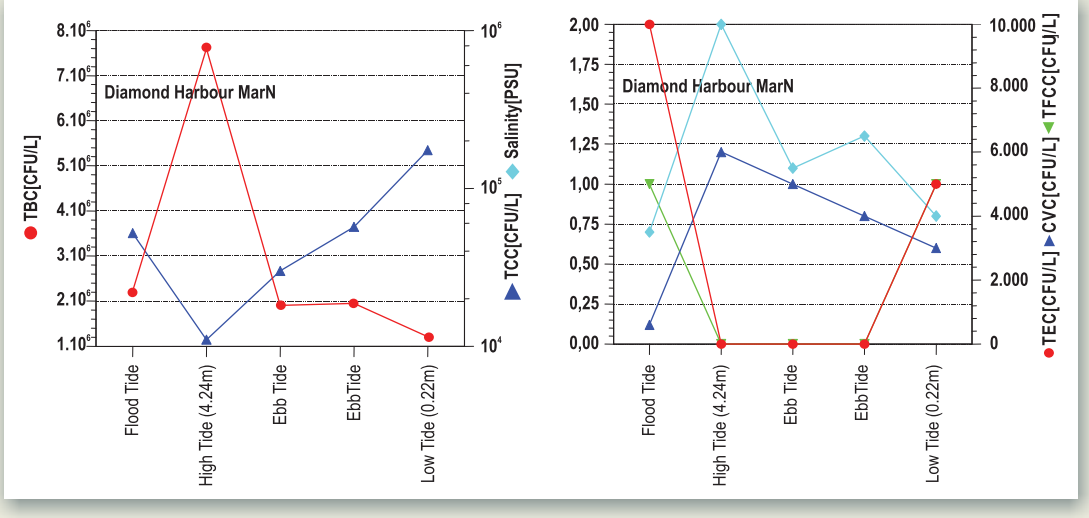


Fig. I. Tidal influence on bacterial disposition at Diamond harbour field station

- 82% of the isolates are found to be *V. cholerae* non O1. So far 12% aquatic *V. cholerae* O1 isolates have also been identified from study sites. Serological identification showed the predominance *of V. cholerae* O1 Ogawa serotype.
- Molecular analysis of *V. cholerae* O1 isolates has revealed that 55% of the isolates possess both ctx and tcp gene. Among those ctx/tcp positive *V. cholerae* isolates,other toxin regulatory genestoxR, zot, RJ, LJ, aldA etc. have also been detected.
- Some of the *V. cholerae* non O1& non O139 isolates have been identified harbouringctx gene.El Tor type ctxB is predominant among the ctx positive *V. cholerae*.

- Antibiotic sensitivity profile revealed that most of the environmental *V. cholerae* O1 isolates are highly sensitive against most of the conventional antibiotics (viz. fluroquinolon, cephalosporin, tetracycline etc.).Although a few virulent *V. cholerae* O1 isolates from Kolkata Hooghly sites showed multi drug resistance pattern.
- Apart from *V. cholerae*, *V. parahaemolyticus*; *V. mimicus*, *V. vulnificus* have also been identified from high saline region as well as low saline region.

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

Investigators: A. Palit and B. L. Sarkar

SALIENT OBSERVATIONS:

- Higher water temperature (31°C ± 1.6°C), alkaline pH (⩾ 7.5) are favorable conditions for Vibrio as well as Vibriopghage proliferation.
- 19 (22.9%) samples were found to be positive for *V. cholerae* O1 phage of which 13 were from Howrah and 6 were from Diamond Harbour.
- Altogether 31 (37.3%) samples were harboring *V. cholerae* O1, comprising of 27 (87%) Ogawa and 4 (13%) Inaba. Mostly (20) from Howrah in comparison to Diamond Harbour.
- Altogether, 9 samples have been identified along with the presence of both *V. cholerae* O1 and their phages. In 8 samples, only the presence of *V. cholerae* O1 has been detected but not of *V. cholerae* O1ϕ.
- It has been well observed that the presence of *V. cholerae* O1 and its phage was very low during the summer months (June) and increased gradually and reached its peak in rainy season followed by a steep decline up to winter months.
- 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* O1 as well as their phage community.

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

Investigators: A. Palit and R. J. Lara (ZMT, Germany)

OBSERVATIONS:

- Increase in abundance of total bacterial count (TBC) (~105 to ~ 109 CFU/L) and cultivable Vibrio count (CVC) (~102 to ~ 105 CFU/L) was discernible at both sites with increasing water temperature (17°C-37°C) in summer.
- A combination of seasonal and tide-dependent variation of salinity and turbidity had distinct influence on TBC, CVC and coliform counts.
- At Diamond Harbour, a salinity increase from 0.6 to 7.9 accompanied with a CVC increase of 1X102 to 2X105 CFU/L indicated the effect of higher salinity in water on Vibrio

abundance, whereas greater disposal of untreated sewage into the river counts for higher coliform prevalence at Howrah Bridge.

- Turbidity dependent variation of CVC was statistically significant. Besides seasonal variation, tidal and lunar cycles have a recognizable effect on Vibrio and other bacterial counts in riverine-estuarine water column.
- Vibrio counts in the intertidal surface sediments showed similar seasonal trend as in surface waters in the middle of the estuary, indicating a benthic-pelagic coupling of Vibrio dynamics.
- In addition to salinity variation with tidal cycles, sediment re-suspension from tidal flats plays an important role on Vibrio abundance in riverine water column.

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

Investigator : B. L. Sarkar.

One of the ongoing institutional activities is the phage typing of *V. cholerae* O1 and O139. The strains of *V. cholerae* sent to us from different institutes across the country. During the period under study, a total of 780 strains of *V. cholerae* were received from different parts of

State	No of Strains	Biotype		Serotype		Basu & Mukherjee			New Phage										
		Eltor	Classical	Ogawa	Inaba	T-2	T-4	UT	4	7	13	14	16	17	19	23	24	25	26	27
New Delhi	59	59	-	59	-	49	3	7	-	-	-	1	-	-	-	4	3	2	5	37
Andhra Pradesh	116	116	-	104	12	105	8	3	-	-	2	1	-	-	-	-	3	-	9	98
Gujrat	140	140	-	140	-	135	3	2	3	-	5	-	-	-	-	4	-	5	15	106
Karnataka	83	83	-	83	-	78	5	-	-	-	-	-	-	-	-	4	5	-	8	66
Maharastra	198	198	-	194	4	184	6	8	4	1	4	3	5	3	8	8	5	-	17	132
Madhya Pradesh	21	21	-	19	2	21	-	-	-	-	1	-	-	-	-	-	-	-	4	16
Tamil Nadu	99	99	-	98	1	91	5	3	-	-	5	-	-	-	3	-	3	-	9	76
Goa	14	14	-	10	4	12	2	-	-	-	-	-	-	-	-	-	-	-	2	12
West Bengal	50	50	-	50	-	50	-	-	4	-	-	3	-	-	-	4	-	-	5	34
Grand Total	780	780	-	757	23	725	32	23	11	1	17	8	5	3	11	24	19	7	74	577
Total %		100	-	97.05	2.95	92.95	4.14	2.95	1.41	0.12	2.18	1.02	0.66	0.39	1.41	3.07	2.43	0.9	9.49	73.97

Table 1: Biotype, Serotype and Phage type of V.cholerae strains received during the year 2011 - 12

the country for serotyping, biotyping and phage typing. Of these, 780 (100%) representative strains were confirmed as *V. cholerae* O1 biotype ElTor and were included in phage typing study. It was observed that 757 out of 780 (97.05%) were serotyped under Ogawa and

remaining 23 were serotyped under Inaba. These strains were grouped under type 2 (92.95%) and 4 (4.14%) with Basu & Mukherjee scheme. A total of 23 (2.95%) strains were found to be untypeable with the conventional phage typing scheme of Basu & Mukherjee. Using the new typing scheme, all these strains were found to be typeable and could be clustered into a number of distinct types of which majority were clustered under type 27(73.97%) followed by type 26(9.49%), 23(3.07 %), 24 (2.43%), 13(2.18%) and 4(1.41%) respectively (Table 1) Recently, we published our results (Table 2) which indicated that 11 out of the 28 states were endemic for cholera because the disease occurred in three consecutive years in that given area as reflected by culture positive strains sent from that state to our Phage Typing Unit, NICED.

State	Block I						Block II						Block III					
	1990	1991	1992	1993	1994	1993	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Andhra Pradesh	16	15	2	67	18	28	1	-	-	-	-	-	-	-	-	69	-	45
Arunachal Pradesh	3	-	1	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
Assam	-	7	-	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-
Bihar	-	-	-	-	4	-	-	1	-	1	-	-	-	-	-	-	-	-
Delhi	84	37	160	120	56	31	13	-	57	85	14	24	18	-	227	-	11	-
Goa	4	3	3	14	-	1	-	-	-	4	3	-	-	3	22	-	-	-
Gujarat	61	91	171	29	374	42	79	51	28	70	34	110	58	67	63	172	104	198
Haryana	13	-	17	6	-	-	-	-	-	2	-	-	-	1	-	-	-	-
Himachal Pradesh	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jammu & Kashmir	-	-	-	-	-	2	-	1	5	1	-	-	-	-	-	-	-	-
Karnataka	-	-	1	-	6	54	87	44	71	20	12	2	14	-	3	32	12	30
Kerala	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	-	-
Madhya Pradesh	20	56	31	2	20	29	12	-	12	-	-	-	1	4	-	12	-	4
Maharashtra	234	322	214	401	191	106	293	125	316	45	146	104	113	232	280	259	156	231
Manipur	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mizoram	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-
Orissa	4	23	-	18	-	2	-	-	-	-	2	3	-	-	15	-	-	-
Punjab	43	34	43	5	94	47	1	24	63	81	21	148	127	-	199	37	-	23
Rajasthan	24	12	22	9	82	50	33	58	27	10	27	20	8	-	12	8	2	-
Sikkim	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Tamil Nadu	215	298	93	34	76	107	178	73	131	38	50	67	59	-	112	76	19	70
Tripura	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uttar Pradesh	-	-	-	-	53	-	1	26	4	-	-	-	-	-	-	11	2	2
West Bengal							271	303	378	244	133	129	83	113	303	148	112	161

*Pondicherry (now Poducherry): No strains received: ** Data from surveillance study were available since1996

Table 2: Overview of V. cholerae strains received from different states of India from 1990-2007

Existence of extensive polymicrobial infections in hospitalized diarrhea cases in Kolkata, India as determined by culture-independent real-time PCR

Investigator: R. K. Nandy

Diarrheal cases need to be attended rapidly and effectively to detect the causal etiology for avoiding significant morbidity and mortality as well as to prevent secondary transmissions. This is very important for India as this country contributes about 77% of the child deaths in Southeast Asia and 18% of the global child deaths caused by diarrhoea. In the post-genomic era, culture independent rapid detection assays have been developed and this study is a part to understand inadequacy, if any, to detect enteropathogens in diarrheal stool specimens by culture dependent assay in comparison to culture-independent methods.

Culture-independent identification of etiological agents was performed using newly developed SYBR-Green-based real-time PCR assay targeting *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Campylobacter* spp., *Shigella* spp. and three different pathotypes of diarrheagenic *Escherichia coli*. DNA was extracted from archived 122 diarrheal stool

specimens and used for the detection of abovementioned enteropathogens by real-time PCR. Conventional culture-dependent methods revealed of 122 specimens, 68 contained bacterial enteropathogens. Among these 68 specimens, 59 (86.8%) had a single pathogen and the remaining 9 (13.2%) contained multiple pathogens. Re-analysis of the 68 specimens by culture-independent real-time PCR methods showed that 25 (36.8%) specimens contained single pathogen and 43 (63.2%) specimens contained multiple pathogens. The prevalence of such high levels of multiple pathogens would not have been detected without using real-time PCR.

Of 122 selected archived specimens, 54 specimens were considered as 'no known etiology' by culture-dependent analysis. Re-analysis of these specimens by real-time PCR revealed the presence of single or multiple pathogens among 34 (63%) of these specimens. Estimation of relative pathogen load by real-time PCR in the stool specimens indicated that the inability of conventional culture-dependent methods to detect the pathogens was related to lower colony-forming units of the pathogen, as reflected by lower Ct values.

Polymicrobial infections are common in low-resource countries and that is in stark contrast to what is found in the developed countries. Specimens were collected from diarrheal patients; majority of them came from a low-income group living in conditions of poor hygiene. Detection of multiple pathogens in diarrheal stool specimens indicated gross contamination of the food and water that they consumed. Detection of high levels of polymicrobial infection by real-time PCR indicates that in the settings like Kolkata and its surroundings, where cholera and other enteric diseases are endemic, the concept of one pathogen one disease might need to be re-evaluated.

Analysis of the 3' end of cagA of Helicobacter pylori in India: Evidence for the evolution of this diverse human gastric pathogen

Investigator: A. K. Mukhopadhyay

The gastric pathogen *Helicobacter pylori* chronically infect more than half of human population. Although most infections are asymptomatic, 10-15% of the *H. pylori* infected individuals develop chronic inflammation leading to atrophic gastritis, peptic ulcer as well as gastric adenocarcinoma. This pathogen was classified as a type I carcinogen by the World Health Organization in 1994. However, specific traits that enable a small proportion of this genetically diverse bacterium in the pathogenesis are poorly understood. Studies have shown that infection with H. pylori strains expressing the CagA is associated with gastritis, peptic ulcer disease, and gastric adenocarcinoma. The biological function of CagA depends on tyrosine phosphorylation by a cellular kinase. The phosphate acceptor tyrosine moiety is present within the EPIYA motif at the C-terminal region of the protein. This region is highly polymorphic due to variations in the number of EPIYA motifs and the polymorphism found in spacer regions among EPIYA motifs. The length polymorphism observed at the 3' end of cagA gene of *H. pylori* is of great interest in recent times since higher number of the phosphorylation sites in CagA protein was described to be associated with stronger biological function and disease manifestation.

We studied the polymorphism at the C-terminal end of CagA and also evaluated its

association with the clinical status of the host in West Bengal, India. Our analysis showed that there is no correlation between the previously described CagA types and various disease outcomes in Indian context. Further analyses of different CagA structures revealed that the repeat units in the spacer sequences within the EPIYA motifs are actually more discrete than the previously proposed models of CagA variants (Figures 1 and 2). Our analyses suggest that EPIYA motifs as well as the spacer sequence units are present as distinct insertions and deletions, which possibly have arisen from extensive recombination events (Figure 2). Moreover, we have identified several new CagA types, which could not be typed by the existing systems and therefore, we have proposed a new typing system (Figure 3). Our study strongly indicates that carrying a particular type of CagA is not the only determinant for the disease outcome especially in the developing countries like India, where multiple infections with different CagA primary structures are possible. We hypothesize that a cagA gene encoding higher number EPIYA motifs may perhaps have arisen from cagA genes that encode lesser EPIYA motifs by acquisition of DNA segments through recombination events.

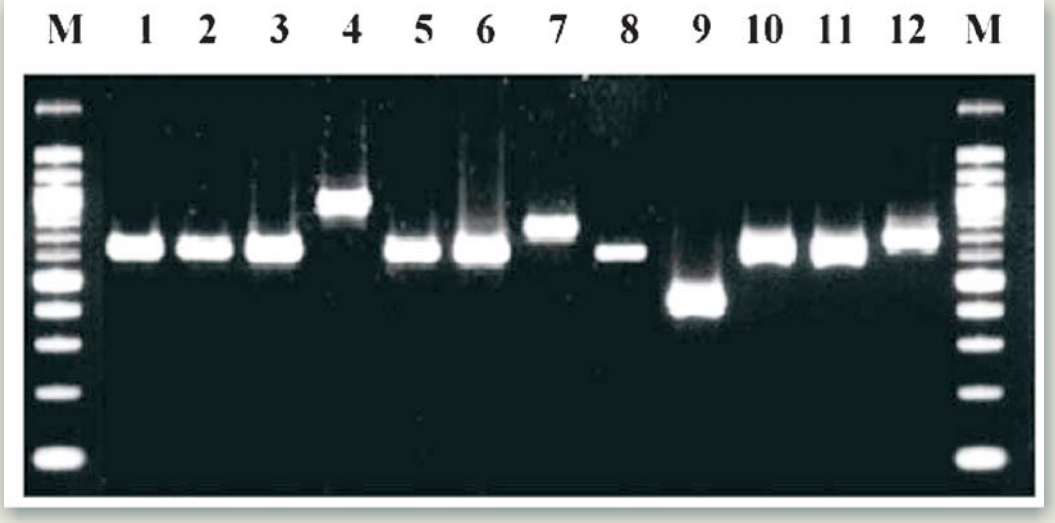


Fig 1

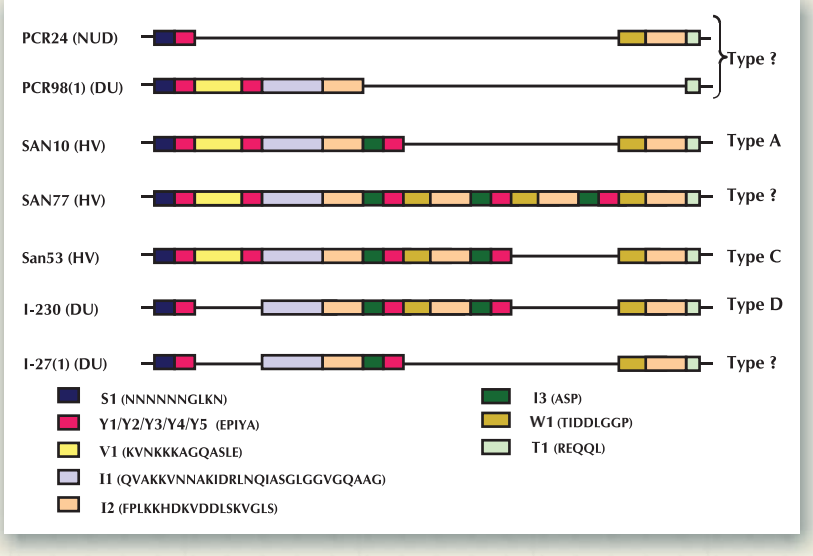


Fig 2

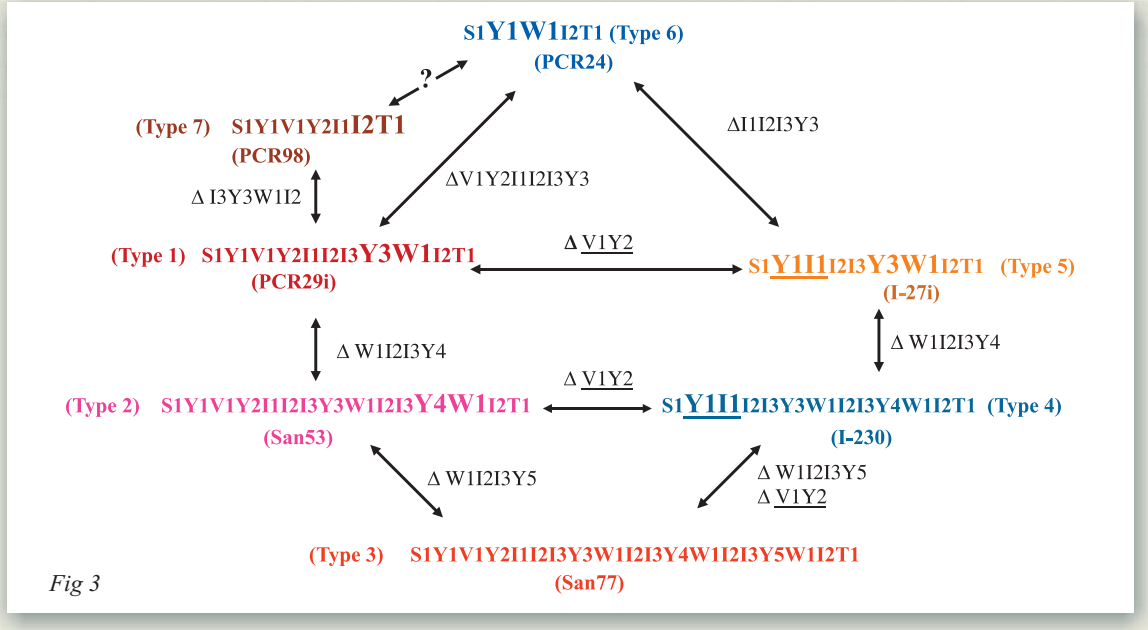


Fig 3

Legend to the figures:

Figure 1: Analysis of the 3' region of the *cagA* gene by PCR. PCR products from a representative group of strains are shown. The sizes of the DNA fragments were confirmed after sequencing of the PCR products. Lane M, 100-bp molecular size markers.

Figure 2: Identification of deletions observed in the repeat region at the C-terminal of CagA of *H. pylori* strains isolated from India. Each motif was assigned a color and the regions deleted were shown in dashed line.

Figure 3: Schematic representation of various types of deletions or insertions among the CagA isolated from WB, India. The segments deleted or inserted are mentioned along with the arrows. The regions where the insertions have occurred are shown in bold and larger front. For type 2 and type 4 CagA, the V1Y2 motifs were inserted within Y1 and I1 and shown in bold, larger front size and also with underscore. As shown in the diagram, almost all the types could be converted to the other types with the occurrence of insertions or deletions.

Haitian Variant strains of *Vibrio cholerae*: did it evolve from Kolkata?

Investigators: A. K. Mukhopadhyay (PI), T. Ramamurthy and R. K. Nandy

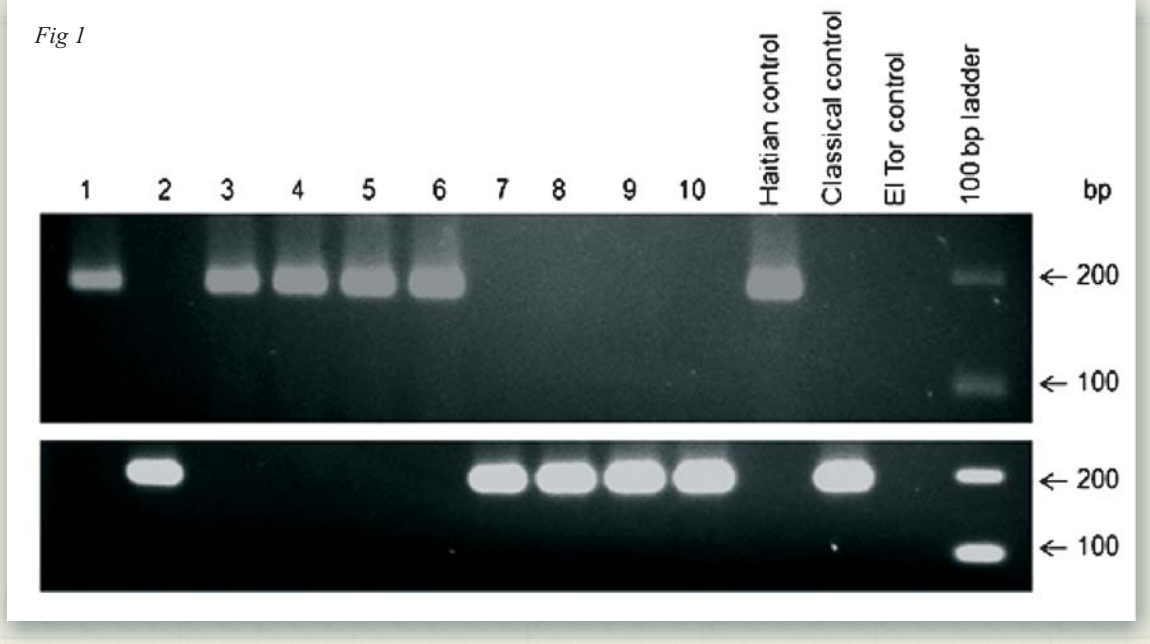
Cholera still continues to be an important cause of human infection especially in developing countries those lacks access to safe drinking water and proper sanitation. The recent devastating cholera outbreak in Haiti, for the first time in almost a century, placed this ancient scourge at the forefront of the global public health agenda. *Vibrio cholerae* O1, the causative agent of this outbreak, contained a unique mutation at the 58th nucleotide of ctxB gene and it has motivated us to investigate the emergence and dissemination of these new variants in Kolkata, India. We developed a double mismatch mutation assay to accurately discriminate the classical, El Tor and Haitian type ctxB alleles and thereby to detect the emergence of new variants of *V. cholerae* O1 (Figure 1). Our chronological study using this newly developed

PCR and sequencing analysis showed that the Haitian ctxB first appeared in Kolkata during April, 2006 and 93.3% strains isolated in Kolkata during 2011 carried the new allele (Figure 2). Dendogram analysis showed a distinct PFGE pattern of the new variant strains when compared with the strains carrying classical ctxB suggesting considerable diversities in genomic content of the new variant strains in Kolkata but they closely matched with the Haitian outbreak strains (Figure 3).

Our results not only signify a cryptic change in the circulating strains in Kolkata but also raise questions about the origin of these variants of *V. cholerae* O1 El Tor. This study clearly indicated that this new type of ctxB (genotype 7) prevailed in Kolkata since April 2006. This finding tempted us to speculate that Haitian type of ctxB may have originated from Kolkata and then disseminated to the neighboring regions like Orissa and other places, although conformation of this hypothesis requires several other epidemiological and experimental validations, and then may have spread incontinently from Nepal to Haiti as shown by recent evidences. It has been hypothesized that the unique genetic composition of the new variants increases their relative fitness, perhaps as a consequence of increased pathogenicity.

Recent reports by several research groups showed a putative link between the strains associated with cholera in Haiti and in Nepal underscoring the speed at which infectious diseases can be transported globally and this situation puts at risk other non-endemic countries also. Implementing a coordinated, integrated multidisciplinary approach is the only efficient way to prevent and contain outbreaks among vulnerable populations living in high-risk areas. Prevention, preparedness, and response all depend upon an effective and holistic surveillance system and are linked and interdependent. We strongly believe that the DMAMA will be an easy and accurate tool for tracking the emergence and dissemination of Haitian variant ctxB in *V. cholerae* O1 isolates and therefore will impart an integral role in understanding the cholera epidemiology around the globe.

Fig 1



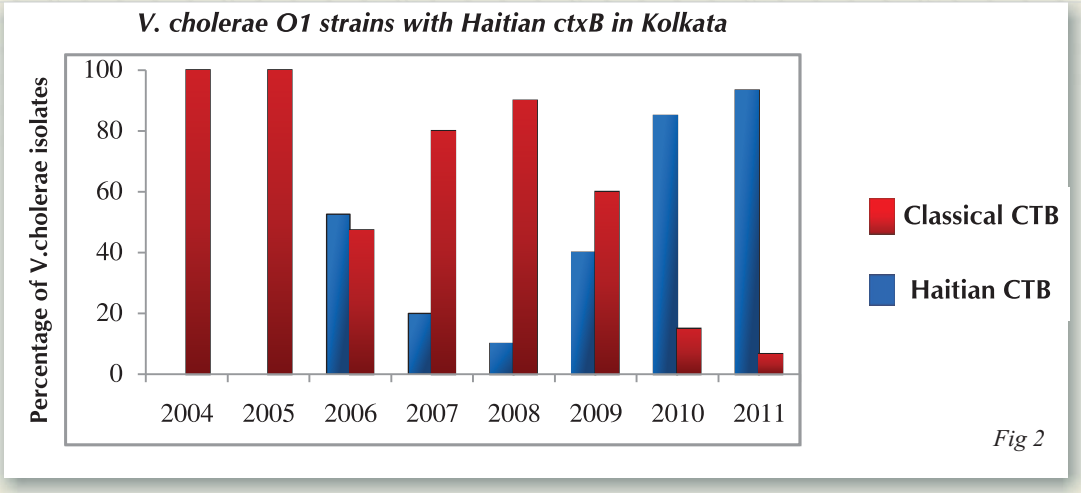


Fig 2

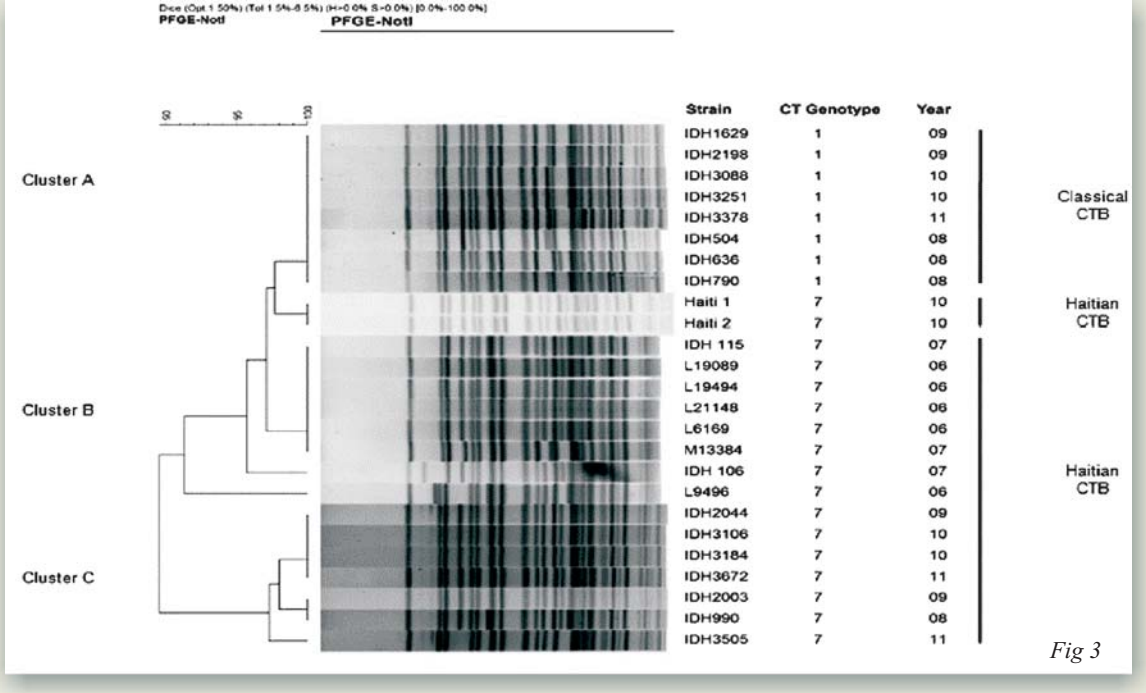


Fig 3

Legend to the figures:

Figure 1: Development of DMAMA-PCR to detect the type of *ctxB* allele in representative *Vibrio cholerae* O1 strains of Kolkata using primers for Haitian *ctxB* allele (upper panel) and classical *ctxB* allele (Lower panel).

Figure 2: Distribution of ctxB allele type in *V. cholerae* O1 strains during 2004-11 in Kolkata. Total 142 strains were tested during study period and *V. cholerae* O1 strain with Haitian type ctxB was first time isolated in Kolkata during April 2006.

Figure 3: PFGE patterns of the NotI digested *V. cholerae* strains from Kolkata and Haitian control strains along with the dendogram analysis using Bionumeric software (Applied Maths, Sint-Martens-Latem, Belgium). Analysis showed 3 distinct clusters with all the *V. cholerae* strains having classical ctxB

(genotype 1) clustered together. All the tested isolates with Haitian ctxB (genotype 7) in 2006-07 and in 2008-2011 however, were found to form two distinct clusters suggesting considerable diversities in genomic content between them.

Transmission of imipenem resistance determinants along the course of an outbreak with NDM-1 *Escherichia coli* in a sick newborn care unit

Investigators: S. Roy, A. K. Singh*, R. Viswanathan*, R. K. Nandy, S. Basu

*Department of Neonatology, Institute of Post-Graduate Medical Education & Research and SSKM Hospital, Kolkata-700020.

An overwhelming proportion of neonatal deaths occur in developing countries. Though the development of sick newborn care units (SNCUs) at rural hospitals in India has made a difference in infant survival rates, infection control still remains a concern. The options for the treatment of nosocomial infections are being severely compromised due to the increasing prevalence of carbapenem resistance in gram-negative bacilli (GNB), limiting the choice of antibiotics to few highly selected and costly ones.

Recently, the emergence of a carbapenemase, designated New Delhi metallo-β-lactamase 1 (NDM-1), has intensified the problem of drug resistance. In November 2009, carbapenem-resistant GNBs were isolated during a point prevalence survey carried out on a single day at a SNCU of a rural hospital in West Bengal, India. Shortly after the survey, a cluster of bloodstream infections in the same unit with carbapenem-resistant *E. coli* was detected. The present study attempted to understand the dynamics between the neonates, the environment and the GNBs with respect to the *bla*NDM-1 gene. In addition it elucidated the mechanism of carbapenem resistance and the presence of other β-lactamases in the context of the *bla*NDM-1 gene.

