वार्षिक रीपोर्ट

Annual Report

2013-2014

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राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

भारतीय आयुर्विज्ञान अनुसंधान परिषद्

National Institute of Cholera and Enteric Diseases

Indian Council of Medical Research

WHO Collaborating Centre for Research and Training on Diarrhoeal Diseases

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P-33, C I T Road, Scheme-XM, Beliaghata, Kolkata-700 010, India

From the Director's Desk

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

Apart from conducting research, NICED has been instrumental in generating trained human resources in scientific fields. Many students obtain their Ph.D. degree from various universities of high repute, by working in the different laboratories of NICED. A thorough and holistic training makes them capable to conduct independent research by themselves in future. Apart from this, a good number of doctors, health practitioners, and Government officials attached with various State Health Systems also get trained through workshops and seminars - events that this Institute conducts on regular basis. Recently, the institute has started collaboration with the Indian Medical Students' Association (IMSA) in organizing Workshops which offers orientation training for research methodologies and ethics as well as exposure to various laboratories of NICED that can be instrumental in creating a critical mass of medical researchers in the country.

I truly acknowledge the sincere contributions from scientists, research fellows and the administrative staff of NICED that have made all these aforementioned achievements possible. Continuous support from the Council and able guidance from the Director General of ICMR has not only helped us in taking this journey but also to sustain good quality research work. At the very last, I would also like to extend my sincere gratitude towards the various national and international funding agencies whose support has helped us undertake various research projects.

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

निर्ईशक की मेज से

यह मेरे लिए खुशी की वात है कि मैं पिछले दो सालों से नाइसेड के विकाश का साक्षी हूँ। इस संस्थान की शुरूआत किराए के चार कमरों के घर से हुई थी जो सेंट्रल कोलकाता में केन्द्रित था। इसके बाद में संस्थान परिवर्तित होकर शहर के उत्तर पूर्वी हिस्से में बेलियाघाटा नामक जगह पर आगया। संस्थान धीरे-धीरे ही स्वास्थ्य अनुसंधान विभाग गर्वमेन्ट ऑफ इण्डिया के तहत ''मेडिकल रिसर्च की भारतीय परिषद'' के प्रीमियर अनुसंधान केन्द्रों में से एक के रूप में अपने आप को स्थापित किया है। हाल ही में नाइसेड एक आधुनिक पशुघर सहित अत्याधुनिक अनुसंधान सुविधाओं के साथ सुसज्जित है। जनादेश के अनुसार नाइसेड का विस्तार किया गया है जिसमें ऑत्ररोग, वैक्सीन रिसर्च के क्षेत्र में अनुसंधान प्रशिक्षण समाज सेवाए शामिल है। एच.आई.वी एडस और अन्य उभरते वायरल रोगों को राष्ट्रीय हित को ध्यान में रखते हुए शामिल किया गया है। मल्टीडीसीप्लीनरी जैसे जन पादिक रोग विज्ञान निष्कर्षो से साथ बेसिकरिसर्च के क्षेत्र में वैक्टेरियोलोजी, भाईरोलोजी, पैरासाइटोलोजी, इम्युनोलोजी, मैलिक्यूलर वाइलोजी और क्लीनिकल मैडिसिन के क्षेत्रों में इन वर्षों में हमारी बहुत मजबुत पकड़ रही है। संक्रमण को दूर करने के लिए और संचालन नियम के विकाश के लिए अलग-अलग तरीके सोचे हैं ताकि उन लोगों की देख भाल अच्छी तरह से हो सके जिन को पहले से संक्रमण है इस पर भी हमारा विशेष ध्यान रहा है।

अनुसंधान आयोजित करने के अलावा नाइसेड वैज्ञानिक क्षेत्रों में प्रशिक्षित मानव संसाधन पैदा करने में महत्व पूर्ण भूमिका निभाई है। विभिन्न विश्वविद्यालयों से आये पीएचडी छात्रों को उनकी पीएचडी डिग्री प्राप्त करने में नाइसेड की विभिन्न प्रयोग शालाओं में काम करके अच्छी ख्याति प्राप्त की है। जो कि यह एक गहन और समग्र प्रशिक्षण उन्हे स्वयं द्वारा भविष्य में स्वतन्त्र अनुसंधान का संचालन करने के लिए सक्षम बनाता है। इसके अलावा डाक्टरों, स्वास्थ्य चिकित्सकों तथा विभिन्न राज्य स्वास्थ प्रणालियों के साथ संलग्न सरकारी अधिकारियों की एक अच्छी संख्या भी कार्यशालाओं और सेमीनारों द्वारा नियमित आधार पर आयोजित कर इस संस्थान के माध्यम से प्रशिक्षित किया जाता है। हाल ही में संस्थान द्वारा भारतीय मैडिकल एसोसिएसन के छात्रों के साथ मिलकर कार्यशालाओं के आयोजन शुरू कर दिए हैं जिन में अनुसंधान के तरीके और नैतिकताविभिन्नप्र योगश ालाओंके लिएज ोखिमके लिएअ भिविन्यास प्र शिक्षणप्र दानक रताहै ।जो दे शमें मैडिकल शोधकर्ताओं को एक महत्वपूर्ण जन बनाने में महत्वपूर्ण भूमिका होगी।