The resistance to carbapenem in these isolates were due to the presence of NDM-1 and not due to other factors like loss of porins. The coexistence of other genes (*bla*CTX-M-15, *bla*TEM-1, blaOXA-1, *bla*CMY-59, *rmtB)* in these isolates were established along with a virulence determinant (*papC)*. The plasmid carrying *bla*NDM-1 was transferable. The time frame of isolation and clonal identity indicated a possible transfer of *bla*NDM-1 from the carbapenem-resistant GNBs to the carbapenem-susceptible *E. coli* which subsequently caused septicaemia. This establishes the promiscuous nature of *bla*NDM-1 and emphasizes the need for early recognition of similar isolates.

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

Investigators: P. Das, A. K. Singh*, S. Mukherjee*, K. Rajendran, D. R. Saha, H. Koley, S. Basu

*Department of Neonatology, Institute of Post-Graduate Medical Education & Research and SSKM Hospital, Kolkata 700020,India

The gut of a neonate is colonized by bacteria immediately after birth. While not all colonization leads to infection, the pathogenicity of the aerobic Gram Negative Bacilli (GNB) may predispose the babies towards infection. In the earlier phases of the study we had been able to establish that there is an association between gut colonization and neonatal sepsis.

In the next phase, to gain an insight into the pattern and to assess factors that could influence colonization by *Escherichia coli*, analysis of the phylogenetic groups and virulence determinants of *E. coli* isolated from the gut of neonates was carried out. The distribution of the phylogroups of *E. coli* isolates recovered showed that A and B1 phylogroups accounted for 71% of the isolates. Isolates of the phylogenetic group B2 were rare. The detection of virulence factors were rare except for aerobactin (iucC) which was detected in 45% of the isolates and was significantly associated with phylogroup B1. Logistic regression established that colonization with phylogroup A was associated with a stay outside the neonatal intensive care unit (NICU) i.e the ward and that of B1 was associated with a stay in the NICU. The evaluation of the effect of *E. coli* of different phylogroups with and without identified virulence determinants on the gut of neonatal mice showed histopathological changes in the mucosa. The severity of the changes could be correlated to the presence of virulence determinants irrespective of the phylogroup.

Studies on Immunogenicity and protective efficacy of *V. cholerae* outer membrane vesicles (OMVs)

Investigator: H. Koley

Studies of OMVs from diverse bacterial strains suggest their roles in the delivery of toxins to host cells, the transfer of proteins and genetic material between bacterial cells, cell-to-cell signals, and the elimination of competing organisms (Kuehn &Kesty, 2005; Mashburn-Warren &Whiteley, 2006).

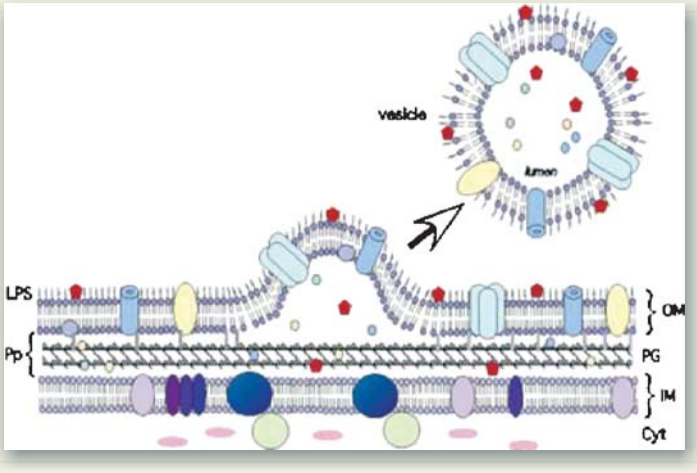


Fig -1 :Both pathogenic and non-pathogenic species of Gram negative bacteria secrete vesicles. Naturally bacterial vesicles are produced by growing cells , not products of cell lysis or cell death.

Because OMVs are essential to bacterial survival and pathogenesis in the host, modulation of vesicle formation and their functions may be a useful objective in relation to the development of antibiotics as well as vaccines (Henry et al., 2004; Lee et al., 2007).

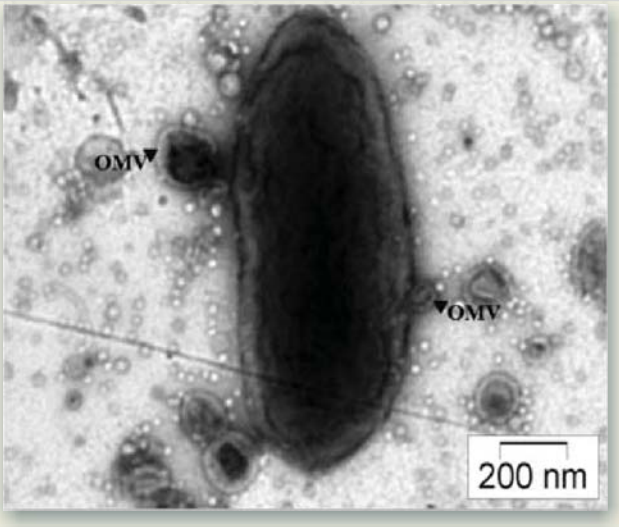


Fig -2 :Electron micrograph of the Outer Membrane Vesicles (OMVs) attached to Vibrio cholerae N16961 strain (20X)

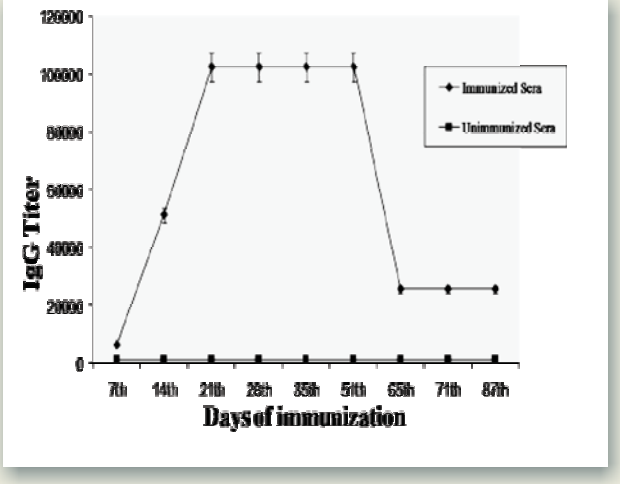


Fig -3 :V. cholerae OMVs are immunogenic. Rabbits were immunized with 4 oral doses of OMVs purified from V. cholerae N16961 strain administered on the days 0, 7, 14 and 21. Serum IgG antibody titer was measured on the days indicated.

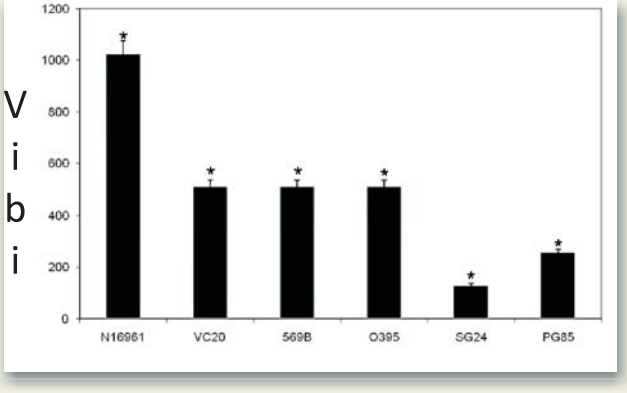


Fig -4:Vibriocidal activity of OMV-induced antibody. Antiserum was collected on day 28 and the homologous and 5 heterologous V. cholerae strains were used for the serum vibriocidal antibody assay.

In the current study, we have thoroughly investigated the safety, immunogenicity and protective efficacy of OMVs derived from *V. cholerae* and their potential use as an orally administered candidate vaccine. We have shown here that oral immunization with purified outer membrane vesicles (OMVs) of *V. cholerae* induce prolonged high rise of protective antibody titer.

Fig -5 OMVs are less reactogenic than the live and heat-killed bacteria. Reactogenicity assay showed the effects on HT2A cell line. HT2A cell line was treated with and without formalin treated V. cholerae, heat killed V. cholerae, live cell of V. cholerae and purified vesicle for 90 minutes.

Fig –6 :OMVs immunised healthy rabbit after challengedwith live virulent V. cholerae strain

The rabbit immune sera showed vibriocidal activities in vitro against the homologous and several heterologous *V. cholerae* strains. In addition, OMV immunization conferred highly significant protective immunity against subsequent bacterial challenges. A total of 30 rabbits were immunized with the OMVs of *V. cholerae* N16961 (El Tor, Inaba) strain.

Rabbits were challenged with the homologous and 5 heterologous strains and each challenged group contained 5 immunized and 3 unimmunized animals. All the immunized rabbits survived bacterial challenges and were healthy after 24 hours except 2 from each group that received the SG24 and SG06 strains, respectively, which developed watery diarrhoea. In contrast, all the unimmunized animals developed cholera-like symptoms with a death-toll of 8 animals within 24 hours of challenge.

This is the first report of the induction of protective immunity by *V. cholerae* OMVs in a rabbit model (Reversible intestinal tie adult rabbit diarrhea or RITARD model) that mimics the human disease. Finally, OMVs were found to be significantly less reactogenic compared with the live and the heat-killed bacteria.

Our studies show that oral immunization with OMVs of *Vibrio cholerae* may induce long-term immunity and may be a useful "non-living" vaccine candidate for the future.

Awards & Honours

S. Dutta

- Invited reviewer for the following journals: Diagnostic Microbiology and Infectious Diseases (DMID), Journal of Applied microbiology (JAM), J of Antimicrobial Chemother (JAC), - BMC infectious diseases-a BMC series journals: - Expert Review of anti-infective therapy, - J of Microbiol and Antimicrobial
- Invited as an expert by the Union Public Service Commission to interview candidates for the Post of Specialist microbiologist Grade II on 28-29 April 2011
- Invited as an expert in the interview to recruit research associates/fellows at various levels for National Innovation Foundation: A DST organization on 1 Feb, 2012
- Invited by the DCGI, Central Drugs Control Organization, Directorate General of Health Services, M/O Health and FW for expert opinion on application forms for new diagnostic kits.

A Palit

- Member, Drinking Water sectional Committee, FAD 25, Bureau of Indian Standards, Ministry of Consumer affairs, Food and Public distribution, GOI, 2011-12.
- Member, Water purification system sectional committee, MHD 22, Bureau of Indian Standards, Ministry of Consumer Affairs, Food and Public Distribution, GOI, 2011-12.
- Reviewer, STS (ICMR),2011-12 programme
- Reviewer of the journals : Epidemiology & Infection, International Journal of Environmental Health Research, Journal of Health Population and Nutrition, Agricultural Research Journal, Egypt

B. L. Sarkar

- Invited as Guest of Honour and delivered lecture as keynote speaker entitled "Water and waterborne diseases with special emphasis on cholera and cholera bacteriophages" at a seminar at Darjeeling, West Bengal on 06 August, 2011.

A. K. Mukhopadhyay

- Invited as an expert to work in the WHO and IVI, Korea sponsored programme entitled "Mass oral cholera vaccination in high-risk populations in Zanzibar: Assessment of effectiveness and herd protection" and to appraise the quality control (QC) and quality assurance (QA) on current practices in the two newly formed laboratories at Zanzibar to isolate, identify and store Vibrio organisms during 11-16 April, 2011.

H. Koley

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

Conferences/ Seminars/Workshops /Trainings Attended/Organised

S. K. Niyogi

- Participated in IBSC General Meeting at Institute of Molecular Medicine (IIM), Kolkata on 21 April, 2011.
- Conducted PhD viva examination at PGI. Chandigarh on 23 July 2011 (Mr Devinder Toor)
- Participated in Intercountry Consultation on Elimination of Kala-azar in the South-East Asia Region held at NICED, Kolkata, India 9-10 November 2011.
- Participated at the 46th Conference on US-Japan Cooperative Medical Science Programme on Cholera and Other Bacterial Enteric Infections held at Hyat Regency Kolkata, India during 13-15 December,2011.
- Conducted PhD viva examination at PGI. Chandigarh on 7 January 2012 (Ms Priyanka Chaudhary)
- Participated in the meeting on Pneumonia Etiology Study in India held at ICMR Headquarters office, New Delhi on 18 January 2012.
- Participated in the Project Review Committee Meeting on "Diarrhoeal Diseases" on 27 February, 2012 at ICMR Hqrs.

T. Ramamurthy

- Presented a talk on "Etiology of enteric pathogens among diarrheal patients admitted at the Infectious Diseases Hospital, Kolkata, India" at the XIII International Congress of Bacteriology and Applied Microbiology of International Union of Microbiological Societies 2011 Congress (IUMS2011) held in Sapporo, Japan from 6- 10 September, 2011.
- Delivered a talk entitled "Foodborne bacterial pathogens and Food safety" at the National Seminar on "Current Issues in Food Safety" at University of Kashmir, Sri Nagar from 27-28 September, 2011.
- Presented a talk on "A glimpse on the emerging enteric pathogens" in the XI Symposium on vector and vector borne diseases of National Academy of Vector Borne Diseases, UNICEF sponsored special session on 'Water borne diseases and sanitation' held at the Regional Medical Centre for Tribals, Jabalpur, from 15-17 October, 2011.
- Delivered a talk on "Characteristics of *Shigella sonnei colicins* and its possible role in determining the existence of other enteric bacterial pathogens" at the 46th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections held in Kolkata, India, 13-15 December, 2011
- Delivered a talk on "Molecular techniques for diagnosis of enteric diseases" during a short-term hands on training on 'Techniques in molecular biology and their application', held at College of Veterinary Sciences, Assam Agricultural University, Guwahati from December 21-23, 2011.
- Delivered a talk on "Etiology of enteric pathogens among diarrheal children: comparative analysis of hospitalized cases and outpatients in Kolkata, India" at the

"Annual Asia-African Research Forum on Emerging and Reemerging Infections 2012" organized by the Japan Initiative for Global Research Network on Infectious Diseases held in Kobe, Japan during 11-12 January, 2012.

- Delivered a talk on "Antimicrobial resistance: much more to manage" in the International Conference on 'Regulatory Network Architecture in Bacteria' at the SASTRA University, Thanjavur, from 9-11 March, 2012.
- Delivered a talk on "Childhood diarrhea: complications and considerations from a Calcutta study" in the Conference on 'Anti-infective Drug Discovery India 2012, at Hyderabad between 20- 21 March, 2012.

S. Dutta

- Poster presentation at the 7th World Congress of the World Society for Pedriatic Infectious Diseases held at Melbourne, Australia on 16-19 Nov 2011. Poster details: Jain P, Nandy S, Mitra U, Dutta S entitled "Increased antimicrobial resistance in non-typhoidal Salmonella stool isolates from children with acute diarrhea in Kolkata, India"
- Participated at the 46th Annual Joint Panel Meeting of US-Jap Cooperative Medical Science Program held in Kolkata India, from 13-15 Dec, 2011 and presented posters with following titles:
  - Nandy S, Ghosh S, Ganai A, Dutta S. "Molecular characterization of *Shigella sonnei* isolates from recent Food Poisoning Outbreak in India and comparison with the sporadic isolates"
  - Dutta S, Holt K, Manna B, Bhattacharya S K, Ochiai L, Ali M, Clemens J and Dougan G. "SNP analysis of *Salmonella enterica* serovar Typhi population from Kolkata, India during a four-year…"
  - Das S, Jain P, Ganai A, Dutta S. "Evaluation of one direct nested PCR method for diagnosis of typhoid fever"

A. Palit

- Presented the following abstracts:
  - Batabyal P, Mookerjee S, Einsporn M, Yamasaki S, Lara RJ, Palit A. "Aquatic environment, bio-geo-ecology and seasonality along the Gangetic delta of West Bengal: Vibrio paradigm" at the 46th Annual Joint Panel Meeting,US-Japan Cooperative Medical Science Program Joint Panels on Cholera and other Bacterial Enteric Infections, in Kolkata during 13-15 December, 2011 pp-226.
  - Fischer P, Einsporn M, Palit A, Batabyal P, Lara RJ, Unger D. " Characterization of nitrogen in estuaries of West Bengal during winter monsoon (dry season)" at German Society for Marine Research, YOUMARES 2.0, 2011, Germany, pp-90.
  - Batabyal P, Mookerjee S, Palit A, Lara RJ, Einsporn M." When pathogens respond to human interaction with waterbodies in West Bengal. India". German Society for Marine Research, YOUMARES 2.0, 2011, Germany, pp-44.
  - Batabyal P, Palit A. "Vibrios of gangetic delta, India: Correlation of physico-chemical parameters and incidence of cholera" at the 13th ASCON,2011,pp-228 (236).

- Organized & participated in Intercountry Consultation on Elimination of Kala-azar in the South-East Asia Region (WHO), during 9-10 November, 2011 at NICED, Kolkata, India. (WHO-SEARO-APW).
- Organized (Member,LOC) & participated in US-Japan Cooperative Medical Science Program Joint Panels on Cholera and other Bacterial Enteric Infections, in Kolkata during 13-15 December, 2011.
- Organized International Workshop on "Bacterial microbiome analysis using 16S-rRNA bacterial database" at NICED, Kolkata, 16-20 December, 2011.
- Organized & participated as joint Principal Investigator in meeting for project work in the DST-DFG (indo- German collaboration) project "Role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal: relation with cholera incidence" along with our international counterparts (German and Japan), 13-17 June, 2011.
- Participated as joint Principal Investigator and presented project progress report of DST-DFG (indo- German collaboration) project "Role of seasonality on the distribution, abundance and diversity of Vibrio organisms in estuaries of West Bengal: relation with cholera incidence "in project evaluation meeting at ZMT, Bremen, Germany (18 June-1 July,2011).

B. L. Sarkar

- Attended the inaugural function of World Environment Day function at New Delhi, sponsored by ICMR on 03 June, 2011.

R. K. Nandy

- Organized US-Japan Cooperative Medical Science Program Panel on Cholera and Other Bacterial Enteric Infections at Kolkata, during 13-15 December, 2011. NICED acted as local host- Participation as Organizing Secretary.
- Attended the 3rd 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 Seattle, USA from 3-5 May, 2011

A. K. Mukhopadhyay

- Presentation of the work entitled "Detection of the traits of Haitian variant strains of *Vibrio cholerae* in Kolkata, India since 2006" in the Annual Asia-African Research Forum organized by the Japan Initiative for Global Research Network on Infectious Diseases in Kobe, Japan during 11-12 January, 2012.
- Presentation of the work entitled "CTX prophage alleles impacting microevolution of pathogenic *Vibrio cholerae* O1 and O139" in the XIII International Congress of Bacteriology and Applied Microbiology of International Union of Microbiological Societies 2011 Congress (IUMS2011) held in Sapporo, Japan from 6-10 September, 2011.
- Received training to the 11th International Advanced Course on Vaccinology in Asia-Pacific Region at the International Vaccine Institute (IVI), Seoul, Korea during 2-7 May 2011.

S. Basu

- Presented a poster at the Indian Sciences Congress Association, Category: Medical Sciences (including Physiology), held on 14 October 2011 in Bhubaneswar, India. Poster details: Roy, S., Singh, AK., Viswanathan, R., Nandy RK., and Basu, S. "*Escherichia coli* causing neonatal septicaemia harboring New Delhi metallo-β-lactamase 1 (NDM-1) along with CMY-59, a novel AmpC β-lactamase".
- Presented a poster at the 46th US-Japan Cooperative Medical Sciences Program Conference on Cholera and Other Bacterial Enteric Infections held in Kolkata, during. 13-15th December, 2011. Poster details: Roy S, Datta S, Viswanathan R, Singh , A K., Basu S. "Carriage and Sepsis in neonates due to Imipenem-resistant *Escherichia coli* producing NDM-1 in a Sick Newborn Care Unit (SNCU)."

H. Koley

- H. Koley, S. Barman, D. R. Saha, R. Kumar, "Oral live transconjugantShigella Vaccine" : International Conference on Molecules to Systems Physiology: 100 Years Journey ICMSP100 Centenary Celebration of the Department of Physiology University of Calcutta 21-23 September, 2011
- J. Mitobe, H. Koley, R. Sinha, G. B. Nair, W. Haruo. "An attempt to develop experimental Shigella Vaccine based on virulence Gene Expression" at the 46th Annual Joint Panel Meeting on Cholera & Other Bacterial Enteric Infections, United States-Japan Cooperative Medical Science Program held during 13-15 December, 2011 in Kolkata , India.
- S. Barman, H. Koley. "An alternative strategy for detection of Shigella invasion". 46th Annual Joint Panel Meeting on Cholera & Other Bacterial Enteric Infections, United States-Japan Cooperative Medical Science Program held during 13-15 December, 2011 in Kolkata, India.
- S. Mitra, S. Barman, D. R. Saha , S. S. Das and H. Koley. "Outer membrane vesicles (OMVs) of *Shigella boydii* type 4 showed protective immune response in mice model" at the 46th Annual Joint Panel Meeting on Cholera & Other Bacterial Enteric Infections, United States-Japan Cooperative Medical Science Program held during 13-15December, 2011 in Kolkata, India.

BIOCHEMISTRY

The long-term interest of the Division of Biochemistry lies in understanding the molecular basis of pathogenesis of diarrheal diseases. The bacterial pathogen and the human host both play active and often complementary roles in pathogenesis of infectious diseases, e.g. infection often requires subversion of the non-specific and specific host defence mechanisms to ensure survival of the bacterial pathogen in an otherwise hostile environment. Ideally, study of the pathogenesis of diarrheal or any infectious disease should encompass identification and structural and functional characterization of bacterial virulence factors and the response of the host to the invading pathogen. However, interest of the Biochemistry Division has so far focussed on bacterial pathogens, e.g.the role of surface proteins of enterotoxigenic *Escherichia coli* in colonization of the human gut or the structure-function relationship of *Vibrio cholerae* hemolysin with emphasis on the mechanism of membrane penetration by the toxin. Recently, we have initiated study of the host signal transduction pathways elicited by bacterial proteins like *V. cholera* chitinase and hemolysin and their relevance to diarrhea in animal models. This is a small beginning towards adopting a holistic approach to understand the complex process of bacterial pathogenesis.

Scientist	:	K. K. Banerjee, Scientist F N. S. Chatterjee, Scientist D
Staff	:	K. C. Pramanik, Technical Officer A (Till August 31, 2011) T. Roy, Technical Assistant (Retired on November 30, 2011)
Research Fellow	:	S. Sabui (SRF) S. Ganguly (SRF) M. Mondal (SRF) A. Debnath (JRF) A. Mukherjee (JRF) S. Mandal (JRF)

The β-prism carbohydrate-binding domain of *Vibrio cholerae* hemolysin (VCC) promotesmembrane-insertion of the toxin by enhancing thermodynamic stability of the β-barrel oligomeric pore.

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

The mature form of VCC is a 65 kDa water-soluble protein that rapidly transforms on the target cell surface into a β-barrel heptamer capable of moving into the lipid-protein bilayer to form a diffusion channel. In addition to the cytolytic domain involved in self-assembly membrane-

spanning, VCC has two contiguous carbohydrate-binding domains of roughly equal size (~15 kDa) at the carboxy-terminus. Proteolytic deletion of the C-terminus β-prismlectin domain causes more than 1000-fold decrease in haemolytic activity toward rabbit erythrocytes and exerts a dramatic effect on biological response of cells sensitive to the toxin, e.g. it causes a switching over of the response of macrophages from apoptosis to activation (Chakraborty et al. 2011: J. Biol. Chem. 286, 34542-34551). The molecular basis of the regulation of biological activity of VCC by the β-prismlectin domain, which is not present in other pore-forming toxins (PFTs) and apparently not essential for its function, is not clear.

Earlier we showed that deletion of the 15 kDaβ-prismlectin domain abrogates specific interaction of VCC with β1-galactosyl-terminated glycoconjugates. To test if this caused a decrease in initial binding of the 50 kDa truncated variant, VCC50 to the target cell, the toxin bound to rabbit erythrocyte stroma was quantified by spectrofluorimetry. The interaction of the toxin with stroma was found to be dominated bypartition equilibrium of the highly amphipathictoxin between the lipid-water interface at the cell surface and the aqueous bulk. The partition coefficient values of 90 and 60% for VCC and VCC50, respectively, were essentially constant within the concentration range of 2.75 to 13.75 x 10-7 M. The almost quantitative, receptor-independent transfer of the toxin from water to the cell surface suggested that interaction of the toxin with specific carbohydrate receptors played at most a minor role in initial binding of the toxin to the cell surface.

Next, we examined if deletion of the -prismlectin domain decreased efficiency of oligomerization of the VCC monomer to the -barrel pore. The question is pertinent because earlier we showed in a 3D cryo-electron microscopic study of the VCC and VCC50 oligomers that their morphologies are significantly different, with the truncated oligomer lacking a 7-fold axis of symmetry (Dutta et al. 2010: J. Bacteriol. 192, 169-178). The association constants for the monomer to the oligomer equilibrium were found to be 8.58 x 1027 M-6 for VCC and 1.89 x 1029 M-6 for VCC50, indicating, in contrary to expectation, that deletion of the lectin domain actually promoted self-assembly. Interestingly, self-assembly was entropically driven ($\Delta S = +1.07$ kJ K-1mol-1 for VCC and + 0.59 kJK-1mol-1 for VCC50) and enthalpically opposed ($\Delta H = +163.02$ kJ mol-1 for VCC and + 10.46 kJ mol-1 for VCC50), indicating that self-assembly caused a significant loss of enthalpically favourable interactions involving the lectin domain. However, the association constant for self-assembly of VCC, but not of VCC50 increases by more than two orders of magnitude in presence of its glycoconjugates ligand, asialofetuin, suggesting that carbohydrate-dependent interaction involving the β-prism lectin domain compensated for the enthalpy loss during oligomerization. In effect, the lectin domain increases thermodynamic stability of the β-barrel oligomer in the lipid-protein milieu of the membrane bilayer.

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

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

Vibrio cholerae O1, a cause of epidemic diarrheal 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* was cultured in artificial sea water medium maintaining optimum environmental conditions. Wild type *V. cholerae* showed a normal growth curve but V. choleraeΔchiA2 isogenic mutant failed to maintain this growth pattern in the same media with same conditions. Number of viable cells also decreases significantly in case *V. cholerae* ΔchiA2 strain compared to wild type *V. cholerae*. In both the cases graph *of V. cholerae* ΔchiA2 complemented with pChiA2-His showed similar growth curve of wild type.

Mice intestinal survival assay showed that *V. cholerae* ΔchiA2 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. cholera* ΔchiA2 compared to wild type strain. *V. cholera* ΔchiA2 complemented with pChiA2-His was able to survive like the wild type strain.

V. cholerae survival in presence of different human intestinal cell lines were studied. Results showed that *V. cholera* ΔchiA2 survived poorly in human intestine than the wild type strain. Wild type strain survival was 4.6-fold higher than the mutant strain based on colony counting. Mutant *V. cholera* ΔchiA2 complemented with pChiA2-His were able to survive in the human intestinal cell lines like the wild type 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 of its involvement in growth and survival in the intestine.

Molecular characterization of Enterotoxigenic *Escherichia coli* colonization factors

Principal Investigator : N.S. Chatterjee
Co-Investigator : T. Ramamurthy

Enterotoxigenic *Escherichia coli* (ETEC) infection is the leading cause of infantile diarrhea in developing countries and an important etiologic agent for traveler's diarrhea. Our lab has been studying different aspects of the colonization factors of ETEC and aims in developing simple methodologies for detection of these factors. Our previous studies have indicated that there are different allelic variants of CS6, caused by point mutations in its structural genes, cssA and cssB, are designated AIBI, AIIBII, AIIIBI, AIBII, and AIIIBII. Recently, we have developed a simple, reliable, and specific mismatch amplification mutation assay based on

real-time quantitative PCR (MAMA-qPCR) method for the first time for the detection of CS6-expressing ETEC, along with the identification of allelic variations. This strategy was very effective and suitable in detecting all the alleles based on their single-nucleotide polymorphisms. Using this assay, we could detect ETEC isolates from various diarrheal samples. We observed that the AIBI and AIIIBI allelic variants were mostly associated with acute diarrheal samples, whereas the AIIBII variants were detected mostly in asymptomatic infections. This assay may help in understanding the association of allelic variants in CS6-expressing ETEC with the clinical features of diarrhea, as well as in ETEC vaccine studies. Previously we have found that pathogenicity of ETEC harboring AIBI and AIIIBI alleles could be correlated with their stronger adherence to intestinal epithelial cells compared to AIIBII in vitro and in vivo assays. Receptor binding analysis revealed that purified CssAIBI and CssAIIBII bound to mucin in a dose-dependent, saturable manner where AIIBII had 4-fold less binding affinity compared to AIBI. Deletion of cssAI or cssBI in wild type ETEC led to marked reduction in their adherence capability with mucin. ΔcssAI ETEC, when complemented with pcssAI or pcssAII, binding was similar to that of wild type ETEC. Similarly, ΔcssBI ETEC, when complemented with pcssBI, binding was similar to that of wild type ETEC. However, a 4-fold reduced adherence was observed when ΔcssBI ETEC was complemented with pcssBII when compared to pcssBI complementation or wild type ETEC. These results suggest that CssB rather than CssA subunit played a crucial role in mucin binding. Presently we are investigating the mechanism of mucin binding by CssB and the role of differential binding ability of CssBI and CssBII subunits in colonization.

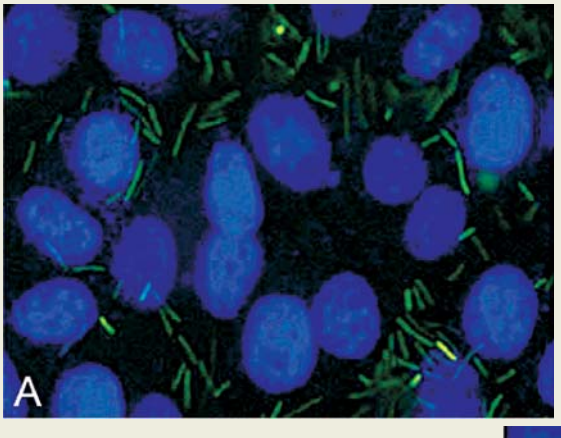
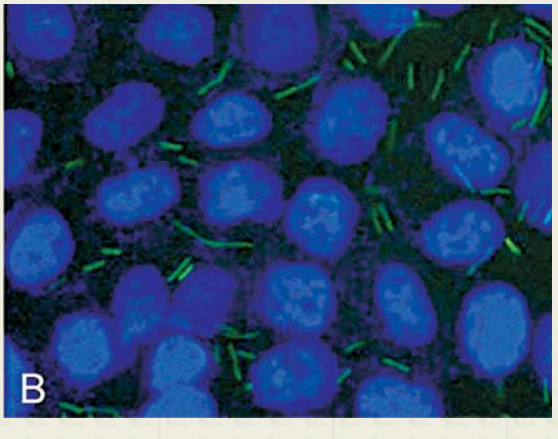


Figure 1 legend: Adherence of ETEC (green) to intestinal cells: (a) AIBI (b) AIIBII



Awards & Honours

N. S. Chatterjee

- Recipient of Fulbright-Nehru Senior Research Fellowship 2011-2012

Conferences/ Seminars/Workshops /Trainings Attended/Organised

K. K. Banerjee

- Presented a paper entitled "The carbohydrate-binding domain of *Vibrio cholerae* cytolysin/hemolysin is critical for its binding to and insertion into the target plasma membrane lipid bilayer" at the 13th International Congress of Bacteriology and Applied Microbiology of the International Union of Microbiological Society, 2011 held at Sapporo, Japan on 6-10 September, 2011.

N. S. Chatterjee

- Delivered invited lecture entitled "Molecules facilitating enteric infection: a biochemist's perception" at the College of Pharmacy, Roseman University, Nevada, USA on 28 October, 2011.
- Delivered invited lecture entitled "Chitin-utilization molecules and host-pathogen interaction: searching a link in *Vibrio cholerae*" at the Department of Biological Sciences, California State University-Fresno, California, USA on 9 March, 2012.

CLINICAL MEDICINE

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

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

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

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

One of the scientists has recently identified a novel virulence protein of Salmonella Typhi, which is a potential candidate for vaccine development because of strong immunogenicity in humans. The scientist reported this discovery in the PNAS, USA (February 22, 2011. vol. 108, no. 8, pp. 3348–3353), which was also highlighted by 'Nature' (1 7 February, 2 0 1 1. Vol 4 7 0, pp. 308).

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

Scientist : U. Mitra, Scientist E
M. K. Bhattacharya, Scientist E
S. S. Das, Scientist D
P. Indwar, Scientist B
(Joined on October 2011)

Staff : A. Pal, Technical Officer A
K. G. Saha, Technician B
S. Turi, Attendant Services
S. Dey, Attendant Services

Outpatient based surveillance of Diarrhoeal Diseases at Dr. B. C. Ray Institute of Pediatric Sciences, Kolkata

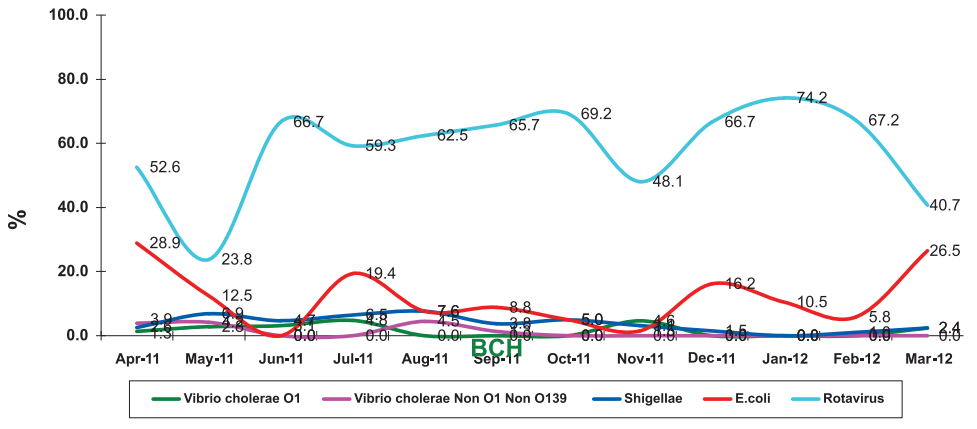
Investigator: U. Mitra

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

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

During January, 2011 to April, 2012 a total of 5122 under five children suffering from diarrhoea attended the Out Patients Department of Dr. B. C. Roy Post Graduate Institute of Pediatric Sciences. Of them, 881(17.2%) children were enrolled in the systematic surveillance system. Sixty two percent children were male and 38% were female. Percentage of children belonging to Hindu and Muslim were 65.6% and 34.3% respectively. Majority of these families (99.9%) had monthly total income of Rs. 2500 to 5000. Around 55.3% children were from surrounding urban areas and 44.7% from rural areas. Majority of the children had the history of loose stools (92.8%), 4.8% had watery diarrhea and 2.4% children had frank bloody diarrhea (dysentery). Fever, cough and vomiting were common presenting clinical features. Majority (99.2%) of children had "No Dehydration", 0.7% children had "Some" dehydration. Severely dehydrated children were not observed in OPD. *Campylobacter Jejuni*, Shigellae, rotavirus and Cryptosporidium were the major sole pathogens. Mixed pathogens were detected from 13.4% diarrhoeal children and no pathogen was detected from 49.8% children.

Isolation (%) of common pathogens of underfive children



Aetiology of diarrhoea of underfive children

Pathogen	BCH	
	Total(%)	Sole(%)
Bacteria		
Vibrio cholerae O1	13(1.5)	7(0.8)
Vibrio cholerae O139	0	0
V. cholerae Non O1 Non O139	10(1.1)	5(0.6)
V. parahaemolyticus	0	0
Vibrio fluvialis	7(0.8)	3(0.3)
Aeromonas spp.	9(1)	3(0.3)
Campylobacter jejuni	107(12.2)	73(8.3)
C. coli	5(0.6)	2(0.2)
Shigellae	30(3.4)	19(2.2)
Salmonella	5(0.6)	3(0.3)
EPEC	19(2.2)	9(1)
ETEC group	21(2.4)	12(1.4)
EAEC	70(8)	47(5.4)
Virus		
Rotavirus	188(59.1)	115(36.2)
Adenovirus	63(19.8)	13(4.1)
Norovirus G1	0	0
Norovirus G2	4(30.8)	2(15.4)
Sapovirus	2(15.4)	0
Astrovirus	0	0
Parasite		
Blastocystis hominis	0	0
Entamaeba histolytica	0	0
Giardia lamblia	21(9.3)	4(1.8)
Cryptosporidium spp.	12(5.3)	5(2.2)
Mixed Pathogen		117(13.4)
No pathogen		436(49.8)
Total Sample		875(100)

Sample not tested

BCH:

Rota+Adenovirus = 557,
Otherviruses = 862,
B.hominis = 565,
Other parasites = 648

Pending Result of Noro, Sapo& Astrovirus as follows:

BCH: Jan-11 to till date

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

Investigator: M. K. Bhattacharya

Completed the single dose Azithromycin in the treatment of cholera in adult which will help the PSC level physician and health worker to manage the huge diarrhoea patients with small manpower, the present scenario of PSC level health care centre.

Corelation of HIV and TB among the seropositive cases in Kolkata

Investigator: M. K. Bhattacharya

Among the HIV seropositives TB infection pulmonary tuberculosis is significantly associated when the CD4 count is $\leqslant 50/\ \mu l$. It may be used as an indicator for early detection of pulmonary tuberculosis among the HIV seropositives.

Toll-like receptor 5 ligands induce a regulatory immune response through Gs-coupled co-receptors activation and Tpl2-independent signals.

Investigator: S. Das

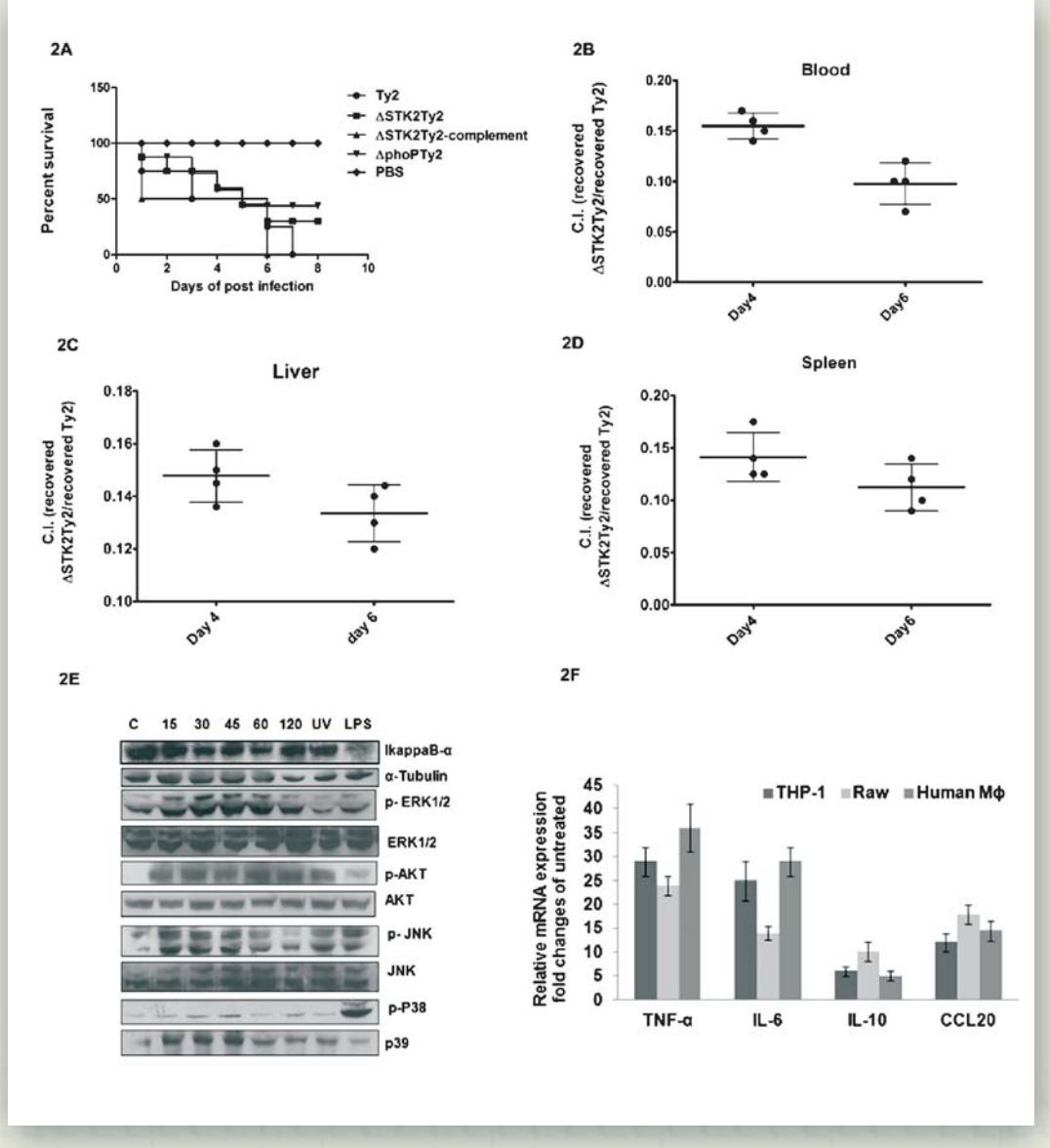
Toll-like receptors (TLRs)-activated Th1 and Th17 responses have been extensively studied. In contrast, TLR-induced regulatory responses are poorly understood. We show that pathogen-derived and commensal flagellins may elicit regulatory responses through Tpl2/Cot-independent activation of ERK and enhanced production of IL10 and TGFβ. These flagellins recruit Gs-coupled receptors, A2A and VPAC1 as co-receptors of TLR5. Signals transduced by TLR5 and the co-receptors converge on c-Src, which activates Rap1-b-Raf-MEK-ERK in MyD88-TRAF6- and cAMP-PKA-dependent mechanisms. Simultaneously, coreceptor-activated signals suppress Th1 cytokines production. Flagellins that generate regulatory responses induce DC tolerogenicity and ameliorate murine experimental colitis through the induction of Foxp3+ T cells. This, to the best of our knowledge, is the first report of Gs-coupled receptor activation by TLR ligands and the regulation of immune response through cross-talk between G-protein signaling and non-receptor tyrosine kinases. This is also the first study to delineate Tpl2/Cot-independent regulation of MEK-ERK signaling pathways by TLR activation signals.

A secreted eukaryotic-like serine/threonine kinase of Salmonella enteric a serovar Typhi (*S. typhi*) activates multiple intracellular signaling pathways, induces pro-inflammatory cytokine/chemokine release and contributes to bacterial virulence in mice.

Investigator: S. Das

Seine/threonine and tyrosine kinases and phosphatases of prokaryotes have recently been shown to play an important role in host-pathogen interactions and virulence of the organisms. We have recently identified a eukaryotic-like serine/threonine kinase (STK) of *S. typhi* whose

expression is upregulated within macrophages. Further studies with wild type, mutant and complemented bacteria showed that STK2 significantly contributes to bacterial virulence in mice, which was comparable to the effects of PhoP, a master regulator of the virulence genes of *S. typhi* (Fig 2A). Only 10-15% of the live bacteria recovered from the blood and visceral organs of mice belonged to the STK2-mutant strain when the animals were infected with equal numbers of the wild type and mutant *S. typhi* strains (Fig 2B-D). In order to explore the underlying mechanisms of STK2-mediated virulence of *S. typhi*, we stimulated human monocytic cell line THP1 with recombinant STK2 and studied the activation of intracellular signaling pathways. The results showed significant activation of NF-kB, ERK MAPK, JNK and Akt (Fig 2E). Pro-inflammatory cytokines (TNFα, IL6 and Ccl20) as well as IL10 was induced in macrophages upon STK2 stimulation. Studies are currently in progress to identify the specific mechanism through which STK2 contributes to the virulence of *S. typhi* in mice.



Award and Honours

S. S. Das

- National Bioscience Award for Career Development, 2011 (Awarded by Department of Biotechnology, Government of India).
- Nominated as member of the Molecular Immunology Forum, India.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

U. Mitra

- HFT Livercon-2011 Organized by Hepatitis Foundation of Tripura held at Agartala during 23-24 April 2011
- 54th Annual State Conference of IPHA held at Kolkata on 16 July, 2011
- 56th Foundation Day Celebration of Indian Public Health Association on 28 September, 2011 at Kolkata
- 46th Annual Joint Panel meeting on Cholera and other enteric infections (United states-Japan Cooperative Medical Science Program) held at Kolkata on 13-15 December 2011
- 55th Annual State Conference organized of IPHA, held at Kolkata on 17 December, 2011

M. K. Bhattacharya

- Attended meeting with Shri Goutam Deb, Hon'ble Minister-in-charge, North Bengal Development Department on 28 November 2011 at Board Room of North Bengal Medical College & Hospital regarding identification of site for establishment of the proposed field unit of NICED at the campus of North Bengal Medical College & Hospital.
- As guest lecturer delivered a lecture on Health Management During Disaster on 7 April 2011 at the Administrative Training Institute, Govt. of West Bengal.
- As guest lecturer delivered a lecture on Health & Hygiene Management to Reduce Urban Risk on 13 July 2011 at the Administrative Training Institute, Govt. of West Bengal.
- As guest lecturer delivered a lecture on Health Management During Disaster on 21 July 2011 at the Administrative Training Institute, Govt. of West Bengal.
- As a chief jury (nominated by DST, New Delhi) for selection of best scientific project in District level Science Exhibition on 29 July 2011 at Indoor Stadium, Jharkhand.
- As guest lecturer delivered lecture on “Etiology of Diarrhoeal Diseases and Importance of Early Diagnosis Management & Prevention” on 12 November 2011 at

the 5th All India Conference on Health Medical Education, Technology and Common Man at the School of Tropical medicine, Kolkata.

- As guest lecturer delivered lecture on Health & Hygiene Management to reduce Urban Risk on 22 November 20011 at ATI, West Bengal.

S. S. Das

- Oral presentation at the 46th Joint Panel Meeting of the US-Japan Cooperative Medical Science Program on Cholera and Other Bacterial Enteric Infections held in Kolkata, India during 13-15 December, 2011.
- Delivered invited lecture at the national level seminar entitled "Research Impact of Thrust Areas identified under CAS Programme of Biochemistry" held at Prof. B.C. Guha Memorial Hall, Department of Biochemistry, University of Calcutta, Kolkata on 7 March, 2012.
- Delivered invited lecture at the Fifth Symposium on Molecular Medicine, Jawaharlal Nehru University, New Delhi 17-18 February, 2012.
- Delivered invited lecture at theMolecular Immunology Forum, Matheran, Maharashtra 10-12 February, 2012.
- Delivered invited lecture at the National Conference on CME in Immunology (organized by West Bengal State University (WBSU) and Indian Immunology Society) at WBSU, Kolkata 5 November, 2011.
- Delivered invited lecture at the CSIR-Indian Institute of Chemical Biology, Kolkata (Organized by Society of Biological Chemists, Kolkata Chapter, 29 September, 2011.
- Delivered invited lecture at the CSIR-Indian Institute of Chemical Biology (IICB), Kolkata (Training Program on Laboratory Safety, 15 September, 2011.
- Organised a workshop entitled "Understanding Genome- A Bioinformatics Approach" at the Biomedical Informatics Center of NICED on 27-29 April, 2011.
- Organised an International Conference on Omics Meets Disease and IIIrd Annual Meeting of Proteomics Society (India) at Saha Institute of Nuclear Physics (SINP), Kolkata on 15-18 December, 2011 (as member of the Organizing committee).

DATA MANAGEMENT

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

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

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

Generation of a database on cholera outbreaks in India

Principal Investigator: B. Manna

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

with the following objectives.

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

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

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

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

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

VI. To determine the possible risk factors for outbreaks

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

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

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

Methodology :

Procedure for data collection :

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

Creation of Database :

A customized Data Entry Programme on Visual Basic at the front end will be developed to feed the necessary data from epidemics and Data will be saved in MS access in the back end. The input variables are as follows:

Time, Place, State, total person affected, attack rate, causative organism, isolation rate, antimicrobial sensitivity of causative organism, treatment management, clinical sign & symptoms, mode of transmission, identification of risk factors for outbreaks

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

Progress so far :

Data collection has been started from different published articles and unpublished documents. About 100 published articles have been collected. Since July, 2009 to May, 2012, a total of 1,383 outbreaks have been reported in Integrated Disease Surveillance Project (IDSP). It has been observed that maximum number of outbreaks occurred during May – July. According to report, West Bengal had maximum number of outbreaks followed by West Bengal, Tamil Nadu and Andhra Pradesh. Data structure has been created and data entry is going on. Study is on progress.

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

Investigator : K. Rajendran

The objectives of the study is to compare the climatic factors with observed infection of diarrhoea and cholera in the Infectious Diseases Hospital, Kolkata and to assess long term changes to develop time series model and mathematical statistical models.

Relational impact sunshine duration associated *V. cholerae* infection :

The disease cholera caused by *V. cholerae* is believed to be influenced by climate variability. Climate is one of the important factors for cholera persistence and spread in Bengal. This part of study is designed to determine sun shine duration on cholera infection in Kolkata. In climatic factors, the difference of RH and temperature [i.e., morning (max)-evening (min)] were used in the analysis. This procedure was relevant to identify the actual causative factors instead of mean factors. The mean factors purposefully have been averted to avoid the influence of high variation in the series. The adopted analytical procedures were linear methods like Seasonal Auto Regressive Integrated Moving Average (SARIMA) and Generalized Linear Model. These models identified periodicity and seasonal patterns of cholera. The GLM furnished an increase of cholera infection during short span of sun shine duration ($<=3$ hrs: $P<0.001$) and highest span of sun shine duration ($>$ 7hrs: $P<0.001$) on the day when rainfall occurs and do not occur, respectively. All these factors are interrelated with other climatic factors. Consistent temperature (29°C) significantly associated with the sun shine duration (3-7hrs: $p<=0.001$) along with variations in relative humidity (12-15%: $P<=0.001$) both rain fall and no rain fall days. Sun shine duration is one of the climate components of cholera infection, which may depend on variation of other factors like, rainfall and relative humidity with consistent temperature. However, the changing temporal patterns of other enteric pathogens were also identified in relation to climatic conditions in Kolkata. When the sunshine duration was less, prevalence of cholera was less but rotavirus infection was high while sunshine duration increases in winter. Over the 16 years (1996-2011) study period, climate significantly fluctuated and the inconsistency was observed. The El Niño and La Niña has definite role in determining the prevalence of diarrhoea in Kolkata.

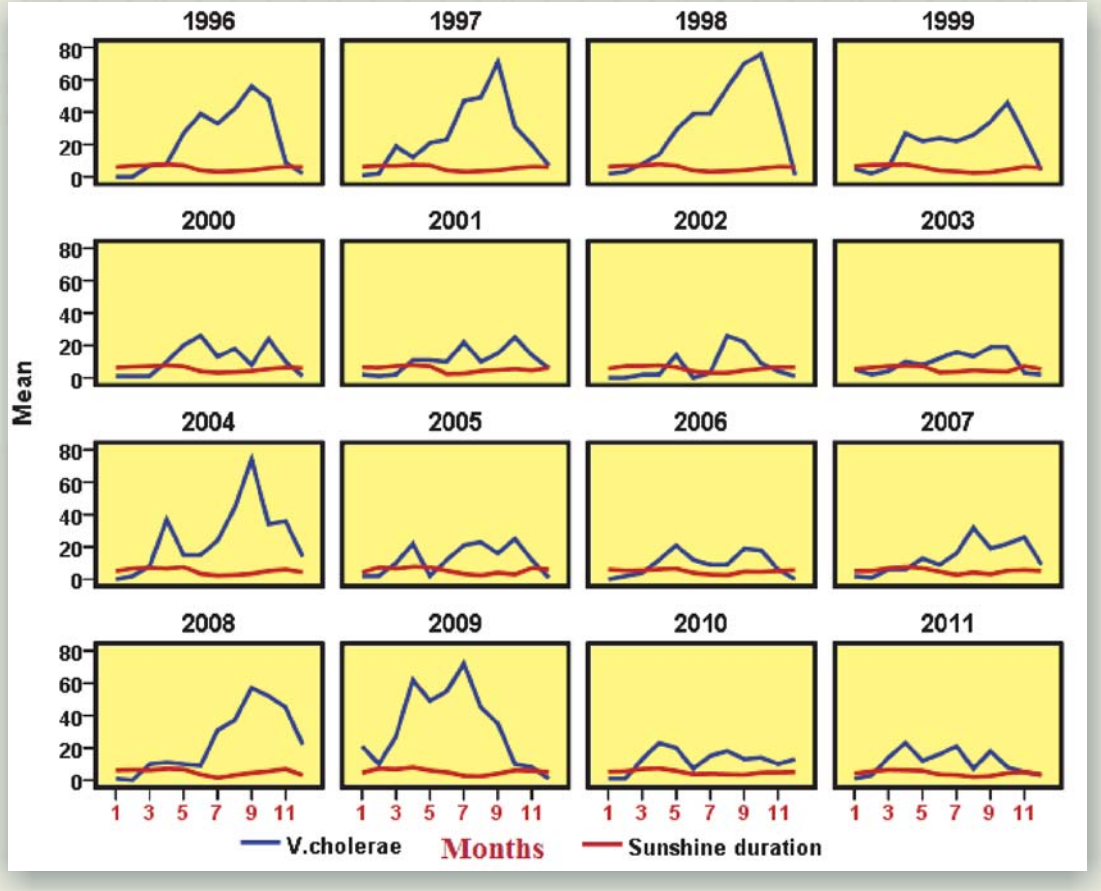


Fig 1: Relational impact of sunshine duration with V. cholerae infection at Kolkata

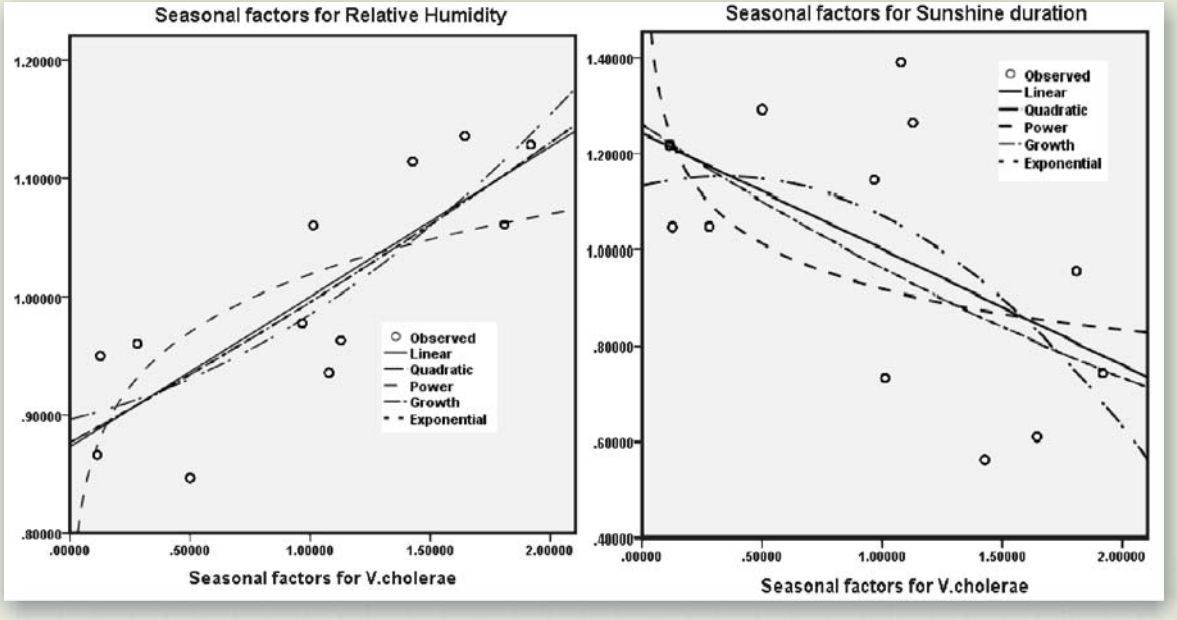


Fig 2: *Regression curve estimation established inverse relation of sunshine in the seasonality cholera infection.*

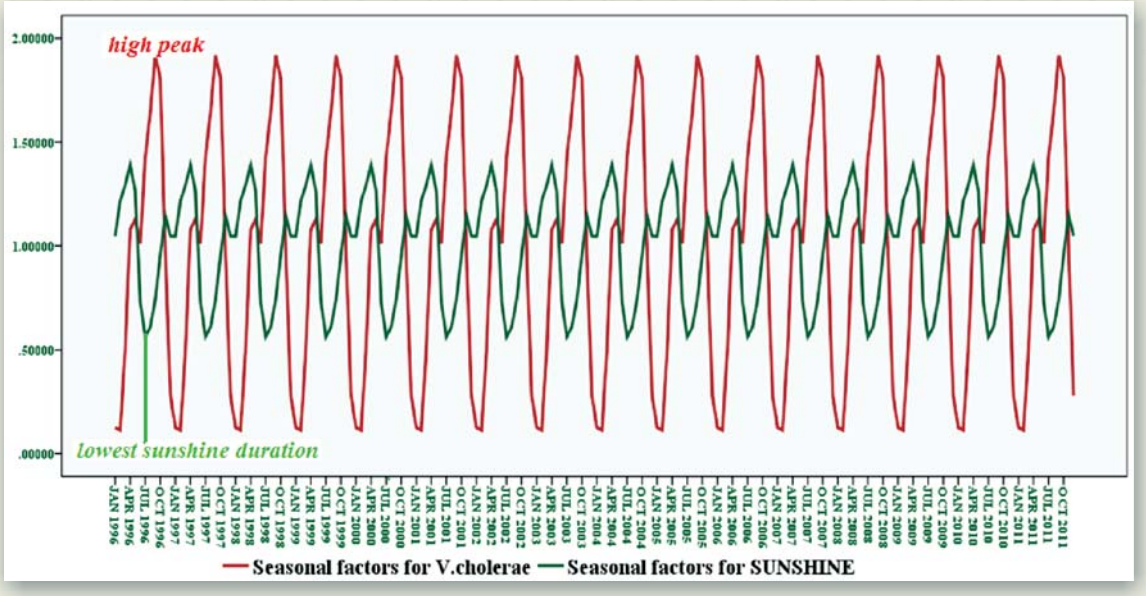


Fig 3: Seasonal decomposition method identified lowest sunshine duration favors V. cholerae infection in the seasonal cholera.

Award and Honours

K. Rajendran

- Invited as plenary Speaker and title of speech was "Climate and cholera: A changing scenario in Kolkata, West Bengal."
- Reviewer of three International Journals

Conferences/ Seminars/Workshops /Trainings Attended/Organised

B. Manna

- Delivered a talk on "Cholera Burden in India" in the Workshop on Estimation the burden of Diseases on 22 May 2011 at Vivekananda Institute of Medical Sciences, Kolkata
- Attended a decision making meeting for the project entitled "Exploration of the Biologic Basis for OPV and Rotavirus Vaccine underperformance in India" held at International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh (ICDDR,B) on 24-25 July, 2011
- Attended the Investigators Meeting for the project " Diarrhoeal Disease in Infants and Young Children in developing Countries" (The Global Enteric Multi Centric Studies) in Philadelphia, Pennsylvania, USA from 30 Nov-1 Dec, 2011
- Probiotic symposium on Health impact of Probiotics- Vision and opportunities in Mumbai from 10-11 December 2011

- Attended and Delivered a talk on "Typhoid Vaccine and Cholera Vaccine trial in Kolkata in the CDSA-OWH (Clinical Development Service Agency – One World Health) Clinical Trial Training Program from 12-17 Dec, 2011 at Chennai
- Delivered a lecture on Ethics in Medical Research in the Foundation Workshop on Clinical & Laboratory Medicine Research from 27-29 March 2012 at Agartala Government Medical College, Agartala

K. Rajendran

- Rajendran K & Ramamurthy T. Climate and cholera: A Changing Scenario in Kolkata, West Bengal" A National Conference on "Climate Change, Oil Spill and Radiation Risk; New Environmental Challenges" Yashwantrao Chavan Pratishthan, Mumbai- 21 during 15 - 16 November, 2011.
- Rajendran K, Sumi A,Manna B, Kobayashi N, Takeda Y, Nair GB & Ramamurthy T. Sunshine duration manoeuvre *V. Cholerae* infection in Kolkata, India: Generalized linear model. The 46th US- Japan cooperative Medical Science Programe Cholera and Other Bacterial Enteric Infections on 13-15 December, 2011. Kolkata, India
- Workshop on "Clinical trial; Design, Analysis: interpretation and Reporting" held during 08-12August, 2011 by Bio-statistics Resource and Training Centre, Christian Medical College, Vellore, India.

ELECTRON MICROSCOPY

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

Cryoelectron microscopy and 3-D image reconstruction

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

Vibriophage research

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

Nanobiotechnology

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

Histopathological studies

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

Scientist : A. N. Ghosh, Scientist F
D. R. Saha, Scientist E

Staff : A. Sarbajna, Technical Officer A
S. Kumar, Technican B
B. R. Mallick, Attendant Services

Cryo-negative staining improves the resolution of *Vibrio cholerae* hemolysin oligomers

Principal Investigator: A. N. Ghosh
Co-Investigator: K. K. Banerjee

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

Symmetry experiment performed on negatively stained HlyA and HlyA50 particles shows that HlyA has C7 symmetry and HlyA50 has no symmetry. C7 and no symmetry were applied to perform 3D reconstruction of HlyA and HlyA50 oligomers respectively. Top view of 3D model of HlyA shows a ring-like structure and bottom view of 3D model shows a bowl-like structure. In addition, this bowl-like domain is attached to the ring-like domain by arm-like domain. There are seven side openings situated just underneath the ring-like domain. The three-dimensional structure further shows that both HlyA and HlyA50 oligomers have a central channel. The resolution of the three-dimensional structure was determined by the FSC curve. The 0.5 value for FSC indicates that the resolution of HlyA and HlyA50 is 16.5 Å and 17.35 Å respectively. The resolution of the three-dimensional reconstruction of HlyA and HlyA50 without cryo-negative staining was 21.8 Å and 22.2 Å respectively. This shows a considerable improvement in resolution of the 3D structures due to the use of cryo-negative stain. Also the structural integrity of hemolysin oligomers remains unchanged in presence of heavy metal (16 % ammonium molybdate solution) staining solution.