मैं सही मायने में मानता हूँ कि वैज्ञानिकों, अनुसंधान अध्येता और नाइसेड के प्रशासनिक कर्मचारीगणों के योगदान से ही हमारी पूर्व कथित उपलब्धियों को संभव बना दिया है। परिषद से निरंतर सहायता और भारतीय आर्युविज्ञान अनुसंधान परिषद के महानिदेशक का निरंतर समर्थन और सक्षम मार्गदर्शनस ेहीअ च्छीग ुणबत्ताके श ोधक ेानाएर खनेकी इ सयात्रामें ह मेंम ददमिलीहै ।अंतमेंमैं र ाष्ट्रीय औरअ ंत्रराष्ट्रीयवित्तीयप ोषणए जेंसीओंक ाभीस ामर्थनमिलाहै जिससेह मेंविभिन्नअ नुसंधानप ारियोजनाओं का कार्य करने में सहायता मिली है। इस सब के लिए मैं सभी का आभारी हूँ।

डः शेखर चक्रवर्ती
पीचडी, एफएनए, एफएनएएससी,
एफएएससी और टी
प्रभारी निदेशक

CONTENTS

RESEARCH ACTIVITIES

Bacteriology

The influence of different physico-chemical factors on the abundance of enteropathogens in river Ganges have been investigated around the cholera endemic regions of West Bengal. The influence of salinity variation with tidal cycles, sediment re-suspension from tidal cycle plays a crucial role on abundance of vibrios in this ecosystem. Significant association ($p<0.05$) between cholera cases and riverine *Vibrio cholerae* O1 abundance was determined, which suggests the role of riverine–estuarine ecosystem in cholera transmission. It was also established that among multiple hosts in the aquatic milieu, enteropathogenic vibrios prefer to colonise on crabs and acquire their virulence during the attachment phase under the influence of chitin.

Several metabolic pathwaysinvolved in *V. cholerae* pathogenesis, have been identified and characterized. The Entner Doudoroff (ED) pathway was identified as obligatory for gluconate (Gnt) utilization by *V. cholerae*, which was also found to be associated with its pathogenesis. Enzymes involved in this pathway are now being targeted for the development of newer drugs/small molecule inhibitors for treatment of cholera infection.

Being the national reference centre, an average 1000 strains of *V. cholerae* are sent to us from 30-40 institutions for biotyping, serotyping and phage typing per year. A study on phage therapy was initiated in response to the emergence of drug resistant *V. cholerae*. The animal study showed significant reduction of *V. cholerae* counts in the gut after inoculation of phages in appropriate doses.

Salmonella enterica serovar Typhi (S. Typhi) has been implicated as a common aetilogical agent for enteric fever in and around Kolkata as compared to S. Paratyphi A. Majority (90%) of S. Typhi and S. Para A isolates were resistant to fluoroquinolones, especially ciprofloxacin, Antimicrobial susceptibility results indicated the usefulness of azithromycin in the treatment of typhoid. Rate of isolation of multidrug resistant (resistant to ampicillin, chloramphenicol, cotrimoxazole) isolates declined over time (~9%). Studies on typhoid diagnostic showed that typhoid diagnosis may be improved by direct detection of typhoid specific genes by qPCR with overall efficiency of 93.6%. When Kolkata isolates were subjected to molecular typing, *Xba1*-PFGE profiles of of S. Typhi showed clonal relatedness with their antimicrobial resistance profiles. The other sequence based molecular typing techniques with better discriminatory ability were also employed to elucidate the variability among isolates having similar pulsotypes.

Shigella spp. secretes lesser extent of outer membrane vesicles (OMVs) during their growth. To increase the in vitro production of OMVs, tolA gene, which was necessary to maintain outer membrane integrity, was disrupted from S. boydii type 4 BCH612 strain. An increase of OMVs secretion was noticed (80mg/500ml culture) by 60% in the tolA mutant in comparison to the wild type (50mg/500ml culture). Additionally, ΔtolA-OMVs played an important stimulatory role on macrophage and epithelial cells by inducing more IL-8, TNF-α, IL-1β, IL-12, IL-18, IL-6, IL-10, IFN-γ and IL-4 secretion than the wild type OMVs. The designated cytokine profile has clearly elucidated the Th1/Th2 based immune response by OMVs of *Shigella*. The ΔtolA-OMVs showed valuable role in the development of next-generation non-living vaccines against shigellosis due to their enhanced immunogenicity than the wild type Shigella OMVs.

Helicobacter pylori, a major cause of peptic ulcers and an early risk factor for gastric cancer is another research focus of this division. Of special interest, are indications that *H. pylori* infection probably increases susceptibility to/or the severity of infection by *V. cholerae*. Conversely, *V. cholerae* infection may facilitate

H. pylori transmission. A simple PCR based assay system for quick detection of hybrid variant *V. cholerae* O1 strains was established, which will help to track the dissemination of these strains.

An emergence of carbapenem-resistant bacteria associated with neonatal gut flora and septicemia necessitated the evaluation of resistance to other antibiotics such as aminoglycosides and fluoroquinolones.New Delhi Metallo- β- lactamase -1(NDM-1) was the only carbapenemase identified in *Enterobacteriacae* along with the other genes such as *rmt*B and *rmt*C for aminoglycoside resistance and *aac(6')-Ib-cr* for fluroquinolone resistance. Most of the isolates were proficient in the transfer of bla*NDM-1* suggesting the potential for its quick spread.

Studies on the prevalence of the virulent clone of *Escherichia coli*, ST-131 in the neonatal gut and septicemic isolates were carried out. Identification of ST131 was low both in the gut and septicemic isolates in comparison to the high percentage of CTX-M-15 producers, which indirectly indicated that the clonal spread of ST131 did not contribute to the high prevalence of CTX-M-15 in Kolkata.

Vibrio parahaemolyticus isolated from the acute diarrhoeal patients admitted in the Infectious Diseases Hospital showed that the frequency of pandemic strains was more than the non-pandemic strains. Serovars O3:K6, O1:K25, O1:KUT were more commonly found. The other serovars such as O3:KUT (6.7%), O4:K8 (6.7%) and O2:K3 (4.5%) were newly detected. The pandemic strains formed two different clades in the PFGE analysis, with one containing the newly emerged pandemic strains in this region.

Heterogenic virulence was detected in an enteropathogenic *Escherichia coli* (EPEC) that expressed heat-labile toxin, specific for the enterotoxigenic *E. coli* (ETEC). This strain harboured *eae* and *elt* genes encoding for attaching and effacing property and heat-labile enterotoxin of EPEC and ETEC, respectively. The *elt* gene was identified in a conjugally transferable plasmid. This plasmid revealed presence of six contigs with the presence of several insertion sequences. A phage integrase gene and the prophages of gp48 and gp49 were detected in the upstream of *elt*AB and in the downstream an urovirulence loci adhesion encoding (*pap*) cluster containing *pap*G, and *pap*C were also identified.

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Awards:

- Sanjucta Dutta received Ph.D. degree from Jadavpur University, Kolkata in 2013
- Suman Nandy received Ph.D. degree from Jadavpur University, Kolkata in 2013.
- Subhasree Roy received Ph.D. degree from University of Calcutta, Kolkata in 2013
- Devarati Dutta received Ph.D. degree from University of Calcutta, Kolkata in 2014.
- Soma Mitra received Ph.D. degree from University of Calcutta, Kolkata in 2014.

Surveillance on diarrheal enteropathogens

Investigators: T. Ramamurthy, M.K. Bhattacharya, B. Manna, T. Krishnan, S. Ganguly

From April 2013 to March 2014, a total of 1226 and 1254 stool samples were collected from the Infectious Diseases Hospital (IDH) and B. C. Roy Memorial Hospital for children (BCRH), respectively. In children below 5 years of age, prevalence of rotavirus was found to be common in both the hospitals (~48%). *Vibrio cholerae* 01 (20%) and *Campylobacter* spp (4.3%) *Vibrio fluvialis* (3.2%) were more in the IDH. In the BCRH, prevalence of adenovirus (15.3%), *Campylobacter jejuni* (13.8%), enteroaggregative *Escherichia coli* (5.6%) and *Shigella* spp (5%) were high. Vibrios remained susceptible for most of the fluoroquinolones. In both the hospitals, most of the *Shigella* strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone. NDM-type carbapenemases were detected in 27 strains of *Vibrio fluvialis* strains isolated from 2011-2013. All these NDM-positive strains were susceptible to azithromycin.

Evaluation and Comparison of Performances of Diagnostic PCRs (Nested and Real time) for Typhoid Fever

Investigators: S. Dutta, S. Das, U. Mitra, D.K. Paul

Rapid diagnosis of typhoid is mandatory for early antibiotic therapy and reducing the mortality. The conclusive diagnosis of typhoid fever by isolation of *Salmonella Typhi* (S. Typhi) in blood culture suffers from poor sensitivity. PCR-based amplification of *S. Typhi* DNA directly in blood has shown the most promising result. The flagellar gene (*fli*C-d) specific for S. Typhi is used as PCR target. The nested PCR (nPCR) and Real-time PCR (qPCR) approach significantly improved the detection rate, but thorough evaluation of the methods is necessary before these are being used routinely. The study was undertaken to evaluate and compare performance ability of various PCR methods (nPCR and qPCR) in our laboratory for typhoid diagnosis.

Analytical sensitivity and specificity of both the PCR methods were determined following standard procedure. A Ct value of ≤ 35 cycles in qPCR was considered positive. The nested PCR was performed using two pairs of primers amplifying *fliC*-d gene with the final product size 343bp. For qPCR the primers and probes were designed based on available *fli*C-d gene sequences of *S. Typhi* CT18 with the product size 156bp. Performances of culture, nPCR and qPCR were determined in the real situation by direct application of the methods on 110 blood samples collected from children with clinically suspected typhoid fever (CSTF). Detection limits of nPCR and qPCR were 0.012pg and 0.015pg of DNA respectively. Considering blood culture as gold standard (n=24), qPCR showed better (79% vs 75%) sensitivity than nPCR and both the methods were 96.5% specific. qPCR was rapid than nPCR (2hr vs 7hr). Performances of culture, nPCR and qPCR were determined considering CSTF cases (n=81) positive for typhoid by any method as true positives and lab confirmed non-typhoidal cases (n=29) as true negatives (Table 1). The sensitivities of the three methods were 29.6%, 74%, 79%, and specificity were 100%, 96.5% and 96.5% respectively (Table 2). The application of qPCR and nPCR directly in blood were comparable for typhoid diagnosis although qPCR is a rapid method.

Table 1. Comparison of results of blood culture, nPCR and qPCR assays for typhoid diagnosis on blood samples collected from 110 clinically suspected typhoid fever cases

Sample categories	Test result and total no.(%) with a positive result in the respective tests			Patient group/no. of cases (%) with suspected typhoid
	Culture	nPCR	qPCR(Ct=35)	
1	+	+	+	16(19.8)
2	+	-	-	3(3.7)
3	-	+	+	30(37)
4	-	-	+	15(18.5)
5	+	+	-	2(2.5)
6	-	+	-	12(14.8)
7	+	-	+	3(3.7)
Total	24(29.63%)	60(74.07%)	64(79.01%)	81(100)
				Non-typhoid[a]
1	-	+	+	1 (3.4)
2	-	-	-	28 (96.6)
Total	0	1 (3.4%)	1 (3.4%)	29 (100)

[a] Confirmed dengue fever (8 cases), malaria (5 cases), culture-positive (*S. Paratyphi* A:2, *Acinetobacter* spp: 2, *Pseudomonas* spp: 2, *Klebsiella* spp: 1) and negative by all tests performed (9 cases) were considered as controls.