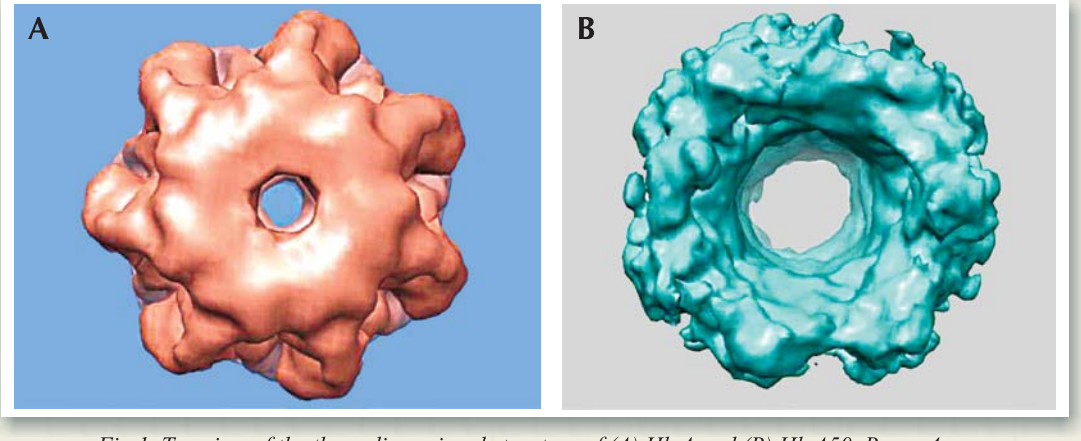


Fig.1. Top view of the three-dimensional structure of (A) HlyA and (B) HlyA50. Bar = 4 nm

Virulence plasmid of Aeromonas hydrophila, macrophage apoptosis and development of systemic infection in mice model

Investigators: D. R. Saha, T. Majumdar and S. Mazumder

Aeromonas hydrophila is a gram negative rod shaped bacteria belonging to the family Aeromonadaceae. This bacterium is both a human and animal pathogen and has been implicated in gastroenteritis, wound infections and septicemia in human. Presence of virulence plasmid has been observed in clinical isolates, but their exact role in disease establishment and virulence is not yet clear. Pathogenic *A. hydrophila* strain AO1 bears a 21 kb plasmid encoding several virulence determinants. Infection studies revealed that wild type isolates bearing 21 kb plasmid were able to disseminate from the intestinal lumen to induce cytotoxicity in BALB/C mice splenic macrophages involving reactive oxygen species generation. Further study using different apoptotic parameters documented macrophage death to be apoptotic in nature. Oral infection with these isolates were able to disseminate from intestinal lumen to peyer's patches, spleen and liver and proliferate efficiently in these organs indicating the initiation of a systemic infection. With plasmid curing, the isolate looses

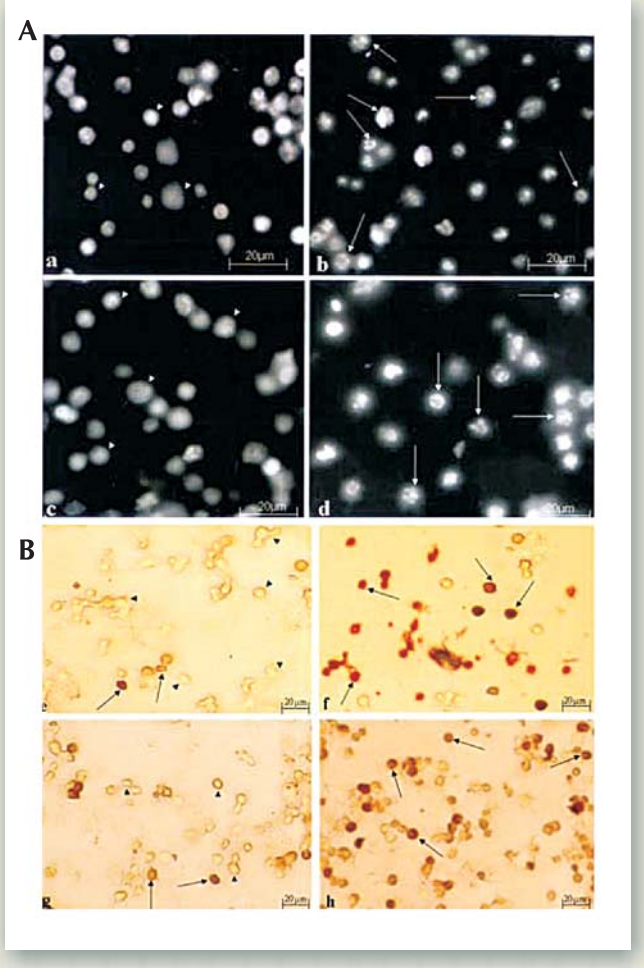


Fig 1. Macrophage apoptosis induced by plasmid bearing A. hydrophila

several virulence attributes including cytotoxic potential. The cured isolates after inoculation also disseminate from intestinal lumen to payer's patches, spleen and liver but never attained the bacterial load recorded with wild type isolates and was rapidly cleared. Transformation of 21 kb plasmid helped the cured bacteria regain wild-type virulence attributes, apoptotic potential and ability to cause systemic infection in mice. Further study of this virulence plasmid will be of immense importance in identifying the factors responsible for the observed cytotoxicity in wild type *A. hydrophila* and also in determining whether the virulence plasmid itself a cytotoxic factor or acts indirectly by regulating the release of different cytotoxic factors in producing systemic infection.

Splenic macrophages infected at a MOI of 50 with Wt-AO1, C-AO1 and T-AO1 isolates were collected 8 h p.i. for Hoechst 33342 staining and TUNEL assay separately and observed under microscope. (▲) indicate apoptotic macrophages and (➞) indicate non-apoptotic macrophages. Panel A Hoechst staining and panel B TUNEL assay. a & e, control macrophages; b & f, Wt-AO1 infected macrophages; c & g, C-AO1 infected macrophages; d & h, T-AO1 infected macrophages. Total of 100 cells were studied in each field and three such fields were included to determine the percentage of apoptotic cells (1 cm = 20 µm).

Award and Honours

A. N. Ghosh

- Delivered invited talk entitled "Packing of DNA determined by Room Temperature and Cryoelectron Microscopy" at the International Conference on Electron nanoscopy and XXXII Annual Meeting of Electron Microscope Society of India held at Defence Metallurgical Research Laboratory, Hyderabad, during 6-8 July, 2011.
- Delivered invited talk entitled "CryoEM of a Novel Cholera phage" at Advances in Electron Microscopy in Virology Research and 3D Imaging: The Road Ahead held at National Institute of Virology, Pune, during 20-21 October, 2011.
- Delivered invited talk entitled "Electron Microscopy Of DNA: protein monolayer technique to cryoelectron microscopy" at Workshop on Electron Microscopy held at Institute of Physics, Bhubaneswar, during 23-25 November, 2011.
- Guest Lecturer, Calcutta University

Conferences/ Seminars/Workshops /Trainings Attended/Organised

D. R. Saha

- Attended 46th Joint meeting and Conference of the US-Japan Panel on Cholera and other Bacterial Enteric infections at Kolkata, India from 13-15 December, 2011

EPIDEMIOLOGY

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

On diarrheal disease surveillance and intervention trials:

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

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

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

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

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

Projects on HIV

On Stigma

A major finding emerging from this piece of work is identification of factors associated with

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

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

Scientist : S. Ghosh, Scientist F
(Retired on 28 February 2011)
D. Sur, Scientist F
S. Panda, Scientist E
K. Sarkar, Scientist E
A. K. Deb, Scientist D
S. Kanungo, Scientist B
Falguni 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, Technician B
A. Chakraborty, Technician B

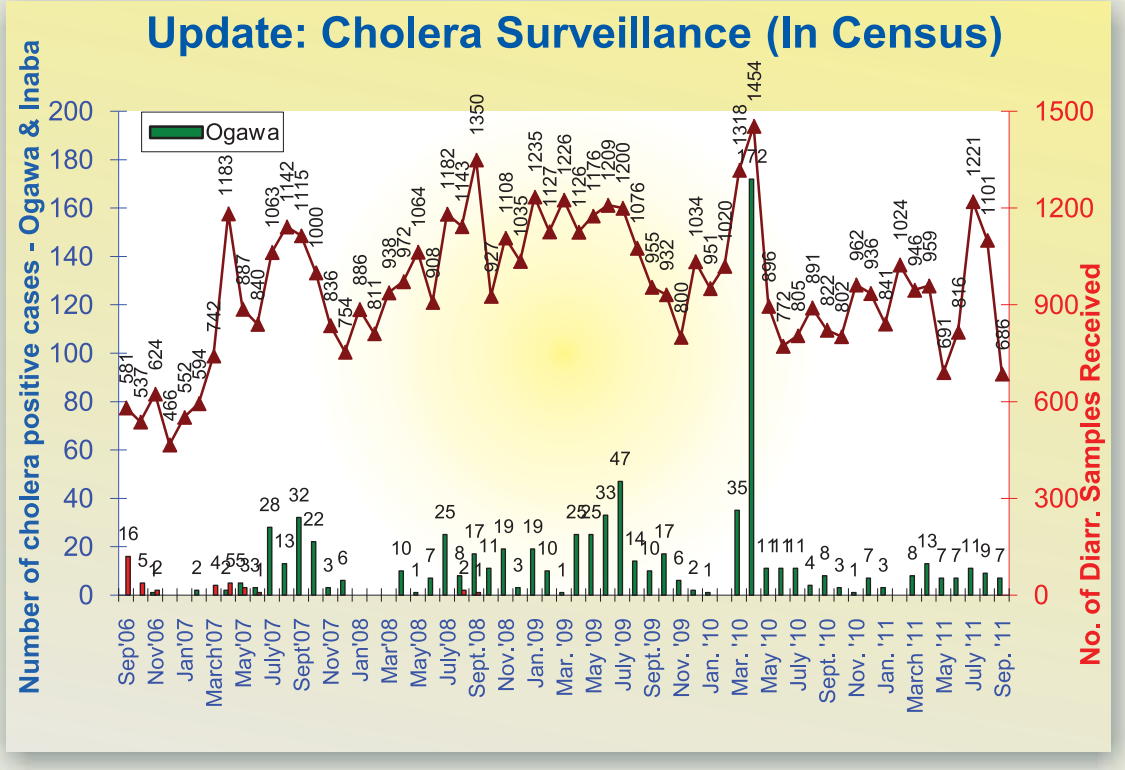
A randomized controlled trial (Phase-III) of the bivalent killed whole cell oral cholera vaccine in eastern Kolkata

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

The study showed that the efficacy of the killed vaccine is ~70%. This culminated in manufacture and licensure of the vaccine in India in 2009. The five years post vaccination surveillance was completed in September 2011. It showed that majority of V. cholerae that were isolated, were *V. cholerae* O1, Ogawa. No *V. cholerae* O139 were isolated.

A total of 58908 diarrhea cases were enrolled in the five years post vaccination surveillance. Among them 795 (1.3%) cholera positive cases were found. The most common effected group was less than 15 years of age.

The graph depicts the diarrhea surveillance and cholera positive cases over the months during the post vaccination surveillance period from September, 2006 to September, 2011.



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

Investigators: D. Sur, S. Kanungo, M. K. Bhattacharya,. B. Manna,. R. K. Nandy
in collaboration with International Vaccine Institute

The primary objective of the study is 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 is 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 is ongoing. Data entry is completed through Remote Data Capturing system (RDCs).

Age group wise Blood Collection

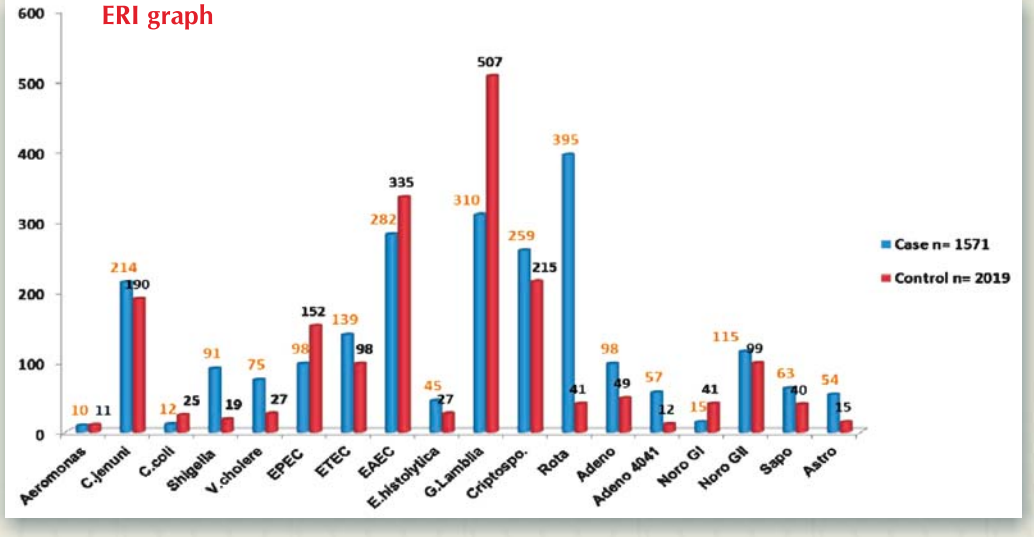
Age Group (Years)	Bleed 1 (Day 0)	Bleed 2 (Day 14)	Bleed 3 (Day 28)	Bleed 4 (Day 42)
1-5	58	53	51	51
6-10	60	58	56	56
11-17	60	60	59	59
> = 18	178	176	174	170
Total	356	347	340	336

Diarrheal Disease in Infants and Young Children in Developing Countries

Investigators: D. Sur, B. Manna, T. Ramamurthy, S. Kanungo, in collaboration with University of Maryland

This project is 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 is the identification of children with diarrhoea in less than 5 years age group along with selection of controls.

Since the beginning of the study in the year 2007, a total number of 2050 Cases and 2500 controls have been identified & enrolled, 332 deaths were identified and 327 verbal autopsies completed.



Exploration of Biological Basis of Underperformance of Oral Polio and Rota Virus vaccine in India.

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

Primary objectives of the study are to determine whether decreased vaccine responsiveness to oral poliovirus or rotavirus vaccines is associated with the presence of tropical enteropathy. Secondary objective is to determine the impact of an inactivated polio vaccine (IPV) boost on systemic (neutralizing antibodies) and mucosal immune responses (shedding OPV vaccine virus) to polio vaccines following vaccination with oral polio vaccine (OPV) in children with and without tropical enteropathy.

The study has been initiated recently in B C Roy Children's Hospital. Total 372 children are planned to be recruited over a period of 2 years.

Health Care Utilization and Attitude Survey regarding diarrhea among parents of children under 5 years: A cross sectional community based study.

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

This study was initiated in 2010. This study will 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.

It will help to prepare an evidence-based assessment (based on disease burden and public perception) of diarrheal disease caused by major indicated pathogens, among children 0-59 months of age, and the public demand for an intervention.

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, B. Manna.

This is an intra-mural project. It is aimed 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.

After regulatory clearances, the project was initiated. Initially 40 randomly physicians were interviewed as pilot, which led to final structure. Preliminarily, the enumeration of the local health care providers practicing in an intervention naïve urban slum and existing area has been completed. 264 (130 in non-naive area and 134 in naive area) health care providers were interviewed, data entry and field verification completed. Analysis of the collected data using SAS version 9.2 was done and scientific communication is under progress.

Sustained commitment of NICED towards intervention development

Investigator: S. Panda

At the behest of the State and central Government, an investigation was carried out to identify the cause of diarrheal outbreak in the coastal district of Purbo-Medinipur, West Bengal following tropical Cyclone AILA in 2009. 'Rapid situation and response assessment' (RSRA) technique and laboratory investigations revealed that antibiotic use, which was being practiced at local level, was inappropriate and *V. cholerae* was primarily responsible for the outbreak. Partnering with the local health authorities helped us in developing an appropriate management guideline and circulate it to all the Block Medical Officers of Health (BMOH). This intervention was able to considerably influence the prescription practice of local physicians towards use of norfloxacin or azithromycin to which the isolates of *V. cholerae* were sensitive. The publication was referred to in one of the articles in 'Emerging Infectious Diseases' issue on Cholera in November 2011. Taking the quality of work into cognizance, 'Save the children UK', West Bengal office requested the concerned scientist of NICED in 2012 to undertake a situation assessment and suggest interventions in selected villages of the

Patharprotima Block in the district of South-24 Pgs, which was devastated by AILA and is still suffering from its aftermath.

Community based epidemiologic study

Principal Investigator: S. Panda (PI),
Co-investigators : A. Deb, T. Ramamurthy, M. Chawla Sarkar, S. Ganguly

A community based study on diarrheal diseases in children ⩽ 2 yr in a rural setting of West Bengal identified factors which could be harnessed upon to develop intervention trial. In children aged ⩽ 6 month in this study incidence rate of rotavirus diarrhoea was 5/100 child-year (95% CI 1.35 - 12); in the age group > 6 to ⩽ 12 month it was24/100 child-year (95% CI 13.8 - 41.4); in children aged >12 - ⩽ 18 month the incidence rate was 24/100 child-year (95% CI 14 - 36) and in the age group >18 - ⩽ 24 month the rate was 19/100 child-year (95% CI 7.5 -39). Most frequent rotavirus genotype detected from 36 diarrheic stool samples in this investigation were G9P[4] (10; 28%), G1P[8] (7; 19%), G2P[4] (5; 14%) and G8P[4] (3; 8%). Bacterial, parasitic and viral co-infections were also identified along with rotavirus in diarrheic stool under this project. The study was completed on 21 May 2011. The information generated through this endeavour is of public health importance as it will not only help in assessing the impact of rotavirus vaccine but also explain its efficacy.

Prevalence of oncogenic HPV in HIV infected female patients in West Bengal

Investigator: K. Sarkar

Burden of both cervical cancer and Human Immunodeficiency Virus (HIV) infection are very high in India. Oncogenic Human Papilloma Virus infection is understood to be altered in presence of HIV infectivity. Hence, a study was carried out to understand the prevalence of oncogenic HPV and their circulating genotypes among HIV positive female patients in West Bengal. In this hospital-based cross-sectional study, 93 known ART naïve

HIV positive females attending a pre-ART registration clinic were selected. The study subjects were interviewed using field-tested questionnaire to study their socio-demographic variables. Following that, cervical cell samples collected from the study population using disposable vaginal speculum & cervical cyto-brush. All samples were tested for the presence of oncogenic HPV using Roche PCR assay to detect specific HPV genotypes. Prevalence of oncogenic HPV was 46.2% (n = 43) among HIV positive female patients.

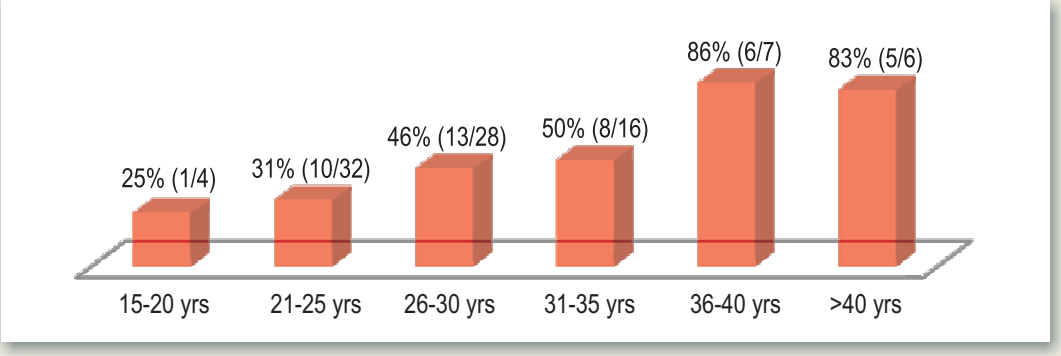


Figure 1: Distribution of oncogenic HPV among HIV infected female patients by age

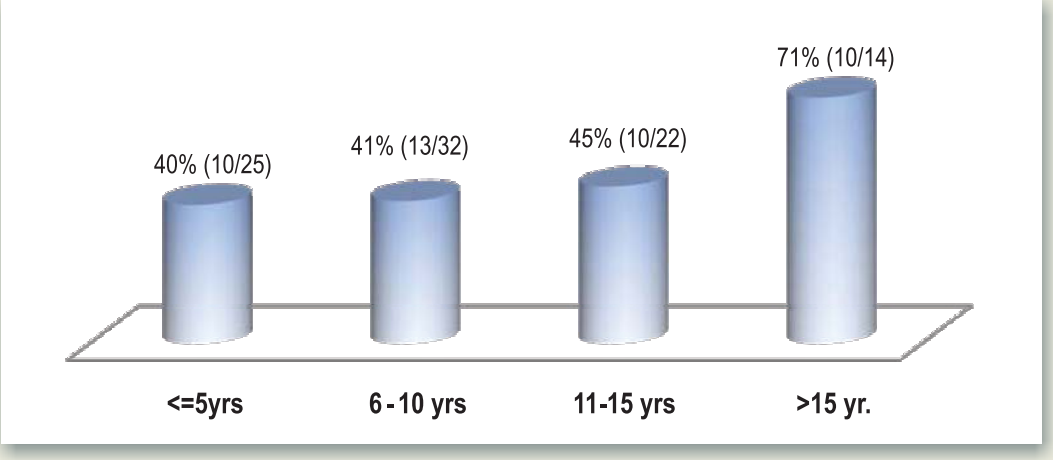


Figure 2: Distribution of oncogenic HPV in relation to sexual duration in HIV+ve subjects

Figure-1 Shows the age-wise distribution of oncogenic HPV among study subjects. It appears that the age group-wise prevalence of HPV has a rising trend till the age of 40 years after which it decreases slightly. About 53% (23/43) of cases with oncogenic HPV were infected with genotypes other than 16, 18 either as single/multiple infections. HPV 18 followed by HPV 16 was the predominant genotypes among HIV positive study subjects.

Figure-2 Shows the distribution of oncogenic HPV in relation to duration of sexual exposure. This revealed that there was an increasing HPV prevalence with increasing sexual exposure duration.

So, oncogenic HPV appears to be related with both age and duration of sexual exposure, which needs to be studied further in-depth. Since most subjects were infected with non-16/non-18 oncogenic HPV genotypes that are not included in currently available HPV vaccines, so, prevention of cervical cancer in HIV positive women must be focused towards early detection of oncogenic HPV with cervical cytological abnormality followed by an appropriate treatment as there is a lack of sufficient evidence of immunogenicity in them (HIV + ve subjects).

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

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

The intramural project is being conducted among under-five children in a selected rural community having a population of more than 27,000 in South 24-Parganas district of West Bengal. The study consists of three components – (a) surveillance for diarrhoeal diseases, (b) case-control study to determine risk / preventive factors, and (c) verbal autopsies. The data collected so far highlighted several areas for possible improvements in the diarrhea prevention and management efforts both at the caregiver and care provider levels that we wish to address through continued research in this area.

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

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

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

(a) to determine the safety of the two-dose killed oral cholera vaccine among infants, and

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

This trial has already been registered with the WHO and the Clinical Trials Registry, India. The study instruments, including the CRFs and consent forms have been prepared and approved for use; all necessary supplies, including the EPI and the study vaccines have also been procured / supplied. Institute of Child Health (ICH), Kolkata has been identified and already approved as a new collaborator for the study. Most of the study staff have been hired and trained (including training on GCP) according to study guidelines. The DCGI approval for the study is being awaited.

Award and Honours

D. Sur

- Invited to talk at US-Japan meet in Kolkata in December 2011. Presented on of the cholera vaccine trial data and the interpretations and implications.
- Invited to speak on the 'Cholera in India and role of vaccination' in several forums of Indian Academy of Paediatrics in Delhi, Bombay, Hyderabad, Goa, Andaman and Nicobar Islands and Kolkata.

S. Panda

- Oral presentation on 'Challenges in providing Anti-Retro Viral (ARV) medicines to

substance users' was made by Dr. Samiran Panda as an invited speaker in the 4th National Conference of AIDS Society of India (ASICON 2011) held at SGPGI, Lucknow during 16- 18 December, 2011.

K. Sarkar

- Elected as Member, National Academy of Medical Sciences (India)

Conferences/ Seminars/Workshops /Trainings Attended/Organised

D. Sur

- Attended South Asian Forum for Health Research (SAFHeR) at New Delhi on 22 February 2012. In the session on 'Discussion on: Regional obstacles as faced by member countries and cross country issues; areas for collaboration / training / capacity building' and spoke on "Cholera"
- Attended meeting on 'A Review meeting of translational research programmes of ICMR Institute/Centre' on 5 March 2012 at New Delhi and presented the translational research activities being conducted at NICED
- Attended a decision making meeting for the project entitled "Exploration of the Biologic Basis for OPV and Rotavirus Vaccine underperformance in India" held at International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh (ICDDR,B) on 24-25 July, 2011
- International Conference on 'Vaccines for Enteric Diseases' on 14-16 September 2011 in Cannes, France and made an oral presentation on "Three year efficacy results from a randomized, controlled trial of a whole-cell oral cholera vaccine"
- Master's Programme in Vaccinology and Pharmaceutical Clinical Development" held in Siena on 27-28 Sept, 2011 and gave lectures on "Cholera : epidemiology, disease and vaccine" and "Typhoid fever - epidemiology, disease and vaccine"
- Investigators meeting for the Global Enteric Multi-Center Study (GEMS) from Nov 30-Dec 1, 2011 and International Strategic Advisory Committee (ISAC) meeting for the Global Enteric Multi-Center Study (GEMS) on 2-3 Dec 2011 at Philadelphia, Pennsylvania, USA.
- Participated as delegate at the symposium," Health Impact of Probiotic -Vision and opportunities, organized by Yakult India Microbiota and Probiotic Science Foundation in association with P.D.Hinduja National Hospital, Mumbai, 10-11 December,2011
- Attended and successfully completed the GCP training held at NICED on 27 February, 2012 conducted by IVI, NICED & Shantha Biotechnics Ltd.

S. Panda

- Conducted a teaching/training session on 'epidemiological research - making claims/inferences' for students of Calcutta Homeopathy Medical College on 13 October, 2011 at the Seminar room of NICED-II building.
- Attended two 'Clinical investigator training forum' - one in July 2011 in Delhi and the other in December 2011 in Chennai.

- Acted as Organizing Secretary in the 3rd CME on Tropical and Infectious Diseases held on 10 March 2012 in Kolkata.
- Acted as a resource person at the Foundation Workshop in Clinical & Laboratory Medicine at the Agartala Government Medical College (AGMC), Tripura sponsored by the Indian Council of Medical Research (ICMR) and co-organized by AGMC and Moving Academy of Medicine and Biomedicine, Pune during 27- 29 March, 2012.

K. Sarkar

- Attended 99th Indian Science Congress held at Bhubaneswar, Orissa, during 3- 7 January 2012 and presented a paper entitled "Oncogenic HPV among HIV infected female population in West Bengal, India"
- Attended an International Conference on Reproductive Health with Emphasis on Strategies for Family Planning held in New Delhi during 19–21 February 2012 and presented a paper entitled `Prevalence of oncogenic HPV in HIV infected female patients in West Bengal'

A. Deb

- Participated as a workshop facilitator in the workshop on "Implementation Research Design, Methodology and Proposal Writing on Infectious Diseases of Poverty" organized by WHO-SEARO, WHO-TDR and supported by the Implementation Research Platform of USAID during 11-15 April, 2011 at Faridabad, Delhi.
- Participated and delivered a presentation on Impact of Climate Change on Health: Lessons from the Global Experience in "Regional Workshop on National Plans for Climate Change and Health" organized by WHO-SEARO, New Delhi during 21-23 September, 2011.
- Participated in the "Consultation Meeting on Etiology of Pneumonia in India" organized by the Division of Reproductive and Child Health, ICMR on 18 January, 2012 at ICMR Hq, New Delhi.
- Attended the "Pneumococcal Symposium" organized by the Health Policy Unit, DBT, India on 19 January, 2012 in New Delhi.
- Attended "Training of Trainers Meeting" for clinical trial investigators organized by the Clinical Development Services Agency (CDSA), THSTI and OneWorld Health (CDSA-OWH) during 19 – 20 July, 2011 at New Delhi.
- Attended a training program on "Qualitative Research Methods" at Indian Institute of Public Health, Delhi (IIPH-D) during 14-17 June, 2011

S. Kanungo

- Attended the CME on Ethical Issues in Clinical Research and Health Care organized by School of Tropical Medicine and Hygiene (STM) in Kolkata 28-29 March 2011, under the auspices of Dept. of Clinical and Experimental Pharmacology,(STM)
- Attended the 3rd Annual meeting of the Global Network on Malnutrition and Enteric Diseases (MAL-ED) and presented the project proposal on " Exploration of Biological

Basis of Underperformance of Oral Polio and Rota Virus vaccine in India" in Seattle, USA from 3-5 May, 2011

- Represented the institute in the 15th National exhibition on theme of "Evolution of India as great nation in the 21st Century" at Bhairab Ganguly College Maidan, Kolkata,7-11 Sep 2011
- Delegate in the Consultative Workshop on WHO Country Cooperation Strategy Development, 14 September 2011 at New Delhi
- Delegate at the symposium," Health Impact of Probiotic -Vision and opportunities, organized by Yakult India Microbiota and Probiotic Science Foundation in association with P.D.Hinduja National Hospital, Mumbai, 10-11 December,2011
- Oral presentation on the topic "Epidemiological pattern of Vibrio parahaemolyticus diarrhea in the urban slums of Kolkata, India" at the 46th Annual Joint Panel Meeting on "Cholera and Other Bacterial Enteric Infections" held in Kolkata 13-15 Dec, 2011
- Attended and successfully completed the GCP training held at NICED on 27 February, 2012 conducted by IVI, NICED & Shantha Biotechnics Ltd.

IMMUNOLOGY

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

Scientist	:	T. Biswas, Scientist E
Staff	:	S. K. Shaw, Technician B N. C. Mondal, Attendant Services
Research Scientist	:	R. Biswas
Fellows	:	S Mukherjee, CSIR-SRF D. Sinha, UGC-JRF A. K. Ghosh, UGC-JRF

Several years now, the Division of Immunology has channeled its resources and manpower to evaluate the capabilities of porin of *Shigella dysenteriae* as an adjuvant and hemolysin (HlyA) of *Vibrio cholerae* as an immunoregulator as furnished in the webpage http://www.niced.org.in/immunology/default.asp.

Study of adjuvanticity of porin of *Shigella dysenteriae* type 1 established the protein primarily as a Toll-like receptor (TLR)2 ligand (see review: Nascimento, L.O., Massari, P. and Wetzler, L.M. (2012) The role of TLR2 in infection and immunity. Front. Immun. 3: 1-17) in association with TLR6 possibly as a heterodimer. The TLR signaling, which was initiated by MyD88 led to NF- κ B-dependent chemokine and type 1 cytokine expression in macrophages and dendritic cells, as well as expression of porin specific IgA, the signature molecule of mucosal immune response in B-1 cell populations. Porin-pulsed macrophages strongly proliferated T cells leading to adjuvant-induced transition of naive T cells to polarized effector Th1 cells. Like B-1 cells, B-2 cells expressed IgG2a and IgA, which were augmented by IL-6, the B cell proliferator. These works lead us to investigate how splenic B-2 cells, categorized as Follicular (FO) and Marginal Zone (MZ) B cell respond to porin. Porin treated FO B cells showed up-regulation of TLR2 and -6, in contrast MZ B cells did not express any of the TLRs. The early activation markers CD69 and CD25 were up-regulated besides CD40 and MHC II (I-Ab) 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 cyktoines by FO B cells and anti-inflammatory cytokine by MZ B cells indicate porin parallely dictates differential maturation of the B cell populations. Summing up, adjuvant activity of porin can

successfully bridge innate signaling with mucosal adaptive immune response, thus largely enhancing the quest for generation of porin-specific memory cells, now unfolding.

In the other project the strategies taken by murine peritoneal cavity (PerC) macrophages under HlyA supervision is being examined in association with 'hemolysin-man' Dr Kalyan K. Banerjee, Division of Biochemistry. *Vibrio cholerae* El Tor O1 and non-O1 strains produce a water-soluble cytolytic exotoxin that has been designated hemolysin (HlyA). The HlyA monomer was purified from the culture supernatant of non-O1 *V. cholerae* strain V2. The extracellular membrane-damaging protein with a native molecular mass of 65 kDa belongs to a subfamily of bacterial virulence factors called pore-forming toxins (PFTs). HlyA exhibits enterotoxicity in experimental animal models and has been implicated in causing diarrhoea during cholera epidemic. Since *V. cholerae* infection occurs in alimentary canal of the host, the PFT is likely to influence the mucosal immune system.

The fully active toxin comprises of a central cytolysin domain followed sequentially by two contiguous lectin domains, a β-trefoil domain and a β-prism domain. The C terminus β-prism lectin domain is susceptible to proteolytic deletion and strongly related to PFT activity. HlyA displays bipartite property while supervising PerC macrophages. The PFT causes profound apoptosis within 3 h of exposure and in parallel supports activation of the defying macrophages. HlyA-induced apoptosis of macrophages remains steady for 24 h and is TLR-independent. Apoptosis is driven by caspase-9 and caspase-7, thus involves caspase-7-mediated mitochondrial or intrinsic pathway. Cell activation is carried forward by time dependent up-regulation of varying TLRs. The β-prism lectin domain of HlyA simulated TLR4 up-regulation by jacalin, a plant lectin homologue. Besides expressing TLR4, the β-prism lectin domain was suggestive of exclusively up-regulating CD86 and releasing type 1 cytokines TNF-α and IL-12. On the other hand, the cytolytic protein domain of HlyA represented by VCC50 up-regulated TLR2, which controlled CD40 for continuity of cell activation. Expression of TOLLIP before TLR2 and TLR6 abrogated TLR4, CD40 and CD86. The work shows that the transient expression of TOLLIP leading to curbing of activation-associated capabilities is a plausible feedback mechanism of macrophages to deploy TLR2 and prolong activation involving CD40 to encounter the HlyA cytolysin domain.

Award and Honours

T. Biswas

- Invited reviewer for Journal of Leukocyte Biology (2011)The Journal of Immunology (2009); Journal of Leukocyte Biology (2010).

Conferences/ Seminars/Workshops /Trainings Attended/Organised

T. Biswas

- Oral presentation entitled "Porin unlocks Toll-like receptor 2 and 6 signalling to bridge innate response with adaptive immunity" in UGC sponsored National Level Seminar on "Rediscovering 100 years of journey on the field of immunology: present status of immunological research in India" organized by Post-Graduate Deparment of Zoology, Barasat Government College and West Bengal State University on 19-20 September, 2011.