Table 2. Determination of diagnostic performances of blood culture, nPCR and qPCR assays for typhoid fever on blood samples collected from 110 clinically suspected typhoid fever cases

Tests	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)	LR+ (95% CI)	LR- (95% CI)	Efficiency (%)
Culture	29.63 (20.0-40.81)	100 (87.94-100)	100 (85.62-100)	33.72 (23.88-44.72)	α	0.70 (0.61-0.81)	48.18[a]
nPCR	74.07 (63.14-83.18)	96.55 (82.17-99.42)	98.36 (91.17-99.73)	57.14 (42.21-71.17)	21.48 (3.12-148.03)	0.27 (0.18-0.39)	80.0[b]
qPCR(=35)	79.01 (68.53-87.27)	96.55 (82.17-99.42)	98.46 (91.69-99.74)	62.22 (46.54-76.22)	22.91 (3.33-157.73)	0.22 (0.14-0.33	83.64[a, b]

[a] $p < 0.0001$ using McNemer test.
[b] $p > 0.5$ using McNemer test.

81 CSTF cases positive by any of the three methods was considered as true positive 29 CSTF cases (20 lab confirmed non typhoid cases and 9 cases negative by all tests) were considered as true negative.

Molecular characterization of serologically atypical provisional serovars of *Shigella* isolates from Kolkata, India

Investigators: S. Dutta, P. Jain, S. Nandy

During 2000-2004, thirteen *Shigella* strains, untypable by commercially available antisera, had been isolated from children <5 yrs of age with acute diarrhea in Kolkata, that were subsequently identified as *Shigella dysenteriae* provisional serovar 204/96 (n=3), *Shigella dysenteriae* provisional serovar E23507 (n=1), *Shigella dysenteriae* provisional serovar I9809-73 (n=1), *Shigella dysenteriae* provisional serovar 93-119 (n=1); *Shigella flexneri* provisional serovar 88-893 (n=6) and *Shigella boydii* provisional serovarE16553 (n=1). In this study, characterization of those provisional serovars of *Shigella* was carried out with respect to their antimicrobial resistance, plasmids, virulence genes and PFGE profiles. The drug resistant study strains (n=10) of *Shigella* possessed various antibiotic resistance genetic markers like *catA* (for chloramphenicol resistance); *tetA* and *tetB* (for tetracycline resistance); *dfrA1* and *sul2* (for co-trimoxazole resistance); *aadA1*, *strA* and *strB* (for streptomycin resistance) and *blaOXA-1* (for ampicillin resistance) (Table). Both class 1 and/or class 2 integrons were present in the resistant strains although their gene cassettes could not be determined. Three study strains were pan susceptible. A single mutation in the *gyrA* gene (Serine-83- Leucine) was present in quinolone resistant four strains. Virulence gene like ipaH (invasion plasmid antigen H) was uniformly present in all the study strains, but *stx* (Shiga toxin) and *set1* (*Shigella* enterotoxin 1) genes were absent. Other virulence genes like ial (invasion associated locus) and *sen* (*Shigella* enterotoxin 2) were occasionally present. Large plasmid of 212kb size and of incompatibility type *Inc*FIIA was present in majority of the study strains (n=10) and diversity was noticed in the smaller plasmid profiles of these strains even within the same provisional serovars. PFGE profile analysis showed presence of multiple unrelated clones among the isolates of provisional *Shigella* serovars (Figure). To the best of our knowledge, this is the first report on the phenotypic and molecular characterization of the provisional serovars of *Shigella* isolates from Kolkata, India.

Sl. No.	*Shigella* provisional serovars (current designation)	No. of isolates	Sample No.	Year of isolation µg/ml)*	R-profile (MIC in	Genes mediating antimicrobial resistance#	Mutations in the QRDR of *gyrA*¶	Presence of Integron (approximate size of gene cassette in kb)	Presence of heavy plasmid (212kb)	Plasmid type by PCR	Virulence genes
1	*S. boydii* E16553 (*S. boydii* serotype 19)	1	BCH-6347	2000	T(96), Q(>32), S(96), Na(>256)	*tetA, dfrA1, sul2, strA-B, aadA1*	S83L	Class 2 (1.3)	present	IncFIIA	*ipaH, ial, sen*
2	*S. dysenteriae* 204/96	3	BCH-2588	2000	T(96),S(96) Q(>32),	*tetB, dfrA1, sul2, strA-B*	nil	Class 2(1.3)	present	IncFIIA	*ipaH, ial, sen*
			BCH-3824	2001	A(>256), C(>256),T(96), Q(>32), S(48)	*blaOXA-1, dfrA1, aadA1 catA, tetB,*	nil	Class 1 (0.5 and 2.5) Class 2(1.3)	present	IncFIIA	*ipaH, ial, sen*
			BCH-4318	2002	T(128), Q(>32), S(96)	*tetB, dfrA1, sul2, strA-B*	nil	Class 2 (1.3)	present	IncFIIA	*ipaH, ial, sen*
3	*S. dysenteriae* 93-119	1	BCH-2834	2000	T(96),Q(>32) S(128)	*tetA, sul2, strA-B*	nil	nil	present	IncFIIA	*ipaH, ial, sen*
4	*S. dysenteriae* E23507 (*S. dysenteriae* serotype 15)	1	BCH-3853	2001	Pan susceptible	nil	nil	nil	present	IncFIIA	*ipaH, ial, sen*
5	*S. dysenteriae* I9809-73 (*S. dysenteriae* serotype 13)	1	BCH-4139	2001	A(>256), C(>256), Q(>32), T(96), S(48)	*blaOXA-1, catA, tetB, dfrA1, aadA1*	nil	Class 1 (0.5 and 2.5), Class 2 (1.3)	present	IncFIIA	*ipaH, ial*

Sl. No.	*Shigella* provisional serovars (current designation)	No. of isolates	Sample No.	Year of isolation µg/ml)*	R-profile (MIC in	Genes mediating antimicrobial resistance#	Mutations in the QRDR of *gyrA*¶	Presence of Integron (approximate size of gene cassette in kb)	Presence of heavy plasmid (212kb)	Plasmid type by PCR	Virulence genes
6	*S. flexneri* 88-893 (*S. flexneri serotype* 7b)	6	BCH-4191	2002	C(>256), T(96), Q(>32) S(128), Na(>256)	*catA, tetB, dfrA1, aadA1*	S83L	Class 1(0.5)	present	IncFIIA	*ipaH, ial, sen*
			BCH-4285	2002	pan susceptible	nil	nil	nil	absent	untypable	*ipaH*
			BCH-5435	2003	Na(>256)	nil	S83L	nil	present	IncFIIA	*ipaH*
			BCH-5449	2003	A(>256), C(>256), T(96), S(96)	*blaOXA-1, catA, tetB, aadA1*	nil	Class 1 (0.5 and 3.5)	absent	untypable	*ipaH*
			BCH-5863	2004	pan susceptible	nil	nil	nil	absent	untypable	*ipaH, ial*
			BCH-6141	2004	C(>256), T(96), Q(>32), S(128), Na(>256)	*catA, tetB, dfrA1, sul2, aadA1*	S83L	Class 1 (0.5)	present	IncFIIA	*ipaH, ial, sen*

*A, ampicillin; C, chloramphenicol; Na, nalidixic acid; Q, co-trimoxazole; S, streptomycin; T, tetracycline

#*bla*OXA-1, ampicillin resistance; *cat*A, chloramphenicol resistnce; *tet*B, tetracycline resistance; *dfr*A1, co-trimoxazole resistance; *sul2*, co-trimoxazole resistance; *aadA1*, streptomycin resistance

¶S, serine; L, leucine

Isolate no.	Serotype	Year of isolation	R-profile	PFGE Pulsotype
BCH-2588	S. dysenteriae 204/96	2000	TQS	P1
BCH-4318	S. dysenteriae 204/96	2002	TQS	P2
BCH-3824	S. dysenteriae 204/96	2001	ACTQS	P3
BCH-2834	S. dysenteriae 93-119	2000	TQS	P4
BCH-4139	S. dysenteriae I9809-73	2001	ACTQS	P5
BCH-3856	S. dysenteriae E23507	2001	pan susceptible	P6
BCH-6347	S. boydii E16553	2000	TQSNa	P7
BCH-5435	S. flexneri 88-893	2003	Na	P12
BCH-4285	S. flexneri 88-893	2002	pan susceptible	P8
BCH-5449	S. flexneri 88-893	2003	ACTS	P9
BCH-5863	S. flexneri 88-893	2004	pan susceptible	P10
BCH-5909	S. flexneri 2a	2004	ACTQSNaCipNorOfxAmc	P13
BCH-6023	S. flexneri 2a	2004	ACTQSNaCipNorOfxAmc	P14
BCH-6152	S. flexneri 2a	2004	ACTQSNaCipNorOfxAmc	P15
BCH-6037	S. flexneri 2a	2004	ACTQSNaCipNorOfxAmc	P16
BCH-4191	S. flexneri 88-893	2002	CTQSNa	P11
BCH-6141	S. flexneri 88-893	2004	CTQSNa	P11

Figure. PFGE profiles of *XbaI* digested DNA of sporadic isolates of provisional serovars of *Shigella* by cluster analysis and comparison with sporadic *S. flexneri* 2a isolates, Kolkata, India, 2000-2004.

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

Investigator: A. Palit

Physico-chemical parameters:

1. During study period, water temperature varied between 17.1^{o}C to 37.2^{o}C, with a mild variation in water temperature has been observed in monsoon season at both sites. pH level of the water samples varied between 7.1 to 8.7.
2. Highest salinity was recorded at Frasergunj ranging between 11.2-29.5 PSU, followed by Kakdwip site (3.6-15.8) and Diamond Harbour (0.0-2.4 PSU). Summer months with a higher wind effect and tidal influence facilitate the higher intrusion of marine saline water that help to increase the salinity level of that riverine aquatic environment. On the other hand, the monsoon time refers to the flood like situation as a result of which salinity level is diminished.
3. Turbidity varied between 35 NTU to 550 NTU (at Howrah Bridge), 40 NTU to 900 NTU (at Diamond Harbour), 128 NTU -563 NTU at Kakdwip and 207 NTU – 484 NTU at Frasergunj. While Howrah site had its peak in monsoon season, Diamond Harbor and Kakdwip site showed its peak in summer months. Lower turbidity at Frasergunj justify the higher salinity.