IMMUNOLOGY

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

Scientist	:	T. Biswas, Scientist E
Staff	:	S. K. Shaw, Technician B N. C. Mondal, Attendant Services
Research Scientist	:	R. Biswas
Fellows	:	S Mukherjee, CSIR-SRF D. Sinha, UGC-JRF A. K. Ghosh, UGC-JRF

Several years now, the Division of Immunology has channeled its resources and manpower to evaluate the capabilities of porin of *Shigella dysenteriae* as an adjuvant and hemolysin (HlyA) of *Vibrio cholerae* as an immunoregulator as furnished in the webpage http://www.niced.org.in/immunology/default.asp.

Study of adjuvanticity of porin of *Shigella dysenteriae* type 1 established the protein primarily as a Toll-like receptor (TLR)2 ligand (see review: Nascimento, L.O., Massari, P. and Wetzler, L.M. (2012) The role of TLR2 in infection and immunity. Front. Immun. 3: 1-17) in association with TLR6 possibly as a heterodimer. The TLR signaling, which was initiated by MyD88 led to NF- κ B-dependent chemokine and type 1 cytokine expression in macrophages and dendritic cells, as well as expression of porin specific IgA, the signature molecule of mucosal immune response in B-1 cell populations. Porin-pulsed macrophages strongly proliferated T cells leading to adjuvant-induced transition of naive T cells to polarized effector Th1 cells. Like B-1 cells, B-2 cells expressed IgG2a and IgA, which were augmented by IL-6, the B cell proliferator. These works lead us to investigate how splenic B-2 cells, categorized as Follicular (FO) and Marginal Zone (MZ) B cell respond to porin. Porin treated FO B cells showed up-regulation of TLR2 and -6, in contrast MZ B cells did not express any of the TLRs. The early activation markers CD69 and CD25 were up-regulated besides CD40 and MHC II (I-Ab) 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 cyktoines by FO B cells and anti-inflammatory cytokine by MZ B cells indicate porin parallely dictates differential maturation of the B cell populations. Summing up, adjuvant activity of porin can

successfully bridge innate signaling with mucosal adaptive immune response, thus largely enhancing the quest for generation of porin-specific memory cells, now unfolding.

In the other project the strategies taken by murine peritoneal cavity (PerC) macrophages under HlyA supervision is being examined in association with 'hemolysin-man' Dr Kalyan K. Banerjee, Division of Biochemistry. *Vibrio cholerae* El Tor O1 and non-O1 strains produce a water-soluble cytolytic exotoxin that has been designated hemolysin (HlyA). The HlyA monomer was purified from the culture supernatant of non-O1 *V. cholerae* strain V2. The extracellular membrane-damaging protein with a native molecular mass of 65 kDa belongs to a subfamily of bacterial virulence factors called pore-forming toxins (PFTs). HlyA exhibits enterotoxicity in experimental animal models and has been implicated in causing diarrhoea during cholera epidemic. Since *V. cholerae* infection occurs in alimentary canal of the host, the PFT is likely to influence the mucosal immune system.

The fully active toxin comprises of a central cytolysin domain followed sequentially by two contiguous lectin domains, a β-trefoil domain and a β-prism domain. The C terminus β-prism lectin domain is susceptible to proteolytic deletion and strongly related to PFT activity. HlyA displays bipartite property while supervising PerC macrophages. The PFT causes profound apoptosis within 3 h of exposure and in parallel supports activation of the defying macrophages. HlyA-induced apoptosis of macrophages remains steady for 24 h and is TLR-independent. Apoptosis is driven by caspase-9 and caspase-7, thus involves caspase-7-mediated mitochondrial or intrinsic pathway. Cell activation is carried forward by time dependent up-regulation of varying TLRs. The β-prism lectin domain of HlyA simulated TLR4 up-regulation by jacalin, a plant lectin homologue. Besides expressing TLR4, the β-prism lectin domain was suggestive of exclusively up-regulating CD86 and releasing type 1 cytokines TNF-α and IL-12. On the other hand, the cytolytic protein domain of HlyA represented by VCC50 up-regulated TLR2, which controlled CD40 for continuity of cell activation. Expression of TOLLIP before TLR2 and TLR6 abrogated TLR4, CD40 and CD86. The work shows that the transient expression of TOLLIP leading to curbing of activation-associated capabilities is a plausible feedback mechanism of macrophages to deploy TLR2 and prolong activation involving CD40 to encounter the HlyA cytolysin domain.

Award and Honours

T. Biswas

- Invited reviewer for Journal of Leukocyte Biology (2011)The Journal of Immunology (2009); Journal of Leukocyte Biology (2010).

Conferences/ Seminars/Workshops /Trainings Attended/Organised

T. Biswas

- Oral presentation entitled "Porin unlocks Toll-like receptor 2 and 6 signalling to bridge innate response with adaptive immunity" in UGC sponsored National Level Seminar on "Rediscovering 100 years of journey on the field of immunology: present status of immunological research in India" organized by Post-Graduate Deparment of Zoology, Barasat Government College and West Bengal State University on 19-20 September, 2011.

PARASITOLOGY

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

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

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

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

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

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

Scientist : S. Ganguly, Scientist C

Staff : T. N. Boral, Technical Officer A
S. L. P. Singh, Technician B

Students : A. Ghosh (SRF)
A. K. Mukherjee (SRF)
K.Das (RA) D. Raj (JRF)
S. Karmakar (JRF)

Non conventional scavenging of ROS: role of pyruvate on oxidative stress management in *Giardia lamblia* or non conventional scavenging of ROS: a novel oxidative stress management pathway in *Giardia lamblia*.

Principal Investigator: S. Ganguly

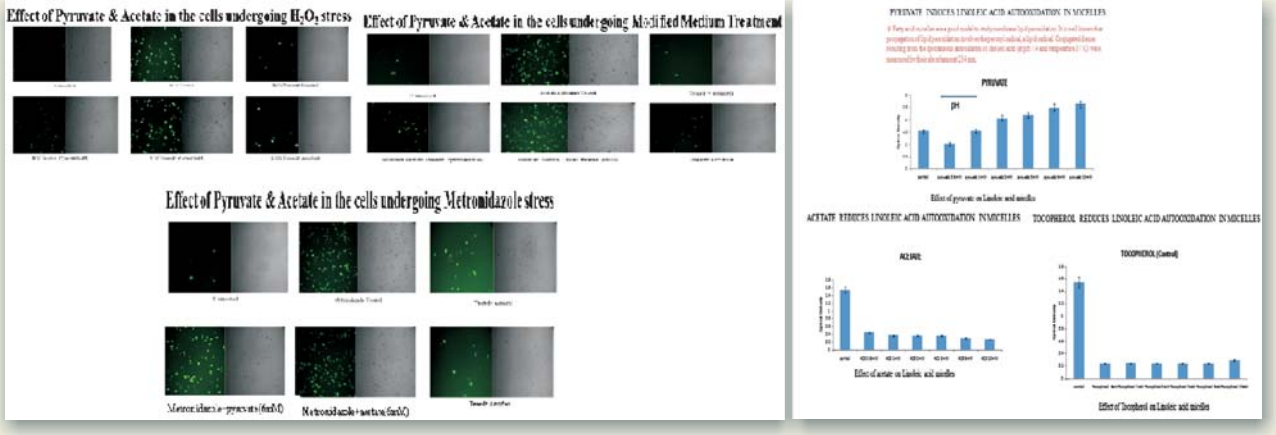
Giardia lamblia is a microaerofilic protozoan parasite which rely on fermentative metabolism for energy production. *Giardia lamblia* have developed a number of antioxidant defense mechanism to reduce the reactive oxygen species generation which are contrary to survival. In this study, the ability of pyruvate, a central component of *Giardia lamblia* energy metabolism, to act as physiological antioxidant was investigated.

With the aid of Dichlorodihydrofluorescein diacetate-based assay, intracellular reactive oxygen species generation by *Giardia lamblia* suspensions was monitored by confocal microscopy and quantitated by FACS analysis. Addition of physiologically relevant concentrations of pyruvate and malate to *Giardia lamblia* cell suspensions was shown to attenuate the rate of hydrogen peroxide induced reactive oxygen species generation.

The upper intestinal lining is well supplied with capillaries, and O_2 concentration there has been measured at 60 μM (Atkinson, 1980). Measurement of O_2 consumption as a function of dissolved O_2 indicates that at low levels (0-50 μM), the organism is capable of scavenging O_2 (apparent Km for O_2 6.4 μM for the trophozoite). Above a threshold of 80 μM O_2, O_2 inhibits its own consumption. Changes in the balance of major fermentation products occur as O_2 concentration is increased. Here we aim to define the limits of O_2 tolerance of this microaerophilic organism, and to characterize the nature of the structural and functional consequences of accumulation of reactive O_2 species.

Acetate, end product in aerobic respiration of *Giardia lamblia* energy metabolism, to reduce membrane lipid peroxidation was also investigated. linoleic acid micelles were employed to investigate the lipid radical scavenging capacity of actate. The TBA Assay was used to study the inhibition of membrane lipid peroxidation by acetate.

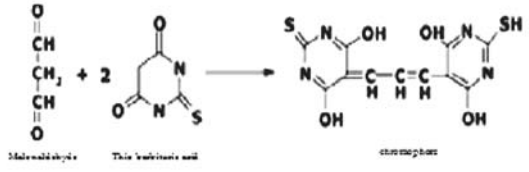
The ability of pyruvate to react with H_2O_2 (and super oxide radical), forming acetate and CO_2 non-enzymically, has long been known (Holleman, 1904). However, whether pyruvate plays a physiological role as an H_2O_2 scavenger in cells is not clear. In this study, we report on the ability of pyruvate, a central component of diplomonad metabolism, to act as an intracellular scavenger of reactive oxygen species in *G. lamblia*. The contrasting physiological roles of pyruvate in oxidative and fermentative metabolism are discussed.



TBA ASSAY

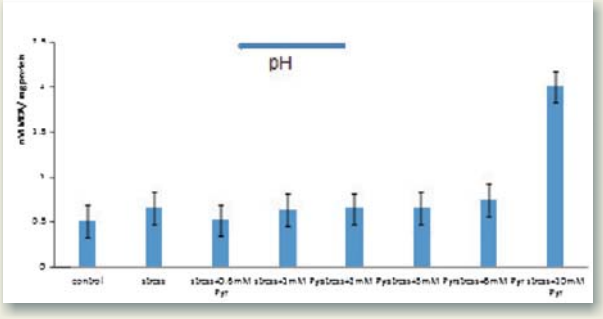
The process of Lipid peroxidation results in the formation of MDA *in vivo*. This is a after product in the sequence of lipid peroxidation reactions (Evans *et al.*,1999; Racl *et al.*, 2004)

The TBA Assay was used to assess the MDA concentration, with some modifications As described in Bar-Or *et al.*, 2001.



Reaction of TDA with MDA forming the 535mm chromophore

TBA ASSAY with Pyruvate



MDA production increases with increasing pyruvate conecntration

TBA ASSAY with Acetate

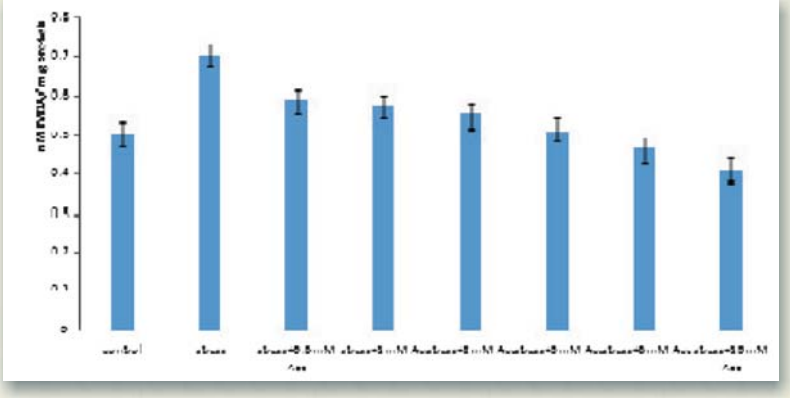
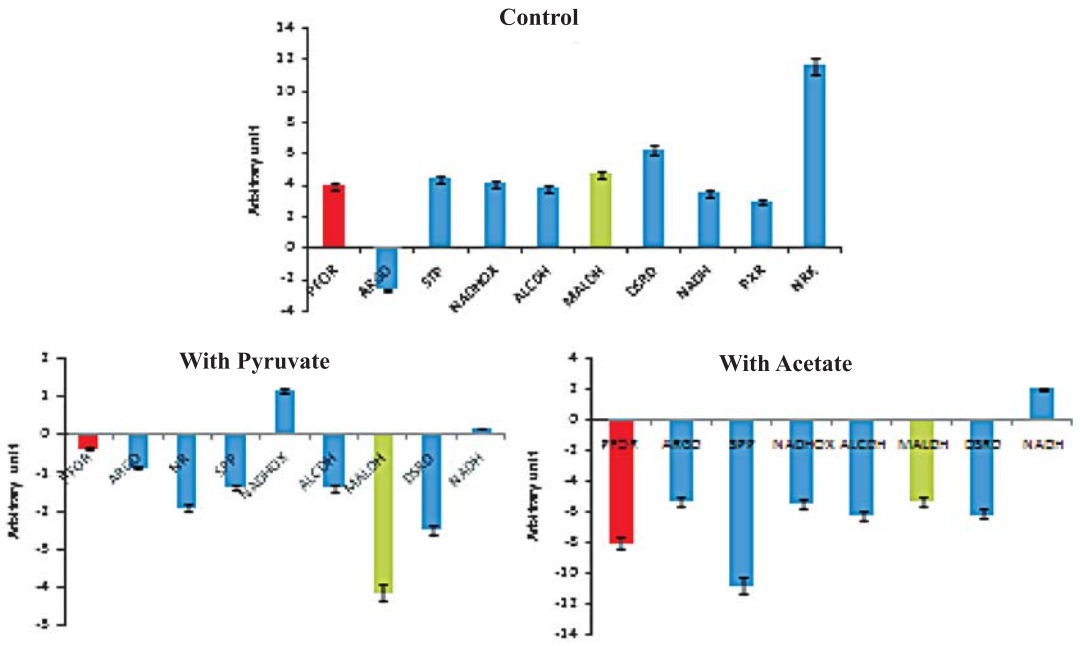


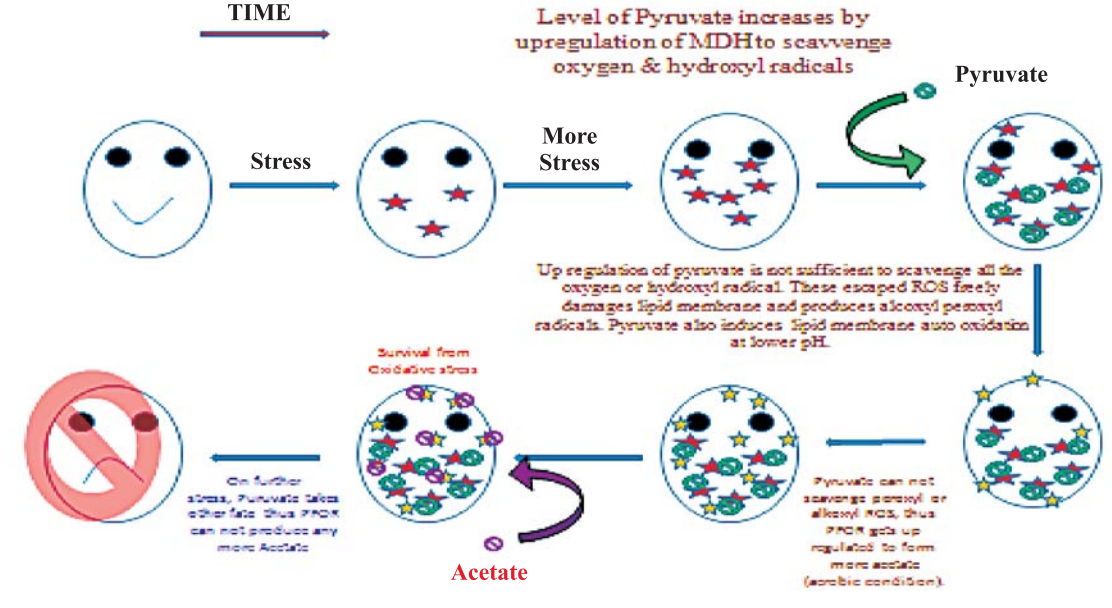
Fig: MDA production decreases with increasing acetate concentration

Differential Expression of H_2O_2 Treated Cells at 3 hr time point



PFOR: Pyruvate ferredoxin oxidoreductase, ARGD: Arginine dehydrogenase STP: Serine Threonine Phosphatase, NADHOX: NADH Oxidase, ALCDH: Alcohol Dehydrogenase, MALDH: Malate dehydrogenase, DSRD: Disulfide reductase , NR: Nitroreductase, ARGD: Arginine dehydrogenase, NADH: NADPH Oxidoreductase,

Hypothesis:



Award and Honours

S. Ganguly

- Recipient of Bharat Jyoti Award for the year 2011.
- Recipient of Bill and Melinda Gates Foundation Keystone Symposia on Drug discovery in Parasitic Diseases Travel award for 2012.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

- Invited participation in TROPACON 2011, at Nagpur, India from 12-14 November, 2011.
- Invited participation and presentation of a paper in National Congress of Parasitology at Chennai, India from 18-20 November, 2011.
- Invited participation in 35th All India Cell Biology Conference in Bhubaneswar, India from 16-18 December, 2011
- Participated and presented a paper in Keystone Symposia on Drug Discovery in Parasitic Diseases held in Santa Fe, New Mexico, USA, from 15-20 January 2012.
- Participated and presented a paper in IV International Giardia and Cryptosporidium Conference 2012 held in Wellington, New Zealand, from 31 January – 3 February, 2012.
- Invited participation in EMBO Global Exchange Lecture Course and Amoebiasis Conference 2012 held at New Delhi and Khajuraho, India from 2-7 March, 2012.
- Invited participant as a resource person in UGC DSA workshop on Advanced tools in Molecular biology in Division of Biophysics and Molecular Biology, University of Calcutta, India from 14-15 Oct, 2011.
- Invited participant as a resource person in DBT STAR college program in modern research in Moulana Azad College, Kolkata, India from 8 – 10 Nov, 2011.

PATHOPHYSIOLOGY

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

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

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

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

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

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

Scientist : M. K. Chakrabarti, Scientist F

A. Pal, Scientist E

Staff : S. Sen Senior Technical Assistant

DBT Ramalingaswami

Fellow : M. K. Hoque

Junior Research Fellows : P. Karmaker

R. Tapadar

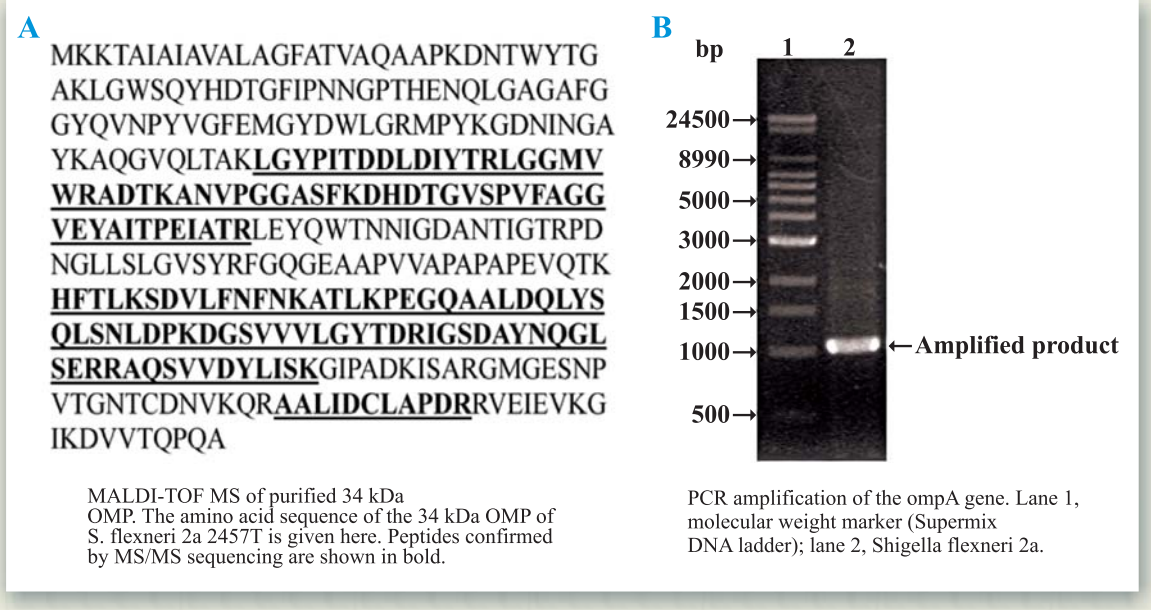
R. Bhowmick

Sk. Irshad Ali

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

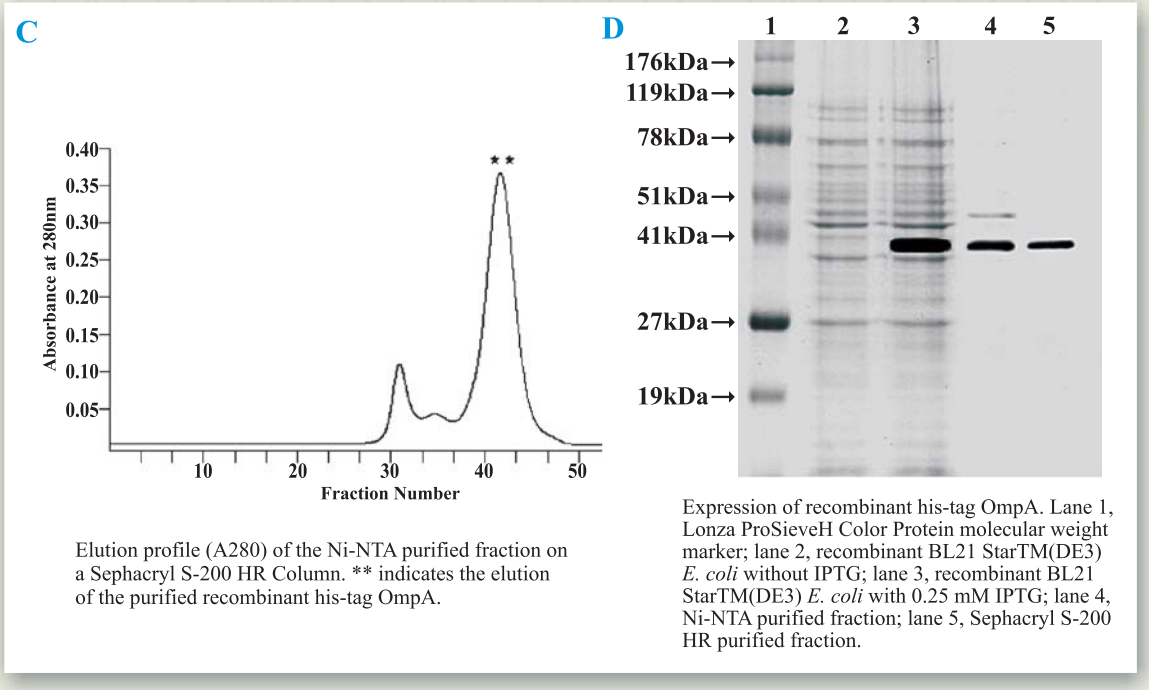
Principal Investigator : M. K. Chakrabarti

We have shown in our earlier studies that the 34 kDa outer membrane protein (OMP) of *Shigella flexneri* 2a is cross-reactive, antigenically conserved among *Shigella* spp., and the epitope is surface exposed on the intact bacterium as well as boost the induction of protective cytokines by macrophages, established itself as a highly immunogenic. We have also evaluated the in-depth mechanism of macrophage activation in response to 34 kDa OMP. We have shown that 34 kDa OMP recognizes TLR2 as a receptor for on macrophages. In addition to TLR2, 34 kDa OMP enhances the expression TLR6 in macrophages. 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. In this reported period we have cloned and overexpressed the 34 kDa protein from *S. flexneri* 2a (N.Y-962/92) genomic DNA, to further characterize the 34 kDa protein, as we are getting very low yield by using traditional purification procedures. For this purpose MALDI-TOF MS analysis of the purified 34 kDa OMP has been performed. MALDI-TOF MS



MALDI-TOF MS of purified 34 kDa OMP. The amino acid sequence of the 34 kDa OMP of S. flexneri 2a 2457T is given here. Peptides confirmed by MS/MS sequencing are shown in bold.

PCR amplification of the ompA gene. Lane 1, molecular weight marker (Supermix DNA ladder); lane 2, Shigella flexneri 2a.



Elution profile (A280) of the Ni-NTA purified fraction on a Sephacryl S-200 HR Column. ** indicates the elution of the purified recombinant his-tag OmpA.

Expression of recombinant his-tag OmpA. Lane 1, Lonza ProSieveH Color Protein molecular weight marker; lane 2, recombinant BL21 StarTM(DE3) *E. coli* without IPTG; lane 3, recombinant BL21 StarTM(DE3) *E. coli* with 0.25 mM IPTG; lane 4, Ni-NTA purified fraction; lane 5, Sephacryl S-200 HR purified fraction.

analysis of the purified 34 kDa OMP of *S. flexneri* 2a shows considerable sequence homology (Identity 65%) with the OmpA of *S. flexneri* 2a. By using the specific primers, the gene of interest has been amplified from *S. flexneri* 2a genomic DNA, cloned in pET100/D-TOPOH vector and expressed using induction with isopropyl thiogalactoside (IPTG)

Studies on proteases of *Vibrio cholerae*

Investigator: A. Pal

Extracellular proteases may play an important role in the pathogenesis of diarrhoea caused by *V. cholerae*. In an earlier study we have shown that hemagglutinin protease (HAP) may play a role in the pathogenesis of ctx gene negative *V. cholerae* non-O1, non-O139 strain (Infection and Immunity, 2006). Two well-characterized proteases secreted by *Vibrio cholerae* O1 strains are hemagglutinin protease (HAP) and *V. cholerae* protease (PrtV). The hapA and prtV knock out mutant, *V. cholerae* O1 strain CHA6.8ΔprtV, still retains residual protease activity. We partially purified the residual protease secreted by strain CHA6.8ΔprtV 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. Our results show the presence of a novel 59-kDa serine protease in a ΔhapAΔprtV *V. cholerae* O1 strain and its role in hemorrhagic response in RIL model (PloS ONE.2010).

Motility is an important virulence factor in many pathogenic species. The role of motility of *V. cholerae* in its ability to cause cholera has not been clearly established. We studied the role of proteases in motility in *V. cholerae* strains. The C6709 wild type *V. cholerae* O1 strain, CHA6.8 (hapA deleted), CHA6.8ΔprtV and CHA6.8ΔprtVΔVC1649 strains were tested for

motility in motility agar. The hapA deleted CHA6.8 strain showed decrease in motility. Interestingly CHA6.8ΔprtV strain showed significant increase in motility (Fig 1). Our results show that prtV may play a role in motility in *Vibiro cholerae* strain.

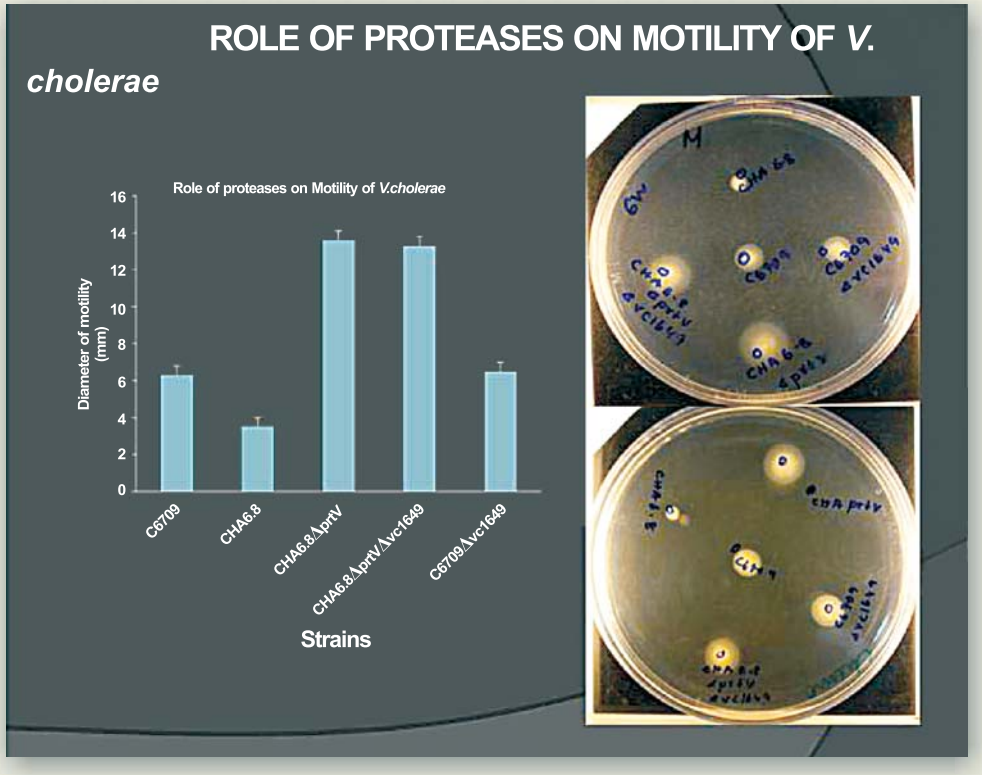


Fig 1. Motility in Vibrio cholerae C6709 and its protease mutant strains in motility agar Diameter of the colony was measured. The CHA6.8ΔprtV strain showed significant increase in motility.

Biofilms generally have been proposed to constitute an environmental refuge for a number of bacterial pathogens and to provide pathogens with an adaptive advantage promoting their environmental persistence. The VC1649 gene showed role in biofilm formation. In both CHA6.8ΔprtVΔVC1649 and C6709ΔVC1649 strain there was significant decrease in biofilm formation (Fig 2).

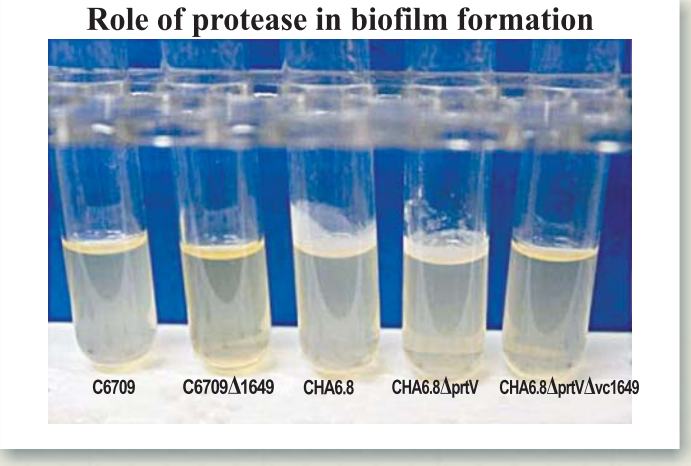


Fig. 2 Biofilm production was qualitatively measured in stationary culture. There was significant decrease in biofilm formation in C6709Δ1649 and CHA6.8ΔprtVΔ1649 strain.

Award and Honours

M. K. Chakrabarti

- General Secretary (HQ), Indian Science Congress Association, 2010-2013.
- Vice-President, The Physiological Society of India, 2010-2014.
- As Convener of the Section of medical and veterinary sciences, 2008-2010, 2010-2012, West Bengal Academy of Science and Technology
- 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.
- Delivered invited lecture on "Vaccine Against Diarrheal Diseases: Present Status" at UGC Refresher course for College and University Teachers, Academic Staff College, Calcutta University, Kolkata on 1 Dec., 2012
- Delivered invited lecture on "Impact of climate change on the Spread of Infectious Diseases" at UGC Refresher course for College and University Teachers, Academic Staff College, Calcutta University, Kolkata on 3 Dec., 2011.
- Delivered invited lecture on "Pathogenesis of diarrhoea caused by heat stable enterotoxin of enterotoxigenic *Escherichia coli*" Medical Science Section, Proc. Indian Sci. Congress on 5 Jan., 2012.
- Delivered invited lecture on 'Zinc and probiotics: Role in diarrhoeal diseases' at the UGC sponsored national seminar on Food and nutrition in community health: Rural perspective of West Bengal, 16 Feb, 2012.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

M. K. Chakrabarti

- 'Studies on the Development of a Vaccine against Shigellosis'. At UGC Refresher course for College and University Teachers, Academic Staff College, Department of Pharmaceutical Engineering, Jadavpur University, Kolkata on 29 April 2011
- Delivered Plenary Lecture on 'Studies on the development of a candidate vaccine against shigellosis' in International conference on Molecules to Systems Physiology from 21- 23 September 2011 as a part of the Centenary Celebration of the Department of Physiology.
- Delivered key note address on 'Pathogenic mechanism of *Escherichia coli* heat-stable enterotoxin (STa)' at seminar of ISCA Jammu Chapter on 10 November2011
- "Development of a candidate Shigella vaccine: A simple approach. 2.12.2011
- P. Karmakar and M.K. Chakrabarti. 'Anti proliferative effect of thermostable direct hemolysin on colon carcinoma cell line' at the second International conference on "Perspectives of cell signalling and molecular medicine" held at Kolkata, India on 8-11 January, 2012.

- N. Mahata and M.K. Chakrabarti. 'Pathogenic mechanism of *Escherichia coli* heat-stable enterotoxin (STa) in COLO-205 cells: Role of cytoskeleton reorganization at the second International conference on "Perspectives of cell signalling and molecular medicine" held at Kolkata, India on 8-11 January, 2012.
- M.K. Chakrabarti. Key note address on 'Diarrhoeal Diseases: Still a Global Health Problem' at UGC sponsored seminar on Rural health and nutrition: recent trend and monitoring at Bajkul College, Midnapur on 20 Jan, 2012.
- M.K. Chakrabarti. Key note address on 'Global Warming and the Spread of Infectious Diseases' at UGC sponsored National Seminar on "Awareness of communicable Diseases at Goaltar College on 24.January 2012.
- M.K. Chakrabarti Lead speaker 'Vaccine against shigellosis: A myth or reality' International conference on Frontiers in biological research as key note speaker on 26 Feb 2012.
- Served as an expert at the 27th MP Young Scientist Congress, Madhya Pradesh Council of Science and Technology on 28-29 Feb., 2012.
- M.K. Chakrabarti. Lead speaker 'Development of a candidate Shigella vaccine: A simple approach' State level seminar on advances of biological science towards sustainable development (UGC Sponsored) at Berhampur Girls' College, Berhampur, 30.03.12

A. Pal

- Delivered a talk on "Studies on a novel serine protease in *Vibrio cholerae*" at the 99th Indian Science Congress held from 3- 7 January at Bhubaneshwar.

VIROLOGY

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

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

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

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

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

Objectives of Division :

1. Molecular characterization of crucial HIV encoded genes with focus on understanding immunogenicity for developing vaccine candidates.

2. Surveillance and disease burden of diarrhoea induced by Enteric Viruses.

3. Molecular phylogenetic analysis of the circulating enteric viruses in and around Kolkata with focus Rotaviruses, Caliciviruses (Norovirus and Sapovirus), Astroviruses, Picobirnaviruses and Adenoviruses.

4. Analysis of the signaling mechanisms during Rotavirus-host cell interactions: Study of host cellular proteins required for viral replication and pathogenesis.

Scientist	:	S. Chakrabarti, Scientist G T. Krishnan, Scientist E M. Chawla-Sarkar, Scientist C B. Ganesh, Scientist B
Staff	:	S. Omesh, Technical Officer A M. Mallick, Technical Assistant K. Sen, Technician C P. De, Technician B B. K. Bera, Technician B Md. M. Hossain, Technician B
Research Scientist/Pool Officer	:	R. Dey
Young Woman Scientist	:	M. Sarkar
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Genetic Characterization of HIV-1 strains among Injecting Drug users in Nagaland, India

Principal Investigator: S. Chakrabarti

It was a community based cross-sectional study and a total of 156 injecting drug users participated in this study voluntarily. All male IDUs of ages 31-40 years were explained about the purpose of this study and requested to participate voluntarily. Experienced social workers interviewed them to study their demography, risk behaviour and risk perception using a field tested questionnaire.

Interview was followed by collection of about 5 ml blood in vacationer containing EDTA. HIV was tested by enzyme linked immunosorbent assay (Immunogenetics, Belgium) followed by tridot assay (Standard Diagnostics, Bioline, Korea). Peripheral blood mononuclear cells were separated from the whole blood by Ficoll-Hypaque gradient centrifugation. DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany, Hilden) according to the manufacturer's protocol.

The HIV-1 strains were subjected to gag and env Heteroduplex Mobility Assay as described earlier. Briefly, the amplicons (460bp, 500bp) for gag (p24-p7) and env (C2-V3) were amplified through nested PCR using standard primers (NIH AIDS Research and Reference Reagent Program, NIH, USA). For gag HMA, 4.5µl of the amplicon of the unknown sample was mixed with 4.5µl of the reference amplicon in presence of 1µl of 10x annealing buffer (1M NaCl, 100Mm Tris-HCl [pH 7.8], 20Mm EDTA). For env HMA, 5µl of the amplicon was mixed with 5µl of the reference amplicon in the presence of 1.1µl of 10x annealing buffer. It was then denatured at 94°C for 2 minutes followed by renaturation by snap freezing in ice to form Heteroduplex molecules. The mixture were then loaded on a 5% Polyacrylamide Gel (for gag-HMA, 5% Polyacrylamide / 20% Urea; 5% polyacrylamide for env) in 1X TBE buffer and electrophoresed at 250 V for 2 hours 30 minutes.

The HIV-1 strains were then subjected to multiregion hybridization assay based on real time PCR method performed using probes designed from eight different genomic regions (p17, pro, rt, int, tat, gp120, gp41 and gpnef) of HIV-1 as described earlier. Briefly, the first round PCR was conducted at ABI9600 Thermal cycler (Applied Biosystems) with outer primers designed for specific genomic regions, 10x PCR buffer, 25mM MgCl2, 10mM dNTPs and 1U AmpliTaq Gold DNA polymerase (Applied Biosystems) in a final volume of 50µl. The second round PCR was carried out in 96 well ABI PRISM 7900HT sequence detection system (Applied Biosystems) using inner primers and probes designed for specific genomic regions in a final volume of 25µl. The probes were labeled with either 6-carboxy fluorescein (FAM) or 6-carboxytetramethylrhodamine (TET) at the 5' end and Black Hole Quencher (BHQ) at the 3' end. The fluorescence intensity was measured by SDS v.2 software (Applied Biosystem, USA). The PCR product generated was checked by conducting dissociation curve analysis through SybrGreen PCR Master Mix (Applied Biosystems) which distinguishes PCR product from primer dimmers of lower thermal stability.

Amplicons of the gag & env gene segments were purified by a QIA quick PCR purification kit (QIAGEN, Germany, and Hilden) and were subjected to cycle sequencing reactions using fluorescent dye-labeled dideoxy 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, NorthCarolina State University) [http://www.mbio.ncsu.edu/Bio edit/BioDoc.pdf]. The edited sequences were Blast searched and further aligned with the reference sequences from different geographic regions available in the HIV database (http://www.hiv.lanl.gov/content/index) for phylogenetic analysis using the Molecular evolutionary genetics analysis software version 4 (MEGA 4).

Out of 18 HIV seropositive samples, the gag heteroduplex mobility assay of all the 18 samples of Nagaland injecting drug users showed 17 samples as subtype C while one as subtype B (nag120) (Table 1). On the other hand, env heteroduplex mobility assay showed 11 samples as subtype C and rest of the 7 samples e.g. nag1, nag12, nag23, nag 57, nag120, nag135 and nag153 as subtype B.

Multiregional hybridization assay was carried out for all the 18 samples (Table 1). The analysis showed that nag 25, nag 33, nag 49, nag 65, nag 81, nag 86, nag 111, nag 113 and nag 157 belonged to subtype C. However, subtype B probe reacted with nag120.

Multigenomic recombination was detected for the samples nag 1, nag 12, nag 23, nag 57, nag 88, nag 135, nag 152 and nag 153. The samples nag 23, nag 88 and nag152 showed both dual probe reactivity and multigenomic recombination pattern.

The B/C recombination pattern with respect to gag (p24- p7) and env (C2-V3) of the recombinant samples was confirmed by phylogenetic analysis. Phylogenetic analysis of the gag (p24-p7) gene of Nagaland injecting drug users with the reference subtype C and subtype B sequences available in the database (http://www.hiv.lanl.gov/content/index) clearly showed that the samples nag 1, nag 23, nag 25, nag 33, nag 49, nag 57, nag 65, nag 81, nag 86, nag 88, nag 111, nag 135 clustered with subtype C HIV-1 strains from Africa and nag 12, nag 113, nag 152, nag 153, nag 157 clustered with Indian subtype C. For nag 120, gag (p24-p7) gene clustered with subtype B strains from China (Fig. 1). On the other hand, phylogenetic analysis of the env C2-V3 gene with other global HIV-1 strains showed that nag 25, nag 33, nag 65, nag 86, nag 88, nag 111, nag 113 formed cluster with subtype C reference sequences from Africa and nag 49, nag 81, nag 152, nag 157 formed cluster with Indian subtype C (Fig. 2). However nag 1, nag 57, nag 135, nag 153 formed a unique cluster close to Thai B sequences and nag 12, nag 23 and nag 120 clustered with subtype B sequences from China and Myanmar.

The Gen- Bank accession numbers for the nucleotide sequences of gag (p24-p7) and env (C2-V3) reported in this paper are EU541498, EU526648- EU526656, EU526658-EU526666, EU526668- EU526669, HM-130667, HQ897949- Hq897962.

Sample No.		MHAbce v.2								HMA	
		p17	*pro*	*rt*	*int*	*tat*	*gp120*	*gp41*	*nef*	p24-p7	C2-V3
1.	nag1	C	NR	C	B	C	B	B	C	C	B
2.	nag12	C	C	C	C	NR	B	B	B	C	B
3.	nag3	C	C	B/C	B	C	B	NR	C	C	B
4.	nag25	C	C	C	C	C	C	C	C	C	C
5.	nag33	C	C	C	C	C	C	C	C	C	C
6.	nag49	C	C	C	C	C	C	NR	C	C	C
7.	nag57	C	C	C	C	C	B	NR	B	C	B
8.	nag65	C	C	C	C	NR	C	C	C	C	C
9.	nag81	C	C	C	C	C	C	C	NR	C	C
10.	nag86	C	C	C	C	C	C	C	C	C	C
11.	nag88	C	C	B	B/C	C	C	C	C	C	C
12.	nag111	C	C	C	C	C	C	C	C	C	C
13.	nag113	C	C	C	C	C	C	C	NR	C	C
14.	nag120	B	B	B	B	B	B	B	B	B	B
15.	nag135	C	C	C	C	C	B	B	NR	C	B
16.	nag152	C	NR	C	B	B/C	C	C	NR	C	C
17	nag153	C	NR	B	C	C	B	B	NR	C	B
18.	nag157	C	NR	C	C	C	C	C	C	C	C

Table 1. Genotyping Results of HIV-1 Positive IDU Samples Based on MHAbce v.2 and HMA. 18 Samples were Subjected to MHA Using Probes Specific for Subtype C, Subtype B and Subtype AE. "NR" Denotes No Probe Reactivity; B/C Denotes Dual Probe Reactivity.

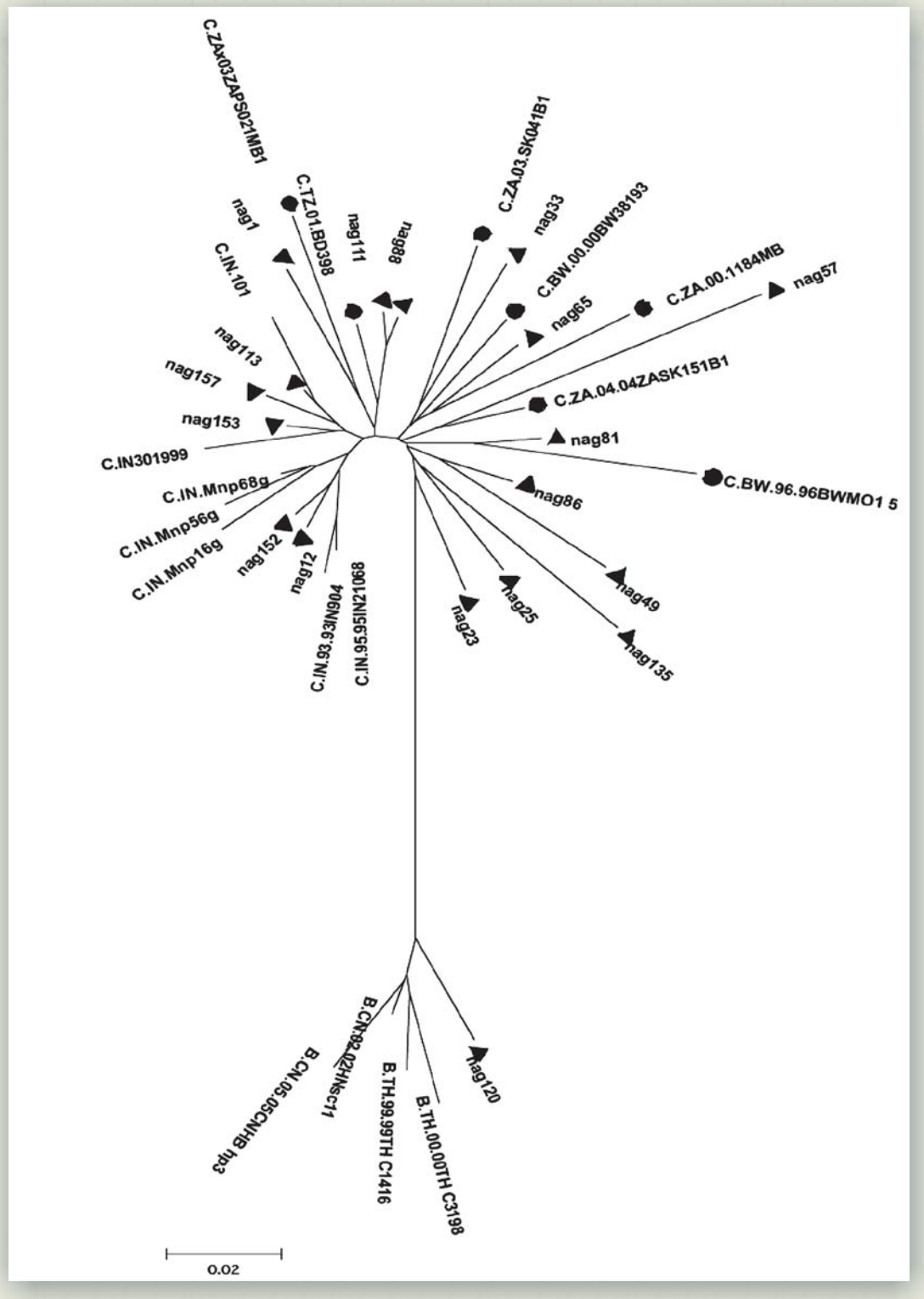


Fig. 1. Phylogenetic analysis of gag gene (p24-p7) of the HIV-1 strains isolated from the Nagaland IDU samples. The Nagaland IDU strains are denoted as "nag"▾ and African strains as "●".

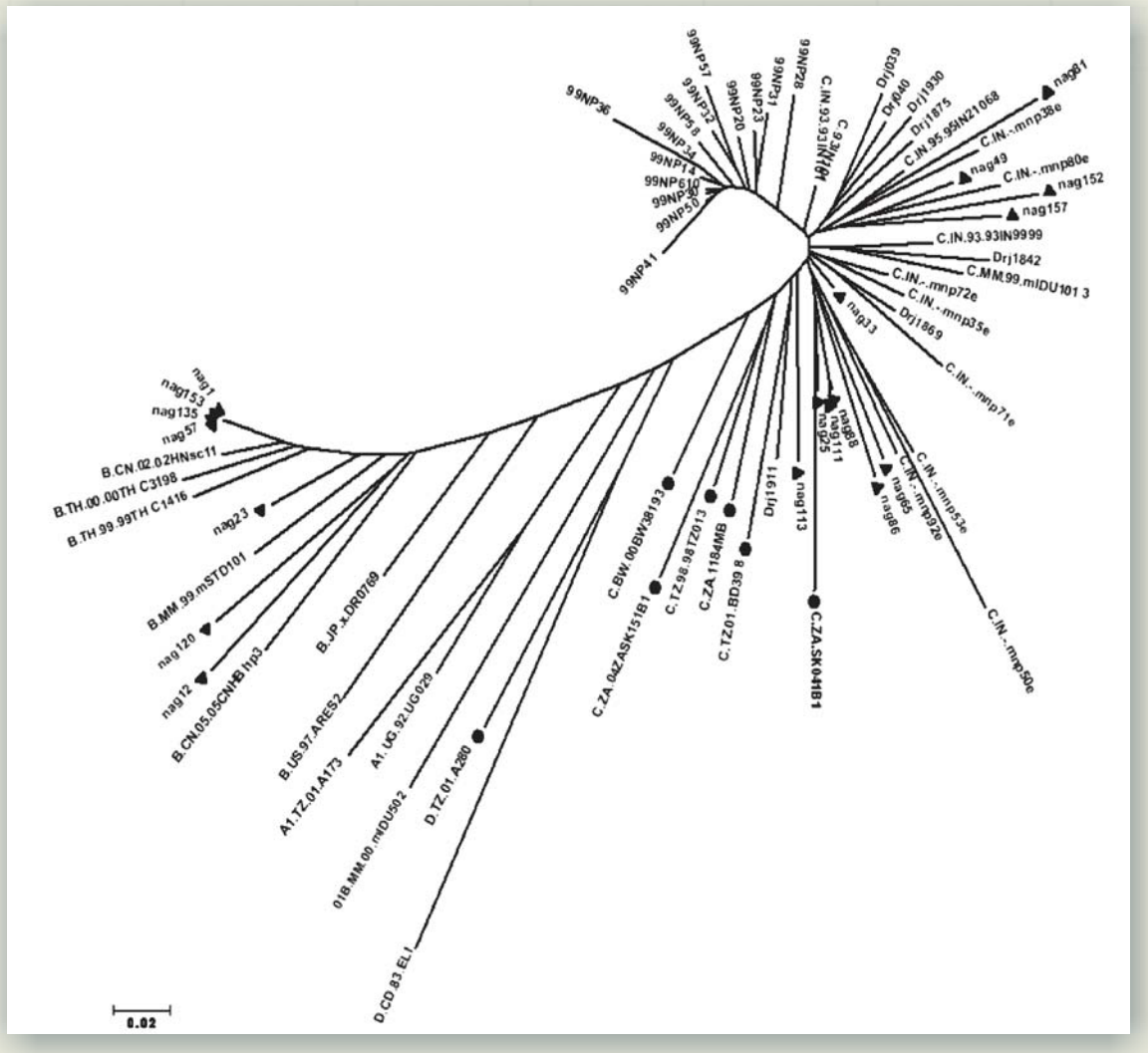


Fig. 2. Phylogenetic analysis of env gene (C2-V3 region) of the HIV-1 strains isolated from the Nagaland IDU samples. The Nagaland IDU strains are denoted as "nag"▾ and African strains as "●".

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

Investigator: T. Krishnan

Viral pathogens viz. Norovirus, Sapovirus and Astrovirus were screened infaecal specimens of diarrhoea cases received in Division of Virology of National Institute of Cholera and Enteric Diseases Kolkata from Dr B.C. Roy Memorial Hospital for Children and Infectious Diseases and Beliaghata General Hospital. The data generated from diagnostic virology experiments is useful for assessment of disease burden owing to the viral pathogens such as Norovirus [Genogroup I & II], Sapovirus and Astrovirus. The molecular epidemiological studies on astroviruses provided interesting information about the genetic diversity amongst

strains of different Astroviruses circulating in Kolkata. Two conserved genomic fragments viz. 289bp of ORF1a and 449bp of ORF2 amplified by RT-PCR showed emergence interesting recombinant strains representing new and novel genetic variants (n = 5) within eight different genotypes of astroviruses known to date [Table 1,2]. HAstV-positive cases with ORF1a [HAstV genotype G2 or G8] and ORF2 [HAstV genotype G1, G2, or G3] were detected as sole or mixed infection among infants, children and adults in Kolkata with severe illness owing to acute gastroenteritis that required hospitalization for treatment between 2007 and 2009. The twelve interesting recombinants were of type HAstV_ORF1a_ORF2 as HAstV_G8_G2 (n = 1), HAstV_G8_G1 (n = 10) and HAstV_G2_G3 (n = 1). Phylogenetic analysis of the ORF1a (289bp) region of the 12 recombinant HAstV strains showed that in eleven instances (2 adults, 9 infants or children) they closely clustered with reference strain of HAstV8 (Yuc 8, AF260508) reported from Mexico and in one instance (child) with HAstV2 Oxford strain L13745 from USA**.** [Figure A, B]

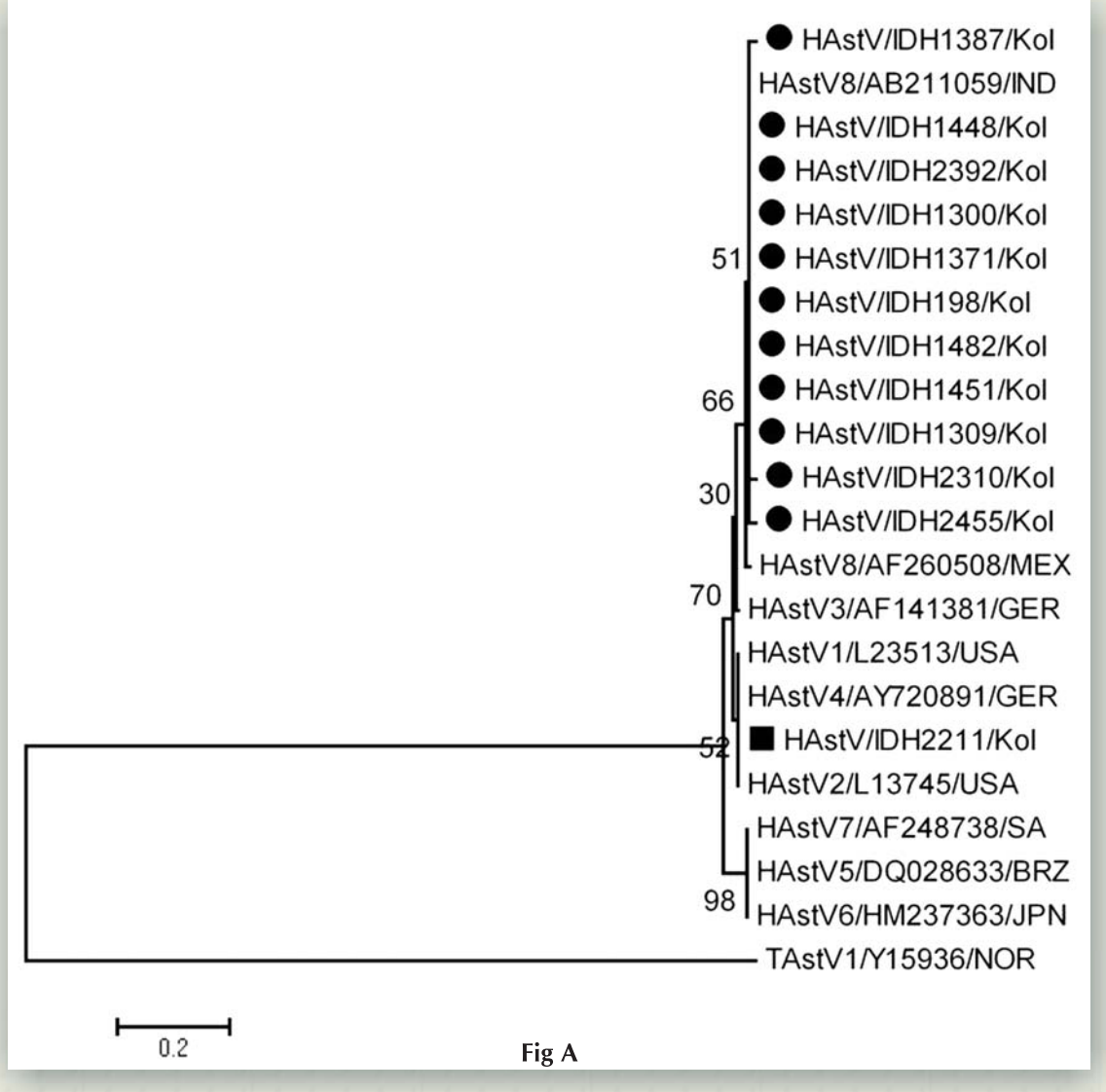


Fig A

Figure-A: Phylogenetic analysis of deduced amino acid sequences (from 289bp fragment of partial ORF1a encoding serine protease) of human astrovirus strains detected in Kolkata, India. The Kolkata strains of HAstVs are indicated by black symbols. Scale bar indicates amino acid substitution per site. Reference sequences were obtained from GenBank under accession nos.HAstV1 (L23513/USA), HAstV2 (L13745/USA), HAstV3 (AF141381/GER), HAstV4 (AY720891/GER), HAstV5 (DQ028633/BRZ), HAstV6 (HM237363/JPN), HAstV7 (AF248738/SA) and HAstV8 (AF260508/MEX). The nucleotide sequences of 289bp ORF1a fragments of the HAstVs from Kolkata (variants marked with bold face) were deposited in DDBJ under accession nos. IDH198/AB607960, IDH1300/AB551381; IDH1309/AB551382; IDH1371/AB551383; **IDH1387/AB551384**; IDH1448/AB551385; IDH1451/ AB607961; IDH1482/AB551386; IDH2211/ AB551387; **IDH2310/ AB551388**; **IDH2392/AB607962**; **IDH2455/AB607963**

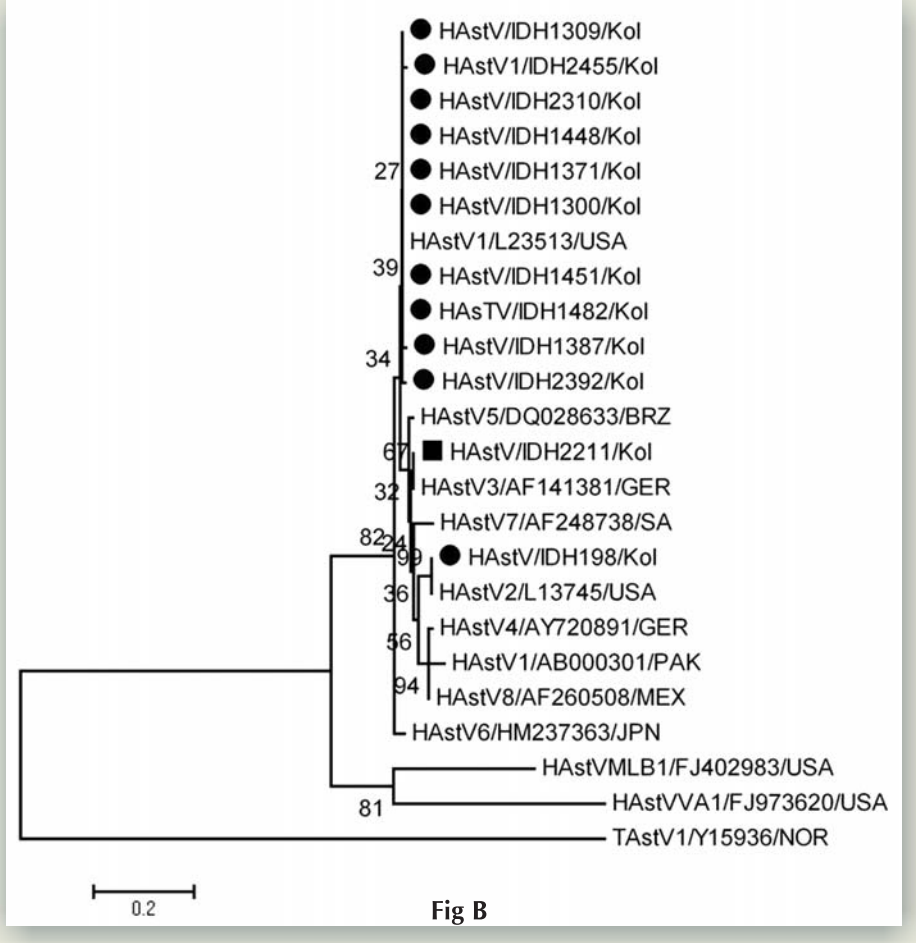


Fig B

Figure-B Phylogenetic analysis of deduced amino acid sequences (from 449bp fragment of the partial ORF2 encoding capsid gene) of human astrovirus strains detected in Kolkata, India. The Kolkata strains are indicated by black symbols. Scale bar indicates amino acid substitution per site. Reference sequences were obtained from GenBank under accession nos. HAstV1 (L23513/USA), HAstV2 (L13745/USA), HAstV3 (AF141381/GER), HAstV4 (AY720891/GER), HAstV5 (DQ028633/BRZ), HAstV6 (HM237363/JPN), HAstV7 (AF248738/SA), HAstV8 (AF260508/MEX), HAstVMLB1 (FJ402983/USA), HAstV1 (AB000301/PAK), HAstVVA1 (FJ973602/USA) and TAstV1 (Y15936/NOR). The nucleotide sequences of 449bp ORF2 fragments of the HAstVs from Kolkata (variants marked with bold face) were deposited in DDBJ under accession nos. IDH198/ AB551371, IDH1300/ AB540662; IDH1309/ AB548400; IDH1371/ AB548401; **IDH1387/AB551372**; IDH1448/AB548402; IDH1451/AB548403; IDH1482/AB551373; IDH2211/ AB548404; IDH2310/AB548405; **IDH2392/AB551374**; **IDH2455/AB551375**.

Table 1. Clinical symptoms associated with astrovirus infections and their severity scores.

Clinical symptoms*	Score	HAstV
Duration of diarrhea (hours)		
0	0	0
1-12	1	1 (8)
12-24	2	1 (8)
24-36	3	**8 (66)**
⩾36	4	2 (16)
Diarrhea episodes /24h		
0	0	0
1-3	1	1 (8)
4-5	2	1 (8)
⩾6	3	**10 (83)**
Duration of vomiting (day)		
No vomiting	0	5 (42)
1	1	**7 (58)**
2	2	0
⩾3	3	0
Vomiting episodes/24h		
0	0	4 (33)
2	1	0
2-4	3	0
⩾5	5	**8 (67)**
Fever (°C)		0
>37.0	0	**10 (83)**
37.1-38.4	1	0
38.5-38.9	2	2 (17)
⩾39.0	3	0
Dehydration		
None	0	0
Some	2	**12 (100)**
Severe	3	0
Catagories based on total		
Mild	0-7	0
Moderate	8-13	**12 (100)**
Severe	14-20	0

The highest numbers for each category are in bold face.
*All patients were hospitalized and received 2 points on the Ruuska and Vesikari score for this outcome.

Table 2 Comparison of conserved amino acids and amino acid changes in positions indicated within the 289bp fragment of astrovirus positives detected during the study and representative strains of eight different genotypes of astroviruses.

AminoAcid	5	10	21	36	43	50	53	55	59	66	81
IDH198	I	V	V	V	K	R	V	D	I	I	N
IDH1300	I	V	V	V	K	R	V	D	I	I	N
IDH1309	I	V	V	V	K	R	V	D	I	I	N
IDH1371	I	V	V	V	K	R	V	D	I	I	N
IDH1387	I	V	V	V	K	R	I	D	I	I	N
IDH1448	I	V	V	V	K	R	V	D	I	I	N
IDH1451	I	V	V	V	K	R	V	D	I	I	N
IDH1482	I	V	A	V	K	R	V	E	I	I	N
IDH2310	I	V	V	V	K	R	V	D	V	I	N
IDH2392	I	V	V	V	K	R	V	D	I	I	K
IDH2455	I	V	A	V	K	S	V	E	I	I	N
HAstV_8	I	V	V	V	K	R	V	D	I	I	N
IDH2211	I	V	V	V	K	R	V	D	I	V	N
HAstV_2	I	V	V	V	K	R	V	E	I	V	N
HAstV_1	I	V	A	V	K	R	V	E	I	V	N
HAstV_3	I	V	A	V	K	R	V	E	I	V	N
HAstV_4	V	I	A	A	R	R	V	E	I	V	N
HAstV_5	V	I	A	A	R	R	V	E	I	V	N
HAstV_6	V	I	A	A	R	R	V	E	I	V	N
HAstV_7	I	V	A	V	K	R	V	D	I	I	N

Non polar: I, V, A; Acidic negatively charged group D, E; Basic positively charged group K, R.
Polar: Acidic negatively charged group N, S

Multisite monitoring of Influenza Virus strains in India, Phase II

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 hospitals in Kolkata (B C Roy childrens Hospital, NRS Hospital and R G Kar Hospital) after obtaining informed consent form from the guardian/parent.