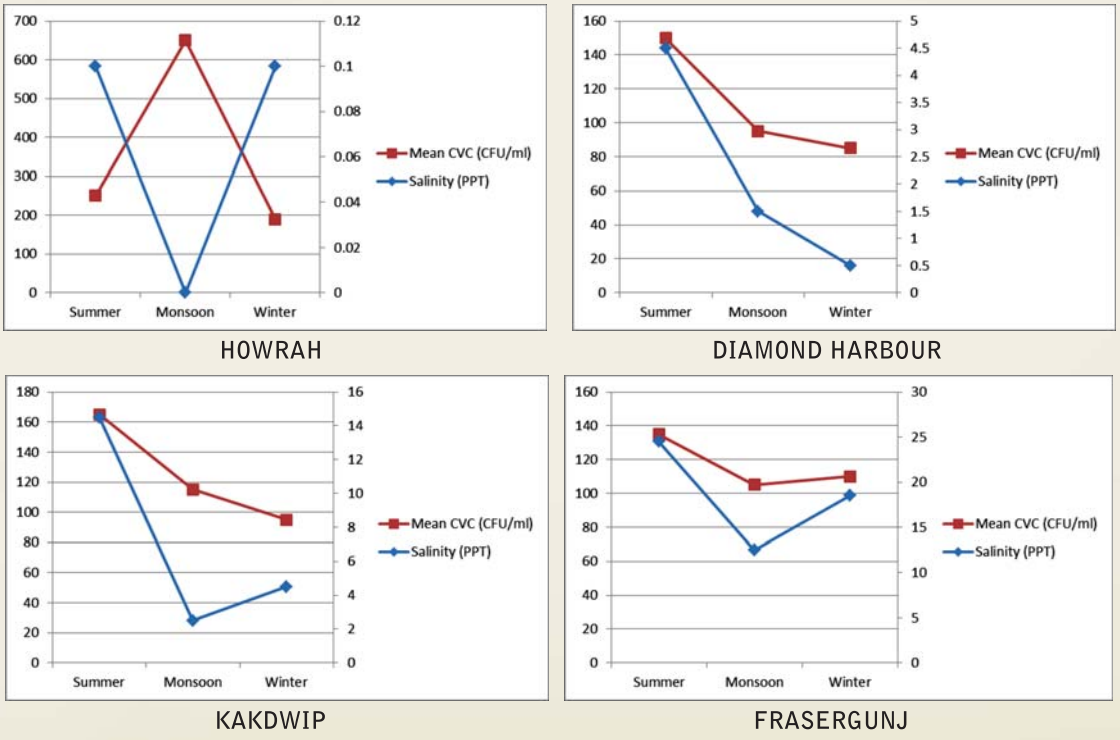


Fig-1. Salinity vs. CVC at four study points

Bacterial Preponderence:

- Inland (Howrah Bridge and Diamond Harbour) bacterial load was upto 10^{7} cfu/mL, estuarine environment had its bacterial preponderence upto 10^{5}cfu/mL. A trend of increase in TBC has been noted at the inland sites during late summer and pre monsoon, where as TBC at the sites nearer to the sea mouth increased during the summer months with the increase in the water remperautre and the intrusion of sea water.
- TCC (Total coliform count) varied between 1cfu/mL to 6500cfu/mL at all study sites with a highest load at Howrah (200 to 6500cfu/mL) and lowest at Frasergunj (1 to 10 cfu/mL).

Surrounding locality, geographical position (away from seamouth), sustained higher anthropogenic intrusion are the major factors for this higher coliform disposition at Howrah Bridge site. The fecal coliform concentration was high at Howrah site (10-4000cfu/mL) than that of other sites (1-90cfu/mL).

- CVC (Cultivable vibrio count) ranged between 1-1000cfu/mL at all the sampling sites, with a higher disposition at Howrah Bridge. The highest CVC disposition at Howrah indicate the factor of indiscremenate sewage disposal along with chunk of microbial pool. At Howrah site we observed a higher peak at "rainy" season in comparison to estuarine sites, where the peak has been observed at "summer" season.

Isolation and Identification of different Vibrio species

I. *V. cholerae, V. parahaemolyticus* seems to be the most prevalent species among all other *Vibrio* organisms.

II. Although higher prevalence of *V. parahaemolyticus* has been detected in high saline (≥15ppt) region or nearer to the sea mouth, *V. cholerae* is predominant in transition zone (3-15ppt) as well as in low saline (≤3ppt) region.

III. *V. mimicus, V. alginolyticus, V. vulnificus* (other than *V.cholerae* and *V.parahaemolyticus*) has also been identified from different zones.

Isolation and Identification of different *Vibriophages*

Altogether 14 samples were positive for vibriophages varied between 1 to 19pfu/mL. Vibriophage preponderance has been increased along with water temperature (during summer) and reached at its peak during monsoon.

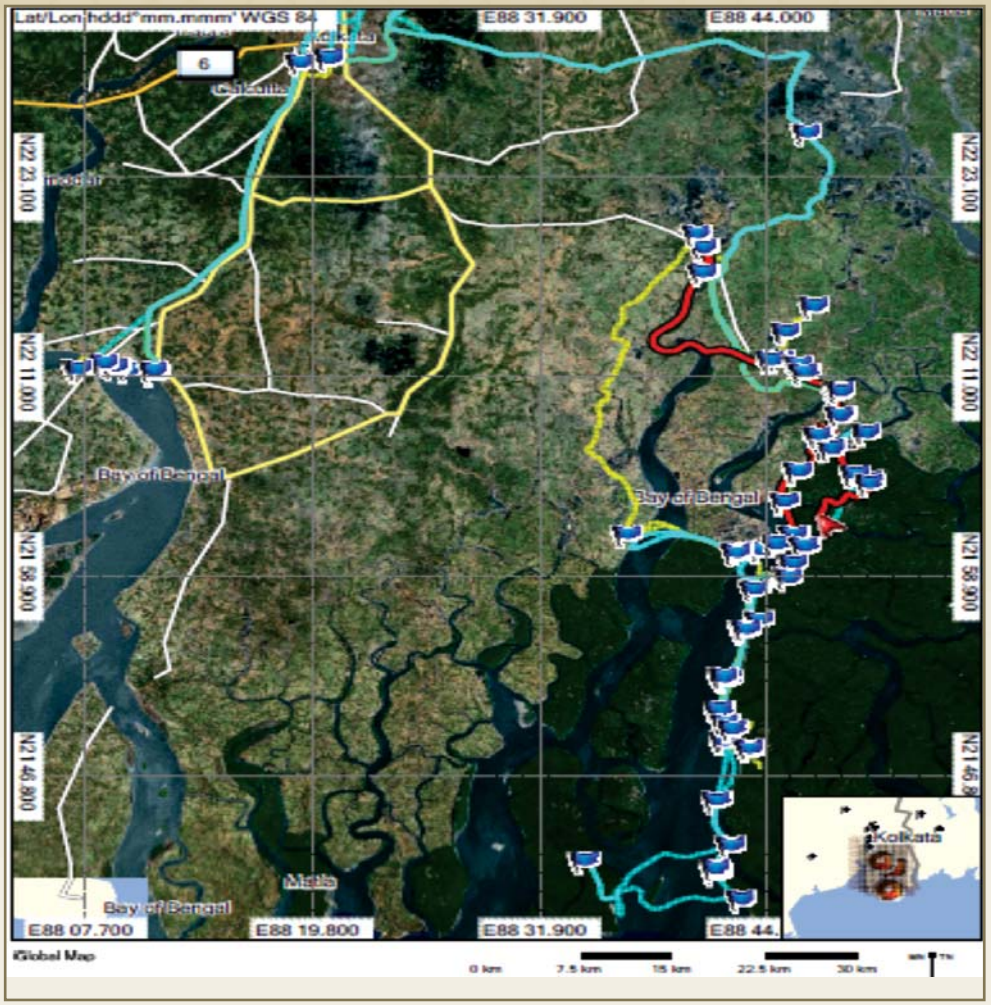
Interpretation:

Seasonal variations coupled with atmospheric oscillations, tidal amplitudes (spring tide, neap tide, etc.), lunar cycle, associated physico-chemical changes are the environmental determinants which are significantly related to the disposition of the Vibrios in particular and the total bacterial preponderance as an entity. The abundance of *Vibrio* and TBC also showed a positive dependence on turbidity which can be influenced by tidal regime, turbulence and flood water runoff. There is a correlation between the seasonal variation patterns of *Vibrio* and its phages in intertidal surface sediments and in surface waters in the middle of the estuary.

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

Investigators: A. Palit, B.L. Sarkar

A total of 148 samples collected from two sites of the Hooghly River (Howrah and Diamond Harbour) were analyzed physico-chemically along with cultivable Vibrio count (CVC), *V. cholerae* 01/0139 and vibriophages. *V. cholerae* 01 was detected in 57 (39% approx.) samples, while 33 (22.6 %) were positive for *V. cholerae* 01 phages. Flood tide,water temperature (31±1.6 °C), and turbidity (=250 nephelometric turbidity unit (NTU) significantly stimulated *V. cholerae* and vibriophage abundance in riverine ecosystem. Solitary existence of *V. cholerae* 01 and phages ($p<0.0001$) in aquatic environment divulges the dominance of either of the entity (*V. cholerae* 01 or *V. cholerae* 01 Φ) on the other. Significant association ($p<0.05$) between Kolkata cholera cases and *V. cholerae* 01 in aquatic environment implies the role of riverine–estuarine ecosystem in cholera transmission.



Interpretation:

A "biomonitoring tool" of physico-chemical stimulants, tidal, and climatic variants has effectively been proposed collating *V. cholerae* and phage dynamics that can forewarn any impending cholera outbreak.

Nationwide screening of phage types of *V. cholerae* 01 and 0139.

Investigator: B.L. Sarkar

The strains of *V. cholerae* sent to us from different institutes across the country. A total of 645 strains of *V. cholerae* were received from different parts of the country during the current year for serotyping, biotyping and phage typing. All the 645 (100 %) strains were confirmed as *V. cholerae* 01 biotype El Tor was included in phage typing study. This year, highest number of strains was received from Maharashtra state. Majority of the strains belonged to Ogawa 576 (89.03 %) followed by Inaba 69 (10.69 %). This year, Inaba strains were more compare to previous year. A total of 40 (6.2 %) strains were found to be untypeable with the conventional scheme Basu and Mukherjee scheme. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (64.03%) followed by type 26 (5.42 %), type 20 (5.73 %), type 23 (4.49 %) and type 7 (2.94 %) respectively. The new phage type encountered were type 7 and type 20 which were absent last year. It has been shown that type 27 was the predominant phage type circulating in this country. During the current year, we did not receive a single strain of *V. cholerae* 0139 for phage typing study from any parts of the country.

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

Investigator: B.L. Sarkar

A total of 151 *V. cholerae*, biotype El Tor strains were incorporated for this study from 1990 – 2012. Majority of the strains were found to be Ogawa (98%) and only 2% strains belonged to Inaba serogroup. According to new phage typing scheme, type 27 was widely distributed throughout the study, followed by type 26, type 24 and type 20 respectively. All except few strains were found to be sensitive to all the common antibiotics e.g. Ampicillin, Erythromycin, Ciprofloxacin, Azithromycin, Doxycyclin, Ofloxacin, Norfloxacin, Ceftriaxone, Streptomycin and Sulfamethoxazole/ Trimethoprim. It has been observed that Ampicillin resistant strains were found in 1998. MAMA-PCR result showed the presence of toxin gene, Classical ctxB in 95.3 % of the strains of *V. cholerae* where as 0.6 % strains were found to be positive for El Tor *ctxB*. Multiplex PCR result showed 88.08% of *ctxA* positive strains and 11.92% strains were found to be *ctxA* negative. The animal experiment result showed representative strains were subjected to rabbit ileal loop experiment. Analysis of fluid accumulation of different *V. cholerae* strains in the rabbit ileal loop model assay was performed to determine the toxicity of the strains. *V. cholerae* strains (VPRL-1, VPRL-3, VPRL-6, VPRL-8, and VPRL-10) of 10^8CFU were inoculated in RIL model. All strains were found to be toxigenic in RIL except VPRL-1. Results were expressed as fluid accumulation per loop length (FA). The fluid accumulation ratio was found to be higher in case of VPRL-10 compare to VPRL-3, 6 and 8 respectively. Preliminary observation revealed that current strains of *V. cholerae* were more toxigenic compare to previous years.