A total of 820 samples were screened during this period of which 163 (19.8%) were positive for Inf A/B. Of 163 samples 135 (16.46%) were typed as H3N2 and 28 (3.41%) as Inf B. Majority of samples were from paediatric population (0-5 yrs) and no correlation with gender was observed. Of 163 Real time PCR samples, 141 were inoculated in MDCK cells for virus culture. Of 141, 61 isolates were obtained. Of 61 isolates, 54 were H3N2 and 7 were Inf B. No pH1N1 strain was found during the period.

Active participation of host chaperone protein HSP90 in modulating rotavirus encoded NSP3 protein.

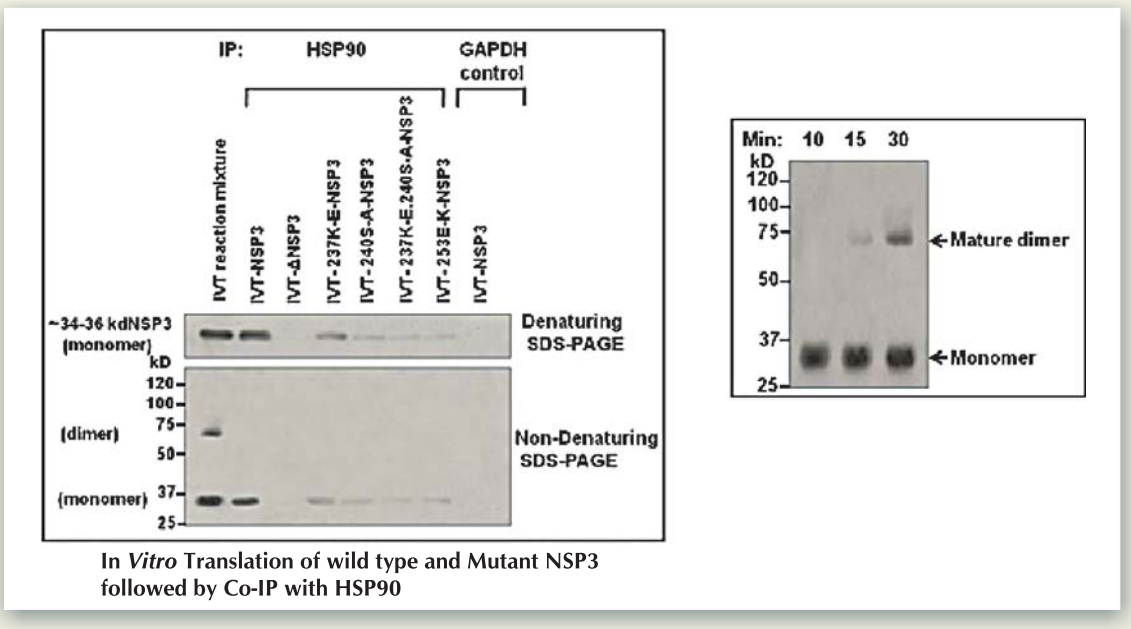
Investigator: M. Chawla Sarkar

Association of HSP90 with rotaviral NSP3 protein is essential for its function : NSP3 protein sequence was analyzed using SMART programme, GAPPED- and PSI- BLAST programmes, Win Gen/Win Pep, and ELM server. A single tetra tricopeptide repeat (TPR) like motif (aa 225-258) was found in SA11 NSP3 protein sequence, within the eIF4G binding domain. Since only a few amino acids were identical (bold letters) with conventional TPR motif we termed it as putative TPR like motif (PTPRLM). To assess whether Hsp90 physically interacts with NSP3 to modulate its function, cell lysates of 293T cells following SA11 infection (8h) were subjected to immunoprecipitation with Hsp90 antibody followed by immunoblotting with NSP3 antibodies. In addition, lysates from cells expressing FLAG-NSP3 in presence or absence of 17DMAG were immunoprecipitated using anti-FLAG antibody followed by immunoblotting with anti-Hsp90. In both experiments, we observed co-immunoprecipitation of Hsp90 with NSP3. However, in presence of 17DMAG (5 μM) significant reduction (~ 3 fold) in co-immunoprecipitation was observed suggesting direct or indirect association between two proteins (D. Dutta et al 2011, J Biol Chem).

To identify the Hsp90 binding region of NSP3, FLAG tagged NSP3 deletion mutants lacking RNA binding domain (aa 1-149) [ΔRB], eIF4G binding domain (aa 206-313) [ΔeIF4GB], dimerization domain (aa 150-240) [ΔDM] and PTPRLM (aa 225-258) [ΔPTPRLM] were generated .

Each of the FLAG-NSP3 mutants was transfected into 293T cells, and cell lysates were immune-precipitated using anti-FLAG antibody, followed by immunoblotting with Hsp90 antibody. Although FLAG-ΔRB-NSP3 co-immunoprecipitated with Hsp90, little Hsp90 was observed in immunoprecipitates of FLAG- ΔDM-NSP3 and in ΔeIF4GB- and ΔPTPRLM–NSP3 transfected cells, no Hsp90 immunoprecipitates were observed. Since FLAG-ΔPTPRLM had full length NSP3, except the PTPRLM region (aa 225-258), this region could be the potential binding site for Hsp90.

Direct interaction of HSP90 c-terminal domain with PTPRLM of functionally immature NSP3 helps its assembly: To determine functional significance of Hsp90-NSP3 interaction, specific point mutations were introduced in pCDNA-NSP3 (K237E, S240A, E253K, K237E & S240A) in addition to a PTPRLM deletion mutant (pCDNA-ΔNSP3). Wild type as well as mutant plasmids were subjected to in vitro coupled transcription and translation (IVT) for 90 min in the presence of Transcend TM biotinylated-Lysyl-tRNA as probe. The IVT products were immunoprecipitated with anti-Hsp90. monoclonal antibody, followed by denaturing SDS-PAGE analysis (Fig1). Immunoblotting with streptavidin-HRP revealed that other than the wild type full length NSP3, mutants of NSP3 poorly coprecipitated with anti-Hsp90 antibody. However, no co-IP of full length NSP3 was observed with internal control GAPDH suggesting that Hsp90 could be associated with NSP3 through interaction with its PTPRLM region. However, when the Hsp90 co-immunoprecipitates were subjected to SDS-PAGE under nondissociating conditions, only the monomeric form of NSP3 but not the dimers were found to be associated with Hsp90 suggesting that Hsp90 may be playing a dynamic role in NSP3 dimerization (Fig 2).



In *Vitro* Translation of wild type and Mutant NSP3 followed by Co-IP with HSP90

Fig 1. Hsp90-NSP3 complex is an intermediate step towards formation of mature NSP3-NSP3 dimer

The molecular epidemiological study of viral diarrhea among hospitalized infants, children and adults in Kolkata.

Principal Investigator: B. Ganesh,

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

Picobirnavirus is currently the only genus in the family "Picobirnaviridae". The name is derived from the Spanish "pico" meaning 'small' with 'birna' for 'bipartite RNA'. The virus particles are 35-41nm in diameter and non-enveloped. The genome consists of two dsRNA segments of 2.3-2.6 and 1.5-1.9 kilobase pairs (kbp), respectively. Segment 1 codes for capsid

protein and segment 2 encodes RNA-dependent RNA polymerase. Picobirnaviruses [PBVs] have been detected both from diarrheic and non-diarrheic hosts, worldwide. The nucleic acid based molecular assay; reverse transcription-polymerase chain reaction (RT-PCR) was carried out using specific primers to amplify small fragments of the bisegmented genome of genogroup I and genogroup II picobirnaviruses represented by the Chinese strain (1-CHN-97) and US strain (4-GA-91), respectively. The amplicons were sequenced and sequence data was analysed to understand the phylogenetic relationship of the picobirnaviruses. There are few reports of human picobirnaviruses from different countries as well as occurrence of PBVs in feces of different animals. The information that has been gathered showed remarkable genetic diversity among picobirnavirus from Kolkata and other PBVs hitherto reported uptil now.

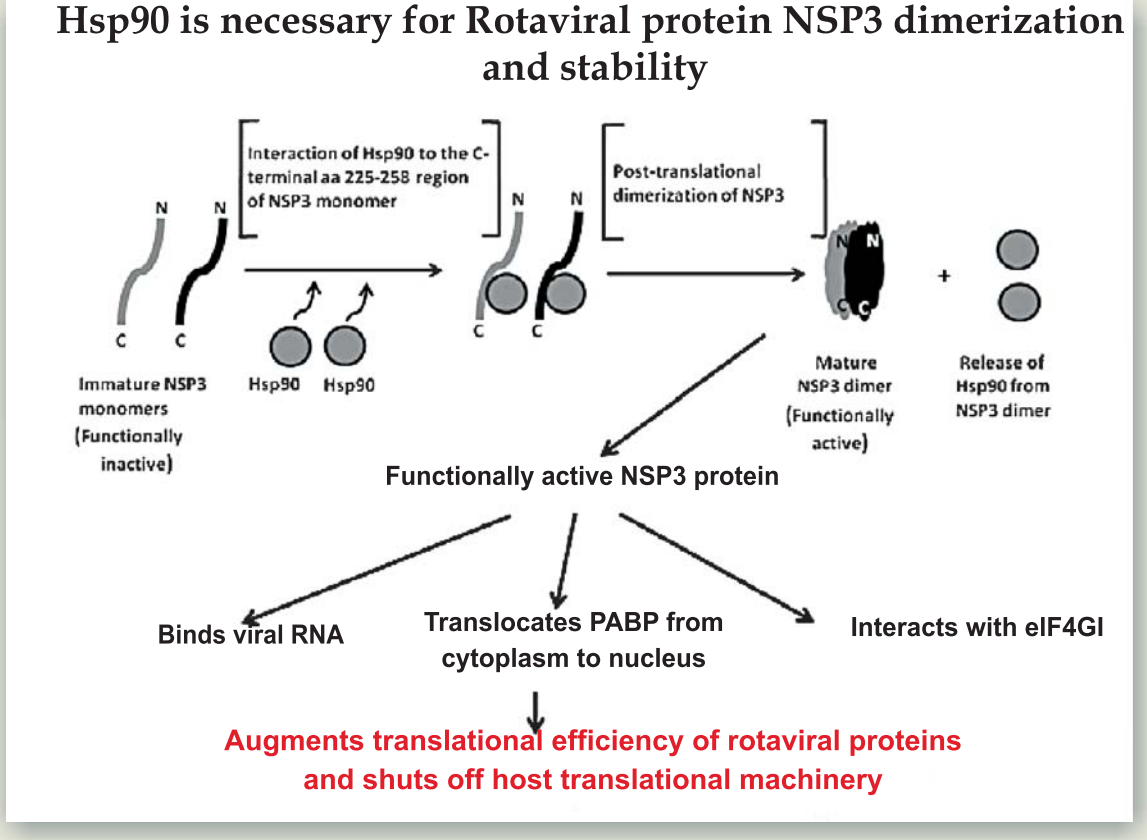
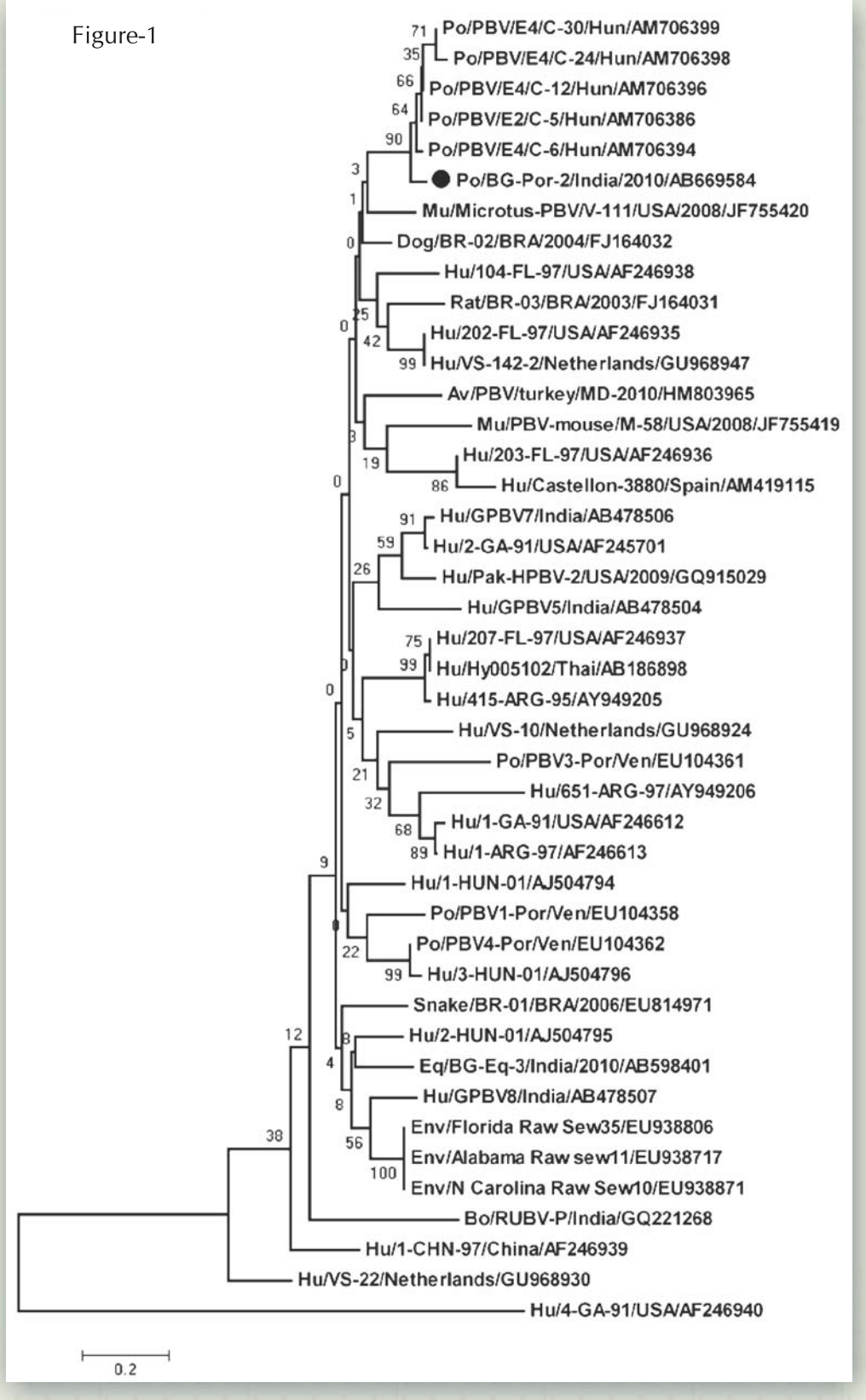


Fig 2. Schematic diagram showing role of Hsp90 in NSP3 biogenesis

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 in Kolkata. Surprisingly, we found that the sequence data of Kolkata (Indian) porcine PBV strain genetically related to European PBV strains and environmental PBV strains of North America [Figure-1]. This 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

Figure-1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Po/PBV/E4/C-24/Hun/AM706398
Po/PBV/E4/C-12/Hun/AM706396
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Po/PBV/E4/C-6/Hun/AM706394
Po/BG-Por-2/India/2010/AB669584
Mu/Microtus-PBV/V-111/USA/2008/JF755420
Dog/BR-02/BRA/2004/FJ164032
Hu/104-FL-97/USA/AF246938
Rat/BR-03/BRA/2003/FJ164031
Hu/202-FL-97/USA/AF246935
Hu/VS-142-2/Netherlands/GU968947
Av/PBV/turkey/MD-2010/HM803965
Mu/PBV-mouse/M-58/USA/2008/JF755419
Hu/203-FL-97/USA/AF246936
Hu/Castellon-3880/Spain/AM419115
Hu/GPBV7/India/AB478506
Hu/2-GA-91/USA/AF245701
Hu/Pak-HPBV-2/USA/2009/GQ915029
Hu/GPBV5/India/AB478504
Hu/207-FL-97/USA/AF246937
Hu/Hy005102/Thai/AB186898
Hu/415-ARG-95/AY949205
Hu/VS-10/Netherlands/GU968924
Po/PBV3-Por/Ven/EU104361
Hu/651-ARG-97/AY949206
Hu/1-GA-91/USA/AF246612
Hu/1-ARG-97/AF246613
Hu/1-HUN-01/AJ504794
Po/PBV1-Por/Ven/EU104358
Po/PBV4-Por/Ven/EU104362
Hu/3-HUN-01/AJ504796
Snake/BR-01/BRA/2006/EU814971
Hu/2-HUN-01/AJ504795
Eq/BG-Eq-3/India/2010/AB598401
Hu/GPBV8/India/AB478507
Env/Florida Raw Sew35/EU938806
Env/Alabama Raw sew11/EU938717
Env/N Carolina Raw Sew10/EU938871
Bo/RUBV-P/India/GQ221268
Hu/1-CHN-97/China/AF246939
Hu/VS-22/Netherlands/GU968930
Hu/4-GA-91/USA/AF246940
0.2

and environmental samples too. The manuscript communicated to the Indian Journal of Virology is accepted with minor revision.

Additionally, for an ongoing Institutional study; during April' 2011 to Febrauary 2012, a total of 529 diarrheic stool samples from hospitalized infants, children and adults were collected from the ID & BG Hospital, Kolkata. The fecal samples were tested for rotavirus and human adenovirus by commercially available immuno-chromatography test according to the manufacturer's instructions. Rotavirus and Human adenovirus was associated with 18.3% and 7% of the cases in all age groups, respectively. Detection and molecular characterization of Calicivirus (Norovirus GI and GII, Sapovirus) and Astrovirus were also performed using RT-PCR assays. The detection of Norovirus GI with the primer set: G1SKF and G1SKR (330bp); Norovirus GII with the primer set: G2SKF and G2SKR (387bp); Sapovirus with the primer set: SLV5317 and SLV5749 (434bp) and Astrovirus with the primer set: PreCap1 and 82B (719bp).

Award and Honours

S. Chakrabarti

- Elected as Fellow of Indian National Science Academy(FNA)
- Received Prof S C Seal Memorial oration award from Indian Public Health Association, Kolkata Chapter
- Guest faculty of West Bengal University of Health Sciences
- Member, Board of Studies; MSc (Biotechnology); West Bengal University of Health Sciences
- Member, Ph D Committee; West Bengal University of Health Sciences
- Guest Faculty of Vidyasagar University; Department of Physiology and Community health
- Examiner, MSc(Biotechnology), University of Calcutta, M Sc(Medical Biotechnology), West Bengal University of Health Sciences.
- Member, Project Review Committee on HIV/AIDS, ICMR, New Delhi
- Member, Expert Committee on H1N1; Department of Health & Family Welfare, Govt of West Bengal
- Chairman, Technical & Purchase Committee, National Institute of Biomedical Genomics
- Member, National HIV Drug Resistance Committee, National AIDS Control Organization, Govt of India
- Member of the Board of Studies, of University of Hyderabad

T. Krishnan

- Awarded Bharat Jyoti award and Certificate of Excellence for scientific contribution by India International Friendship Society in November 2011.

- Selected to Editorial Board of World Journal of Clinical Infectious Diseases (WJCID) from April 2011.
- Selected to Editorial Board of ISRN Microbiology from June 2011.
- Associate Editor for the journal BMC Infectious Diseases and four for the journal .ISRN Microbiology.
- Reviewed eleven research publications for scientific journals viz. Archives of Virology;; Infection Genetics and Evolution; Int. J. Phys Sciences; PLOS Pathogens; Journal of Medical Virology; Intervirology; Frontiers of Medicine.
- Invited to deliver a lecture on "Emerging viruses in relation to viral gastroenteritis" during Two day National Seminar on Microbiology: Development and Challenges in the Field of Basic and Applied Research, organized by Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur Math, Howrah, West Bengal and Bose Institute, Kolkata on 7, April 2011.
- Invited to deliver a lecture on "Moving ahead to meet ethical challenges in our health settings: Promoting good ethical climate in Kolkata, India" at Joint Centre for Bioethics, University of Toronto, on 29 February 2012.

B. Ganesh

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

Conferences/ Seminars/Workshops /Trainings Attended/Organised

S. Chakrabarti

- Attended the 6 th IAS conference on " HIV Pathogenesis, Treatment and Prevention" and present a poster on " Molecular characterization of human immunodeficiency virus type 1 detected among female sex workers(FSW) and injecting drug users(IDUs) from Eastern and North-Eastern and parts of India" held in Rome, Italy during 17-20 July, 2011
- Attended the Indo US Workshop on Measuring Human Immune Responses held in Guargaon, during 31 October- 2November, 2011.
- Participated as Temporary Adviser to the Regional Director in the Inter-country consultation on elimination of Kala-azar in the South-East Asia Region, Kolkata, during 9-10 Nov. 2011.
- Attended the Annual Conference of the Association of Medical Biochemist of India-eastern zone (AMBICON-eastern zone) held on 12-13 November, 2011 in the Department of Biochemistry, NRS Medical College and Hospital, Kolkataand delivered a talk on"HIV/AIDS: present scenario"
- Attended the International Symposium on "Vaccines: from Discovery to Translation" in

New Delhi held during 14-17 November, 2011 organized by DBT, New Delhi.

- Acted as Chairperson of the National Conference on Interface of Science & Environment: Emerging Public Health Challenges & the 13th Annual Meeting of Society for Science and Environment at the Centenary Hall, University of Calcutta on 26 November, 2011.
- Attended Probiotics Symposium on "Health Impact of Probiotics – Vision and Opportunities" held in Mumbai from 10-11 December, 2011.
- Attended the 46th Joint Meeting and Conference of the US-Japan Cooperative Medical Science Program Panel on Cholera and Other Bacterial Enteric Infections at Hyatt Regency, Kolkata during 13-15 December, 2011.
- Delivered the Professor S.C. Seal Memorial Oration 2010 on "Prevention of HIV/AIDS : Dream or Reality?" to be organized by the Indian Public Health Association on the occasion of their 55th Annual Conference at 11-00 am on 17 December, 2011 at National Institute of Cholera and Enteric Diseases, Kolkata.
- Delivered a talk on "Molecular characterization of HIV in eastern and north-eastern part of India" at the National Symposium on Veterinary Medical Sciences on Health, Environment and Food Security organized by Bengal Veterinary College, Faculty of Veterinary and Animal Sciences, BCKV and West Bengal University of Animal & Fishery Sciences, Belgachia, Kolkata on 10 January, 2012.
- Acted as Chairperson in one of the Sessions of the 2nd International Conference on Perspectives of Cell Signaling and Molecular Medicine held at Bose Institute, Kolkata on 10 January, 2012.
- Delivered a Keynote address entitled "Diarrhoeal Disease: Solvable Enigma" at the UGC sponsored national seminar on "Modern Biology and its Impact on Public Health" held at Dinabandhu Andrews College, Kolkata on 18 January, 2012.
- Chaired a Session at the 12th Congress of the International Society for Ethnopharmacology (ISE) at Science City Convention Centre, Kolkata on 18 February, 2012.
- Attended the International Conference on Frontiers in Biological Researches as Chief Guest and delivered a talk on "Prevention and Cure of HIV/AIDS: Dream or Reality" during 26-27 February, 2012 organized by Department of Physiology with Community Health, Vidyasagar University, Medinipur, West Bengal.
- Chaired a session at the 5th FIMSA Congress on "Adjuvant & Vaccines" held on 16 March, 2012, New Delhi.

T. Krishnan

- "Phylogenetic analysis of etiological agents associated with viral gastroenteritis among children and adults in Kolkata, India" on 1 October 2010 at the International Conference on Frontiers in Biological Sciences held in Department of Life Sciences, National Institute of Technology, Rourkela from 1-3 October 2010.
- "Improved viral diagnosis by molecular detection of different viral etiological agents

among acute watery diarrhoea cases, in Kolkata, India". Presented by colleagues at United States-Japan Cooperative Medical Science Program (CMSP) sponsored 14th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim held in Penang, Malaysia during 4-6 October 2010.

- "Norovirus surveillance among viral gastroenteritis cases in Kolkata, India: genetic diversity of Region C of capsid gene fragment" at the 58th Domestic Congress of Virology held in Tokushima, Japan from 7-9 November 2010
- "Viral Gastroenteritis: Emerging viruses" on 26th February 2011 at the National Seminar on Ecology and Environment Management: Indian Scenario organized as a part of Golden Jubilee Celebrations of the Sri Venkateswara College, University of Delhi [South Campus) from 24-26 February 2011

M. Chawla Sarkar

- "Influenza A virus encoded matrix protein modulates cellular apoptosis for efficient viral infection". U C Halder and M Chawla- Sarkar. 2nd International Conference on Perspectives of Cell Signaling and Molecular Medicine", Bose Institute, Kolkata, India, 8-11 Jan 2012.
- "Role of rotavirus encoded non structural proteins in modulation of cell death during infection" R Bhowmick, U C Halder, P Bagchi and M Chawla-Sarkar. 2nd International Conference on Perspectives of Cell Signaling and Molecular Medicine", Bose Institute, Kolkata, India, 8-11 Jan 2012.
- "Cellular chaperone Heat Shock Protein-90 positively regulates rotavirus infection by modulating the virus encoded Non structural protein -3". Dipanjan Dutta, Nobumichi Kobayashi, and Mamta Chawla-Sarkar. IUMS 2011 (XV International Congress of Virology), Sapporo, Japan 11-16 Sept 2011.

B. Ganesh

- Balasubramanian Ganesh, Shovan Das, Nataraju SM, Mihir K Bhattacharya, Ramamurthy T, Niyogi SK, Mrinmoy Ghosh, Triveni Krishnan. "The incidence of picobirnavirus infections in infants, children and adults hospitalized for acute gastroenteritis in Kolkata, India" at the 99th Indian Science Congress, held at Bhubaneswar during 3-7 January 2012.
- Madhusudhan Pativada, Nataraju SM, Ganesh B, Nobumichi Kobayashi and Triveni Krishnan. "Detection and molecular characterization of Human Astrovirus infections in infants, children and adults hospitalized for acute gastroenteritis in Kolkata, India" at the 99th Indian Science Congress, held at Bhubaneswar during 3-7 January 2012.

services

OUTBREAK INVESTIGATIONS

Water and Environmental samples :

During the focal diarrheal/cholera outbreaks (2011-12) in different southern districts of West Bengal, microbial analysis and examination of samples of potable water sources, from different districts of West Bengal as well as Municipal wards under Kolkata Municipal Corporation (KMC) 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 Parganas, S. 24 Parganas, Hooghly and Kolkata as well as from endemic and epidemic affected Municipal wards under the Kolkata Municipal Corporation and its adjoining areas. During the period under report, 47 samples had been received from various sources of which 39 had been found to be positive for fecal coliforms and 8 for presence of *V. cholerae* (Table 1).

Sl No.	District / s	No. of samples received	Source					Coliform Positive samples	*V. cholerae* Positive samples
			Tap	Tube well	Pond	Unknown	Stored		
1.	South 24 Parganas	2		1	1			1	1
2.	North 24 Parganas	24	9	14			1	22	3
3.	Kolkata	12	3	1	3	4	1	11	3
4.	Hooghly	2	1	1				1	
5.	Howrah	7	5	2				4	1
	Total	**47**	**13**	**17**	**4**	**4**	**2**	**39**	**8**

Table 1: Analysis of water / environmental samples

Vibriophage Reference Laboratory

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

Biomedical Informatics Centre

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

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

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

Present manpower:

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

Public Health Laboratory Division

Activities of the newly created Public Health Laboratory Division include

i. 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 National AIDS Control Organization (NACO), Government of India.

ii. Counseling and Testing for HIV under the Integrated Counseling and Testing program funded by West Bengal State AIDS Prevention and Control Society.

iii. Estimation of HIV for the states of Andaman & Nicobar Islands, Chattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal as Regional Institute (East) for HIV Sentinel Surveillance funded by National AIDS Control Organization (NACO), Government of India.

iv. Evaluation of HIV, HBV and HCV assay kitsfor the Consortium of National Reference Laboratories for Kit Quality priject, funded by National AIDS Control Organization (NACO), Government of India.

v. Molecular assayof HIVemploying Dry Blood Spot (DBS) samples for infant and young children under the age of 18 months for Early Infant Diagnosis project funded by National AIDS Control Organization (NACO), Government of India.

vi. Enteropathy and micronutrient assay for the project entitled "Exploration of the Biologic Basis for OPV and Rotavirus Vaccine Failure in India" (short named PROVIDE)

in the Clinical Trial Laboratory funded by International Vaccine Institute. Additionally, biochemical and hematological assay for cholera vaccine trials

Staff of Public Health Lab 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)
Ms. P Bhaumik (Tech. Asst.)
Ms. C Das (Attendant/Servical)

Quality Assurance for HIV Testing

NICED has been functioning as a national resource from the very beginning of HIV testing in the country since 1986. NACO under the Ministry of Health and Family Welfare, Government of India funds the HIV National Reference Laboratory of the Institute since 1992. The activities are as following.

- EQAS and Panel Sera preparation for SRL from states of Eastern North-Eastern India.
- Referral for confirmation of HIV testing results of the samples received from different SRLs.
- Training for Doctors, 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.

EQAS Programme:

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

Referral Services

Amongst the responsibilities allotted to the National Reference Laboratory at NICED, Referral Service is of utmost importance. NACO-NRL, NICED has been entrusted with the responsibility of verifying results for all discordant samples sent by State Reference Laboratories and several Hospitals. The samples tested, result communicated within the turnaround time (TAT) of 5 working days, analyzed the root cause of discordance and trained the referring lab for improvement and technical capacity building.

Testing of DBS for HSS:

Assessing the quality and competence as a specialized lab NACO designated NRL at NICED as a testing center for Dry Blood Spot Samples (DBS) collected from five remote states from northeast and eastern part of the country for HIV sentinel surveillance.

Name of SRLs	Samples received by NRL, NICED from SRLs in four Quarter: April 2011- to January 2012				No of Concordant Result at NRL	No. of Discordant Result at NRL
	April 2011	July 2011	October 2011	January 2012		
G.B Pant Hospital Andaman & Nicobar Islands	00	00	04	06	10	00
Rajendra Institute of Medical Science, Ranchi, Jharkhand	00	08	10	10	28	00
MGM Medical College, Jamshedpur, Jharkhand	07	07	05	07	23	03
Patuliputra Medical College, Dhanbad, Jharkhand	15	13	21	18	66	01
SCB Medical College, Cuttack, Orissa	18	13	13	12	66	01
VSS Medical College Burla, Orissa	13	16	16	14	59	00
MKCG Medical College, Beharampur, Orissa	15	17	15	14	61	00
Guwahati Medical College, Guwahati, Assam	07	16	00	00	23	00
Assam Medical College, Dibrugarh, Assam	03	08	06	03	20	00
Silchar Medical College, Silchar,Assam	03	04	03	05	15	00
NEIGRIHMS, Shillong, Meghalaya	14	45	07	09	75	00

Table 1: External Quality Assurance for SRLs under NACO NRL, NICED, Kolkata

Source of Samples	No. of sample Tested	No. of sample Positive
WEST BENGAL		
1. Command Hospital	56	55
2. B. M. Birla Heart Research Center	06	05
3. Woodlands Multispecialty Hospital Ltd.	07	06
4. Ruby General Hospital	01	01
Total -	**70**	**67**
OTHER STATES		
Assam	03	02
Meghalaya	03	01
Orissa	12	01
Andaman & Nicobar Islands	35	00
Total -	**53**	**04**
GRAND TOTAL-	**123**	**71**

Table 2: Referral Service for SRLs of Eastern & North-Eastern states and other institutions.

Name of the State	No. of samples Received	No. of samples Rejected	No. of samples Tested at NRL
Chattisgarh	1196	33	1163
Meghalaya	235	01	234
Mizoram	1620	04	1616
Nagaland	2526	18	2508
Tripura	1182	00	1182
Total Samples	6759	56	6703

Table3: Testing of DBS (for High Risk Group) samples, HSS 2010 for the states of Chattishgarh, Meghalaya, Mizoram, Nagaland and Tripura

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 in NICED in 2008. It has grown gradually not simply with large client load, 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 psychological support
- Link people with other HIV prevention, care and treatment services.

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.

The ICTC unit of NICED caters to all HIV-testing needs of in-patients and outpatients of the Infectious Diseases Hospital,Beliaghata, as well as from various NGO's, CBO's, RNTCP and the general public. Health Camps organized by some NGO's were also successfully conducted during the tenure of the ICTC here. Additionally students from different university are trained for counseling.

Apart from counseling, testing and ART delivery (as Post exposure Prophylaxis) the ICTC also functions as an auxiliary unit for orientation and training of health-care personnel at NICED. This ICTC unit provided training to health care workers of AMRI Hospitals, Salt Lake.

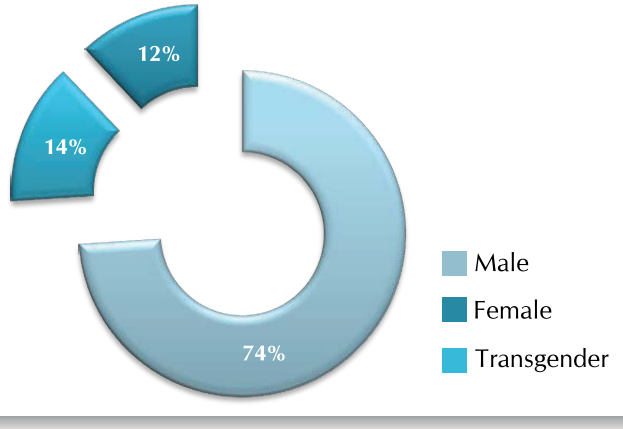
Estimation of HIV as Regional Institutefor HIV Sentinel Surveillance

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.

Monthly distribution of clients at ICTC, NICED' 2011-12

Month	Total Client	Negative	Positive
April	89	85	4
May	93	89	4
June	98	95	3
July	115	110	5
August	104	100	4
September	229	227	2
October	33	33	0
November	88	87	1
December	61	61	0
January	40	38	2
February	80	77	3
March	105	98	7
Total	1135	1100	35

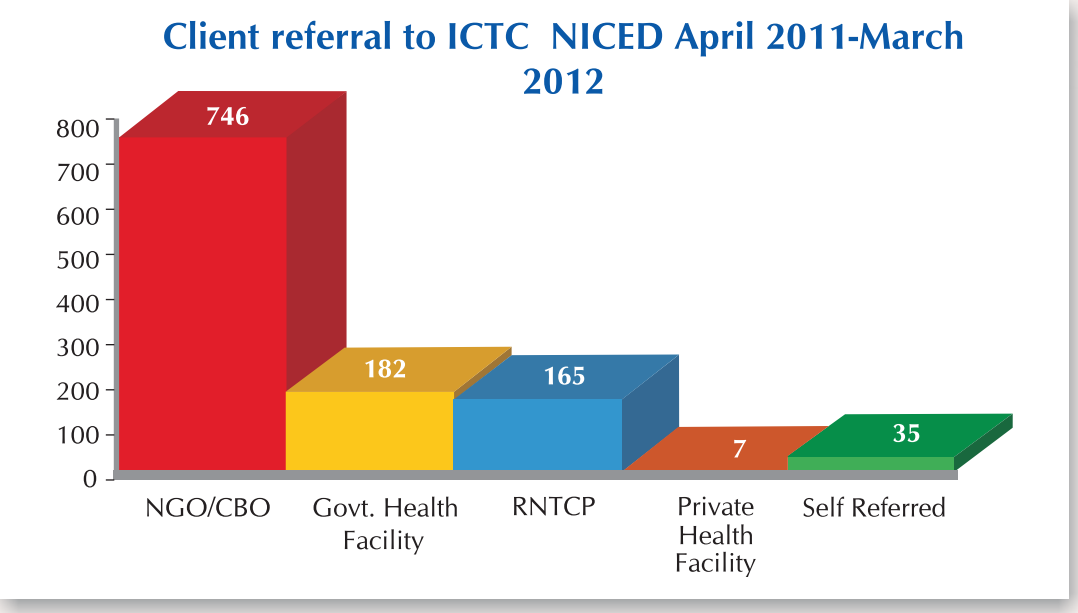
Sexwise distribution of clients at ICTC unit, NICED for the period of April'11 -March'12



Spectrum of Activities

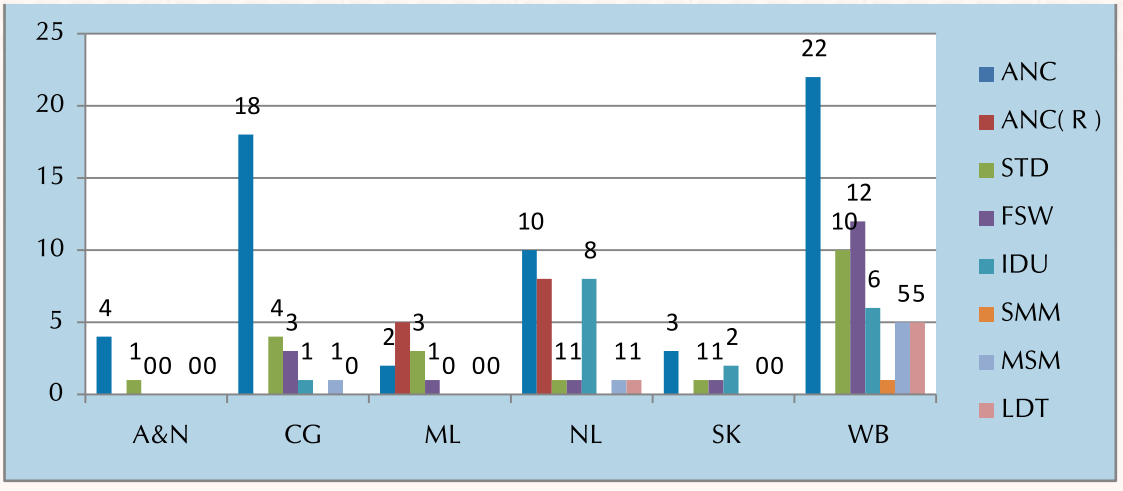
- Technical support & guidance to SACS in overall planning & implementation of HSS activities in Andaman & Nicobar Islands, Chhattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal, facilitating smooth implementation of HSS activities by liaisoning with the concerned state authorities and addressing 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.

HIV Sentinel Surveillance Sites



	ANC	ANC(R)	STD	FSW	IDU	SMM	MSM	LDT	TOTAL
A&N	4	0	1	0	0	0	0	0	5
CG	18	0	4	3	1	0	1	0	27
ML	2	5	3	1	0	0	0	0	11
NL	10	8	1	1	8	0	1	1	30
SK	3	0	1	1	2	0	0	0	7
WB	22	0	10	12	6	1	5	5	61
Site Type Totals	59	13	20	18	17	1	7	6	141

New Sentinel Sites validated by Regional Institute from HSS 2010 Round

Chhattisgarh

1. (FSW) JankalyanSamajikSansthan, Rajnandgaon
2. (FSW) Chetna Child & Women Welfare Society, Raipur
3. (MSM) SamtaMahilaMandal, Raipur
4. (FSW) Samarpit, Bilaspur
5. (IDU) AdarshNavyuvakMandal, Korba

Meghalaya

1. (IDU) Manbha Foundation, Shillong

Nagaland

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

West Bengal

1. (ANC)-Aranghata BPHC(Composite)

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

Monitoring visit/Training/Meeting/Workshop

Purpose	Venue/Place	Date
Chhattisgarh HRG site monitoring visit	Chhattisgarh	11 April 2011 - 13 April 2011
Sikkim HRG site monitoring visit	Sikkim	17 May 2011 - 19 May 2011
Meghalaya HRG site monitoring visit	Meghalaya	18 May 2011 - 20 May 2011
West Bengal Site Personnel Training for HSS 2010 (HRG Round)- 1st Batch	WBSAPCS, Kolkata, West Bengal	23 May 2011 - 25 May 2011
West Bengal Site Personnel Training for HSS 2010 (HRG Round)- 2nd Batch	WBSAPCS, Kolkata, West Bengal	26 May 2011 - 28 May 2011
West Bengal Site Personnel Training for HSS 2010 (HRG Round)- 3rd Batch	WBSAPCS, Kolkata, West Bengal	29 May 2011 - 31 May 2011
Nagaland HRG site monitoring visit	Nagaland	8 June 2011 - 12 June 2011
West Bengal HRG site & NBMCH-SRL monitoring visit	West Bengal	15 June 2011 - 31 Aug. 2011
Post Surveillance Review Meeting	NIHFW, New Delhi	3 Feb 2012 - 4 Feb. 2012
SIMS: Data matching and Finalization workshop	NIHFW, New Delhi	14 Feb 2012 - 19 Feb. 2012

Evaluation of HIV, HBV and HCV assay kits

Consortium of National Reference Laboratories for Kit Quality started functioning with the mandate to develop and operationalize a system of evaluation of the quality of diaginstic HIV, HBV and HCV kits for ensuring the predefined quality for the laboratories across the country.

Four National Reference labs (NARI-Pune, NCDC-New Dedhi, NICED-Kolkata and NIMHANS-Bangalore) started the journey together and now a functional consortium of the laboratoriesfollowing the same Standard Operating Procedure (SOP) and using the same panel developed by the consortium labs.

NARI has been designated as secretariet for the consortium and coordinating all the functions.

Kit Evaluation done by NICED Consortium Lab for different purchaser during April 2011 to March 2012.

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

- For United Nation Office for Project Services (UNOPS).

Total Kit Evaluated by NICED Lab during April 2011 to March 2012

Type of Kit	No of Batch/ Lot evaluated
HCV ELISA	07
HCV Rapid	02
HIV ELISA	03
HIV Rapid	04
HBsAg ELISA	01
HBsAg Rapid	01

- For West Bengal State AIDS Prevention & Control Society (WBSAP & CS)
- For Andhra Pradesh State AIDS Prevention & Control Society. (AP SACS)
- RITES, Govt of India.

Molecular assay of HIV for infant and young children under the age of 18 months.

Molecular detection of HIV 1 by DNA PCR for infant and young children under the age of 18 months is the cornerstone in the efforts to significantly reduce HIV related morbidity and

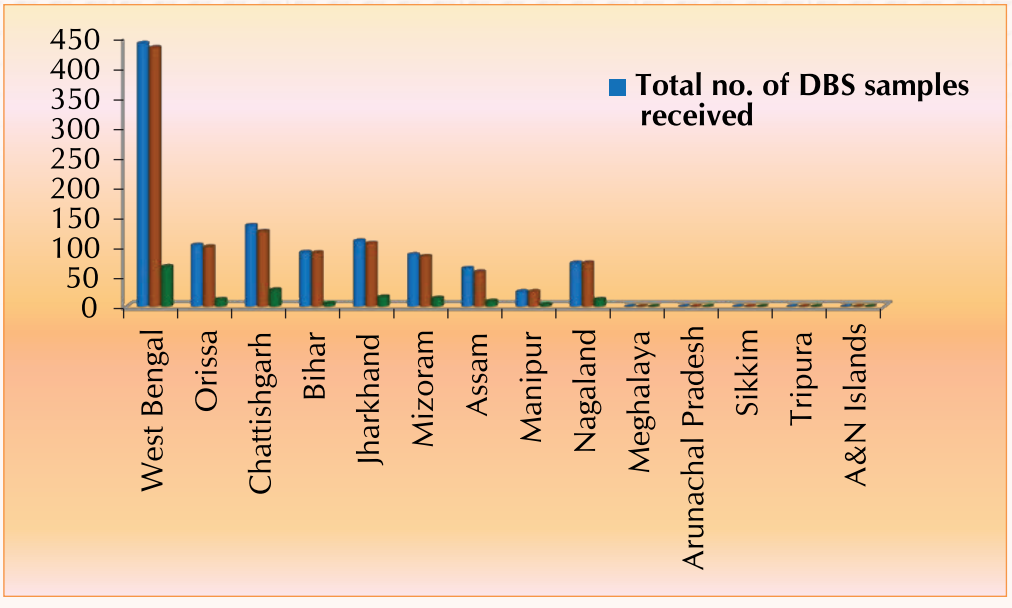
mortality in infants. The diagnosis of HIV infection in infants and children younger than 18 months is different from that in adults due to trans-placental transfer of maternal antibodies from mother to child during pregnancy, childbirth and breast feeding. Hence HIV-1 DNA PCR testing is recommended 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 Chattishgarh. 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. Presently, 116ICTCs are involved in collection of DBS samples in 14 states under NICED and 28 linked ART centres are collecting Whole Blood Samples from infants reactive for DBS-HIV-1 DNA PCR. Different

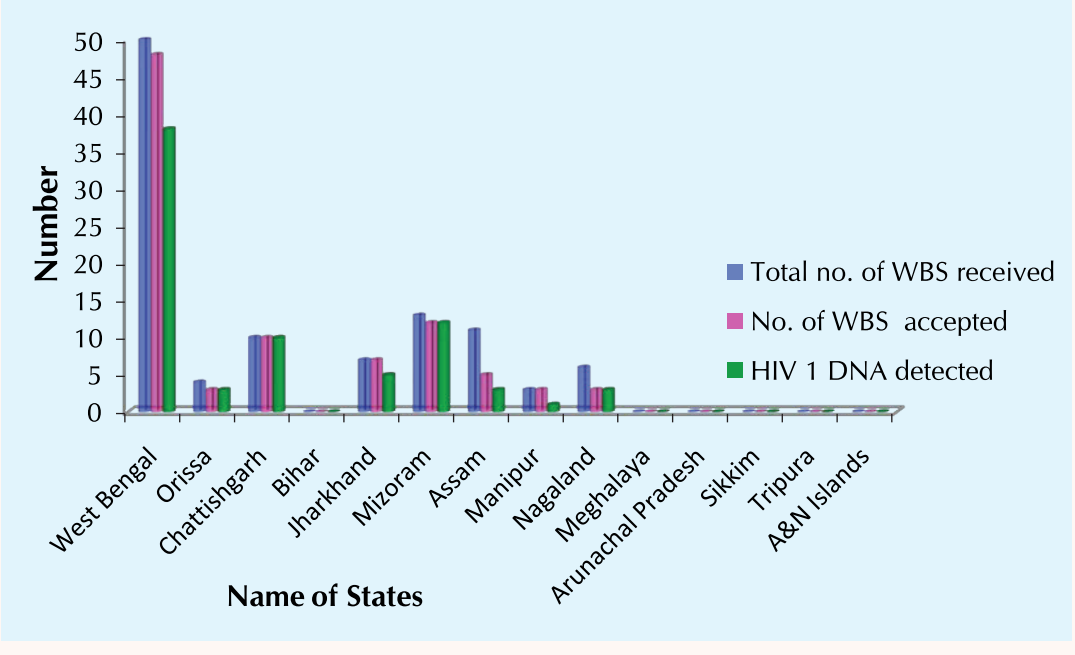
Status of DBS and Whole Blood Samples received at NICED from April'2011 to March'2012.

Name of state	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	440	433	67	50	48	38
Orissa	103	100	12	4	3	3
Chhattisgarh	136	126	28	10	10	10
Bihar	91	90	5	-	-	-
Jharkhand	110	106	16	7	7	5
Mizoram	87	84	14	13	12	12
Assam	64	58	9	11	5	3
Manipur	25	25	3	3	3	1
Nagaland	73	73	12	6	3	3
Meghalaya	-	-	-	-	-	-
Arunachal Pradesh	-	-	-	-	-	-
Sikkim	-	-	-	-	-	-
Tripura	-	-	-	-	-	-
A &N Islands	-	-	-	-	-	-

testing algorithms (algorithm A: for < 6months and algorithm B: for 6- 18 months)have been followed for two different age group of HIV exposed infants in this EID program for detection of HIV-1 DNA. A total of 1129DBS and 104 Whole Blood Samples received at NICED for the period ofApril.2011 to March 2012.



Status of Whole Blood samples received at NICED from April'2011 to March'2012.



Status of DBS and Whole Blood Samples received at NICED from April'2011 to March'2012.

All DBS DNA PCR reactive specimens are further confirmed by 2nd HIV-1 DNA PCR performed with Whole Blood samples.

Training Conducted for technical capacity building

As a leading research institute in India, it has always been our goal to educate minds on the relevant field. We have been organizing workshops to train staff of each of our 11 SRLs. Apart from this; we also conduct Summer Training for students from different Universities in the country.

Organizing Training / Workshops	
i) **Workshop on Calibration, Biosafety and SIMS:** Venue : NICED, Kolkata Date : 28-30 June, 2011 Participants : 38	Provided hands on training for all technical staff on calibration, Biosafety and SIMS from NRL-NICED, RRL-NICED, NRLonQ-NICED, SRL of Orissa, Jharkhand, GMCGuwahati, GMC-Agartala and State AIDS Control Society of West Bengal, Orissa, Sikkim, A & N, Jharkhand.
ii) **EQAS workshop:** Venue : NICED, Kolkata Date : 14 July 2011 Participants : 09	Hands on training for quality assurance and testing of proficiency testing panel in the State Reference Labs of A&N, AS, JH, MG and OS.
iii) **EQAS workshop:** Venue : G. B. Pant Hospital. Port Blair, A & N Islands Date : 2-4 August 2011 Participants : 48	Hands on Training for Counselor, Lab-technician and Medical Officer from different ICTCs attached to G. B. Pant Hospital, Port Blair on Quality Assurance in ICTC, External Quality Assessment Scheme under NACP-III of NACO, Sample packaging & transporting.
iv) **Consortium Workshop:** Venue : NICED, Kolkata. Date : 20-22 Sept. 2011 Participants : 13	Panel Characterization workshop for Consortium members (NICED, Kolkata ; NIMHANS, Bangalore; NCDC, Delhi and NARI, Pune) for preparing new panel under NRLon Q for evaluation of diagnostic kit.
v) **EQAS workshop:** Venue : Silchar Medical College & Hospital, Silchar, Assam. Date : 31st October & 1st November 2011 Participants : 33	Hands on Training for Counselor, Lab-technician and Medical Officer from different ICTCs attached to SMCH, Silchar, on Quality Assurance in ICTC, External Quality Assessment Scheme under NACP-III of NACO, Sample packaging & transporting.
vi) **EQAS workshop & Panel Distribution:** Venue : NICED, Kolkata Date : 23 December 2011 Participants : 07	Hands on training for quality assurance and testing of proficiency testing panel in the State Reference Labs of A&N, AS, JH, MG and OS and distribution of proficiency testing panel to the SRLs.
vii) **Workshop for EID, West Bengal** Venue : NICED, Kolkata Date : 24th June, 2011 Participants : 57	Review cum Reorientation Training on Early Infant Diagnosis
viii) **Jharkhand** Venue : Jharkhand SACS Date : 7th July, 2011 Participants : 22	Review cum Reorientation Training on Early Infant Diagnosis.
ix) **Mizoram** Venue : Civil Hospital, Aizawl Date : 15th -16thDec., 2011 Participants : 25	Reorientation/Hands-on Training on Early Infant Diagnosis In Mizoram

Our objective is to generate awareness about inherent characteristics of the virus, detection, testing and necessary Prophylaxis. The trainings are conducted along these lines with emphasis laid on Good clinical laboratory practice & Bio-safety which is a pre-requisite ensuring wellness of any laboratory and its staff.

Conferences/ Seminars/Workshops /Trainings Attended/Organised

Posre presentation:

P Ghosh, S Biswas, M K Saha. Profile of clients tested positive in an integrated counselling and Testing centre of Kolkata, India at the 10th IntConf AIDS Asis Pacific (ICAAP 10) 2011, Busan, Korea.

nicer training

TRAINING ACTIVITIES

The Division of Training and Extension (a WHO collaborating center for research and training on diarrhoeal diseases) is actively engaged in the following salient activities in the reported (2011-12) period:

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

- Intercountry Consultation on Elimination of Kala-azar in the South-East Asia Region (WHO), during 9-10 November, 2011 at NICED, Kolkata. India.
- US-Japan Cooperative Medical Science Program Joint Panels on Cholera and Other Bacterial Enteric Infections at Hotel Hyatt Regency, Kolkata during December 13-15, 2011(Member, LOC).
- International Workshop on "Bacterial microbiome analysis using 16S-rRNA bacterial database" at NICED, Kolkata during December 16-20, 2011.
- Training of Final year BHMS students of National Institute of Homeopathy, Kolkata, (Orientation training programme),13th October,2011

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

- SAC meetings on 19-20th August, 2011 at NICED, Kolkata
- Biosafety committee (IBSC) meeting
- Organizing meeting for GCLP training 2011.
- IVI training programmes.
- Meeting of NACO from time to time through the year viz. induction training programmes for Medical Technologists
- Observance of National Science Day,2011
- 50th Anniversary celebration of NICED,18th February,2011
- Alumni Day celebration of NICED,18th February,2012
- Seminars and oration lectures organised by ISCA, Kolkata chapter & NICED, Kolkata
- Oration lecture of Indian Science Congress Association, Kolkata chapter
- NACO Regional Institute meeting for HIV surveillance
- Arrangement of meeting for Dr. Rita Colwell, Dr. Colin Stein, Dr Nozaki, DR. Alessandro Craviato etc
- NICED Scientific forum.
- Assistance for Annual session meeting of Calcutta University and National Academy of sciences, Allahabad.

II. Prepared the following documents for the institute:

- Compilation and submission of Annual report of WHO Collaborating Center for research and training on diarrhoeal diseases (2011-12).
- Highlight of the Institute for DBT
- Highlight of the Institute for ICMR Centenary celebration (Publication & Information directorate)
- Training modules of different workshops
- Document for the Institutional Scientific Audit
- WHO Workshop Planning & Budget
- Report of the Training Programme to WHO (SEARO)

III. Prepared following documents for ICMR Head Quarter:

- Highlights of the Diarrhoeal Diseases Research carried over by the Institute
- Highlights of the Institutional activities for ICMR Annual Report
- Reports of celebration of Technology day for ICMR
- Documents for foreign participation in R&D activities of the Institute
- Documents for National Institute of Science Communication & Information Research
- WHO training details with prospective budget estimate conducted by NICED
- Documents on activities undertaken by NICED in the context of global climate changes on diarrhoeal diseases and cholera.

IV. Submission of WHO-collaborating centre Annual report, administrative and A/C related documents for WHO and Institutional profile.

V. Training programmes:

- Training for M.Sc. Biotechnology of students from Bishwabharati University
- Training programme for students of National Institute of Homeopathy,13th October,2011
- Training programme for Microbiology students of Calcutta University.
- Training programme for Microbiology students of Kalyani University
- Training programme for M.Sc.(Zoology) students of Kalyani University, 2011.
- Training programme on Clinical Tropical Medicine, Optional Module for Master's programme in International Health.
- NACO workshops.

- Training Programmes of Immunization strengthening Project for mid level managers of the districts of Eastern states Andaman & Nicobar islands, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Orissa, Sikkim, Tripura, West Bengal as per programme schedules.

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

- For Indian Science Congress Association, Kolkata chapter for observance of Science Day, 2011.
- Meeting public health officials with dr. Eric Mintz, Dirrhoeal disease Epidemiology from CDC, Atlanta, West Bengal State AIDS prevention and control Society meeting on DBS and whole blood sample collection at NICED, Kolkata, coordinated by WBSAP&CS.
- For Indian Science Congress Association, Kolkata chapter for observance of World Environment Day.

Staff : A. Palit, Scientist "E" and I/C
Mr. R.J.Mukherjee, (Technical Officer-A)
Mr. A.Jana, (Technician -B)
Mr. A.Roy, (Technician -B)
Mr. S.Adhikary, (Attendant Services)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events

US Japan Cooperative Medical Science programme

The 46th Annual Joint Panel Meeting of the US-Japan Cooperative Medical Science Programme on Cholera & Other Bacterial Enteric Infections was held on 13-15 December 2011 in Kolkata. Organization of this meeting was an integral part of Golden Jubilee celebration by NICED (February 2011- February 2012) and Centenary Celebration of ICMR (November 2010 November 2011).

About 160 participants joined this meeting. The main focus of this International Conference was an opportunity to share the latest scientific information and lessons learned regarding enteric diseases, and also to discuss and identify potential areas for collaborative research.

Scientific Advisory Committee (SAC) Meeting

39th SAC meeting was held at NICED on 19-20 August, 2012 at the NICED. Prof. N. K. Ganguly was the chairperson of the meeting.

extramural
PROJECTS

EXTRAMURAL PROJECTS

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

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

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

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

3. **Title :** Elucidation and analysis of Biological Function(s) of *Helicobacter pylori* Restriction-Modification systems.

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

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

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

5. **Title :** Determine the Immune Response to Novel Conserved Shigella Protein Antigens in Patients with Recent Onset of Shigellosis.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

13. **Title :** Surveillance for Dengue Fever in Eastern Kolkata.

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

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

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

15. **Title :** Diarrheal Disease in Infants and Young Children in Developing Countries .

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

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

P.I.	:	Dr. D. Sur.

Funding agency : Bill and Melinda Gates Foundation.
Duration : 12 months.

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

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

18. **Title :** Technical Assistance Support to NACO – Baseline/Impact assessment study on HIV in IDUs in Punjab.

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

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

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

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

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

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

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

22. **Title :** Analysis of rotaviruses and their interactions with the host: A Viral Proteomics Approach.

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

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

P.I. : Dr. S. S. Das.
Funding agency : ICMR.

Duration : 2006 onwards.

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

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

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

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

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

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

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

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

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

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

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

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

publications

PUBLICATIONS

1. **Ali, M., D. Sur, D. R. Kim, S. Kanungo, S. K. Bhattacharya, B. Manna, R. L. Ochiai, and J. Clemens.** 2011. Impact of Vi vaccination on spatial patterns of typhoid fever in the slums of Kolkata, India. Vaccine **29**:9051-9056.

2. **Banerjee, R., A. Roy, F. Ahmad, S. Das, and S. Basak.** 2012. Evolutionary patterning of hemagglutinin gene sequence of 2009 H1N1 Pandemic. J.Biomol.Struct.Dyn. **29**:733-742.

3. **Barman, S., R. Kumar, G. Chowdhury, S. D. Rani, T. Wajima, T. Hamabata, T. Ramamurthy, N. G. Balakrish, Y. Takeda, and H. Koley.** 2011. Live non-invasive *Shigella dysenteriae* 1 strain induces homologous protective immunity in a guinea pig colitis model. Microbiol.Immunol. **55**:683-693.

4. **Barman, S., D. R. Saha, T. Ramamurthy, and H. Koley.** 2011. Development of a new guinea-pig model of shigellosis. FEMS Immunol.Med.Microbiol. **62**:304-314.

5. **Bhattacharya, M. K., T. N. Naik, M. Ghosh, S. Jana, and P. Dutta.** 2011. Pulmonary tuberculosis among HIV seropositives attending a counseling center in Kolkata. Indian J. Public Health 55:329-331.

6. **Bhattacharya, K., S. K. Niyogi, and S. K. Choudhuri.** 2011. Role of a novel copper chelate in modulation of resistance by time and dose-dependent potential on the growth of tetracycline-resistant *Vibrio cholerae* O1. Int.J.Antimicrob.Agents **38**:182-183.

7. **Bhattacharya, S. D., S. K. Niyogi, S. Bhattacharyya, S. Fitzwater, N. Chauhan, A. Sudar, and S. Mandal.** 2011. High rates of colonization with drug resistant *hemophilus influenzae* type B and *Streptococccus pneumoniae* in unvaccinated HIV infected children from West Bengal. Indian J.Pediatr. **78**:423-429.

8. **Bhuiyan, N. A., S. Nusrin, M. Ansaruzzaman, A. Islam, M. Sultana, M. Alam, M. A. Islam, A. Cravioto, A. K. Mukhopadhyay, G. B. Nair, J. C. Mwasna, and H. P. Endtz.** 2012. Genetic characterization of *Vibrio cholerae* O1 strains isolated in Zambia during 1996-2004 possessing the unique VSP-II region of El Tor variant. Epidemiol.Infect. **140**:510-518.

9. **Biswas, A., R. Panigrahi, A. Banerjee, M. Pal, B. K. De, S. Chakrabarti, and R. Chakravarty.** 2012. Differential pattern of pre-S mutations/deletions and its association with hepatitis B virus genotypes in Eastern India. Infect.Genet.Evol. **12**:384-391.

10. **Biswas, S., D.Lala, J.A.Teixeira da Silva, K.K.Sen, and M.K.Saha.** 2011. Development and Evaluation of Colonic Drug Delivery of Aceclofenac using Pectin and Guar Gum. Intl.J.Biomed.Pharma.Sci. **5**:79-84.

11. **Chakraborty, D. C., G. Mukherjee, P. Banerjee, K. K. Banerjee, and T. Biswas**. 2011. Hemolysin induces Toll-like receptor (TLR)-independent apoptosis and multiple TLR-associated parallel activation of macrophages. J.Biol.Chem. **286**:34542-34551.

12. **Chatterjee, R., A. Ghosal, S. Sabui, and N. S. Chatterjee**. 2011. Three dimensional modeling of C-terminal loop of CssA subunit in CS6 of enterotoxigenic *Escherichia coli* and its interaction with the 70 KDa domain of Fibronectin. Bioinformation. **6**:307-310.

13. **Chattopadhyay, D., H. Mukherjee, P. Bag, D. Ojha, A. K. Konreddy, 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.

14. **Chowdhury, G., G. P. Pazhani, G. B. Nair, A. Ghosh, and T. Ramamurthy**. 2011. Transferable plasmid-mediated quinolone resistance in association with extended-spectrum beta-lactamases and fluoroquinolone-acetylating aminoglycoside-6'-N-acetyltransferase in clinical isolates of *Vibrio fluvialis*. Int.J.Antimicrob.Agents **38**:169-173.

15. **Chowdhury, P., D. Pore, N. Mahata, P. Karmakar, A. Pal, and M. K. Chakrabarti**. 2011. Thermostable direct hemolysin downregulates human colon carcinoma cell proliferation with the involvement of E-cadherin, and beta-catenin/Tcf-4 signaling. PLoS.One. **6**:e20098.

16. **Clemens, J., S. Shin, D. Sur, G. B. Nair, and J. Holmgren**. 2011. New-generation vaccines against cholera. Nat.Rev.Gastroenterol.Hepatol. **8**:701-710.

17. **Das, M., R. K. Nandy, T. S. Bhowmick, S. Yamasaki, A. Ghosh, G. B. Nair, and B. L. Sarkar**. 2012. *Vibrio cholerae* typing phage N4: genome sequence and its relatedness to T7 viral supergroup. Intervirology **55**:185-193.

18. **Das, P., A. K. Singh, T. Pal, S. Dasgupta, T. Ramamurthy, and S. Basu**. 2011. Colonization of the gut with Gram-negative bacilli, its association with neonatal sepsis and its clinical relevance in a developing country. J.Med.Microbiol. **60**:1651-1660.

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20. **Deb, A. K., S. Kanungo, M. Deb, and G. B. Nair**. 2012. Impact of climate change on health and strategies for mitigation and adaptation. WHO South-East Asia J.Pub.Health **1** :8-19.

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23. **Dutta, P., U. Mitra, S. Dutta, K. Rajendran, T. K. Saha, and M. K. Chatterjee**. 2011. Randomised controlled clinical trial of Lactobacillus sporogenes (*Bacillus coagulans*), used as probiotic in clinical practice, on acute watery diarrhoea in children. Trop.Med.Int.Health **16**:555-561.

24. **Ghosh, A., M. Chawla-Sarkar, and D. J. Stuehr**. 2011. Hsp90 interacts with inducible NO synthase client protein in its heme-free state and then drives heme insertion by an ATP-dependent process. FASEB J. **25**:2049-2060.

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administration

ADMINISTRATION

Administration provides operational support to the Office of the Director through activities, which include procurement and purchase of equipments, chemicals and stationery, fixing of fiscal responsibilities, budget preparation and execution, personnel administration, mailroom functions and supplies and, in short, for the management of human and material resources of the Institute. The primary objective of the Administration of NICED, as in any other research organization is to promote and ensure smooth and uninterrupted execution of the research mandate of the Institute.

Administration performed the following tasks:

- Supervision and coordinate of staff activities
- Recruitment of staff
- Conduct orientation programs for new employees
- Disbursement of salaries and maintenance of leave records
- Preparation of maintenance of budgetary and inventory controls and make recommendations to management
- 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

We have already implemented the following.

- New Pension system in NICED

We are going to implement the following facility.

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.

The Institute is receiving liberal assistance from different Government, non-Government and International Agencies, e.g., IVI, WHO, DST, DBT, CSIR, CDC etc. for conducting more than 60 extramural projects along with Okayama project. Two new buildings have also been built up in I.D. & B.G. Hospital campus under the Institute. The load of work for Administration has tremendously expanded which we have had to manage with our existing staff Over 60 and above Extramural Projects are going on. The total workload is carried out by the Administrative staff of the Institute.

(Subodh Karmakar)
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