Entner Doudoroff (ED) pathway and regulation of virulence determinants in *Vibrio cholerae*

Investigators: R.K. Nandy and H. Koley

The Entner Doudoroff (ED) pathway has been identified as obligatory for gluconate (Gnt) utilization by *V. cholerae* and deactivation of one of the constituting genes (*edd*) resulted into severe virulence attenuation. Based on bioinformatics analysis, Gnt utilization system in *V. cholerae* has been predicted which included genes for the ED pathway (*edd* and *eda*). Constituting genes of *V. cholerae* Gnt utilization system (*gntP, gntK, gntR, edd* and *eda*) have been shown to be conserve among *V. cholerae* but not with other organisms that possess Gnt utilization system. Further to this, unique genomic organization of these genes were noted in *V. cholerae*. Results generated with different isogenic in-frame deletion mutants (*edd, eda, gntP*) confirmed their obligatory participation in Gnt utilization. Despite the fact that mutation in any of the genes *edd* or *eda* or *gntP* resulted into loss of Gnt utilization by *V. cholerae*, mutational impact on virulence attenuation greatly varied among these mutants. Isogenic, in-frame *gntR* mutant has no role in Gnt utilization *in vitro*, however, this mutation lead to some what decrease in virulence potential. It is evident from this study that instead of functional Gnt utilization and/ or the ED pathway, functionality of the *edd* appeared to be more important in virulence expression and that probably mediates through one of the intermediates KDPG. Therefore, further studies are required for complete understanding on the role of intermediate products, specifically KDPG on *V. cholerae* virulence.

High-level azithromycin resistant *Campylobacter jejuni* in Paediatric Diarrhoea cases in Kolkata, India

Investigator: A.K. Mukhopadhyay

Campylobacter species infection is the leading cause of bacterial enteritis worldwide. Most *Campylobacter* infections cause acute, self-limiting diarrheal disease. However, patients with more severe disease and

immunologically compromised patients need antibiotic treatment. The most common antimicrobial agents used in the treatment of *Campylobacter* infections are fluoroquinolones and macrolides. In India, fluoroquinolone resistance has been increased markedly (more than 85%) in recent years. Resistance towards macrolides varies from place to place. In north India, the macrolide resistance was 6.1% during 2005 and reached to 22.2% in 2013. During 2008-2010, macrolide resistance was only 0.7% in eastern India.

One hundred and sixty-six *Campylobacter* jejuni strains isolated from paediatric diarrhoea cases (children <5 years) at B.C. Roy children's hospital, Kolkata during 2010-2012 were tested for macrolide resistance. About 4% of the isolates (6/166) were macrolide resistant by disc diffusion method. E-strip (Biomeriux) assay with azithromycin indicated that 5 isolates were resistant up to 256 µg/ml. When tested by dilution method, two isolates were resistant to 1000 µg/ml of azithromycin and three others had MICs between 500 and 1000 µg/ml. Macrolide resistance in *Campylobacter* is mainly associated with point mutation(s) occurring within the peptidyl transferase region in domain V of the 23S rRNA gene, the target of macrolides. Sequencing analysis of the V region of 23S rRNA revealed the presence of A2075G transition in all the isolates. The PCR amplified product of V region of 23S rRNA from a highly resistant isolate was electroporated into an azithromycin susceptible C. jejuni strain 81-176 and transformants selected on 10 µg/ml azithromycin plates. 23S rRNA sequence analysis of resistant mutant CJ81-176 (MIC 1000 µg/ml) identified the same mutation found in the parent strain, indicating that the A2075G mutation is responsible for the macrolide resistance in this region. In addition, we also found some colonies having mutation at different position (A2074C) under in vitro condition and may have mutated due to selection pressure. The apparently high frequency of the A2075G mutation among *Campylobacter* isolates is possibly attributed to the biological features generated by this mutation such that, A2075G mutation has a survival advantage over the other mutations. Azithromycin has been recently introduced in India for the treatment of various infections both in children and adults. Antibiotics are available without prescription in India. *Campylobacter* can transfer genes by natural transformation and antibiotic pressure can select for mutations responsible for macrolide resistance. As macrolide resistance is emerging in eastern India, the study and monitoring of macrolide resistance are, in turn, becoming increasingly important. The finding of *Campylobacter* with high level of resistance in this region of India demonstrates the relevance of antimicrobial susceptibility surveillance that will define the proper utility of various drugs in the treatment of different infections.

Antibiotic resistance in neonatal gut and septicemic isolates.

Investigators: S. Basu

An emergence of carbapenem resistance has necessitated the evaluation of resistance to other antibiotics such as aminoglycosides and fluroquinolones. Studies have been carried out in neonatal septicemic isolates to understand whether there is an association of aminoglycosides and fluroquinolone resistance determinants along with carbapenem resistance. New Delhi Metallo- β- lactamase -1(NDM-1) was the only carbapenemase isolated in Enterobacteriacae and co-resistance to other genes such as *rmt*B and *rmt*C for aminoglycoside resistance and *aac*(6')-Ib-cr for fluroquinolone resistance were observed along with NDM-1. Most of the isolates were proficient in the transfer of NDM-1 and cotransmission of the other determinants was also observed. The study indicated that NDM-1 was not associated with any particular clone or plasmid type. This suggests the potential for *bla*NDM-1 to spread quickly. New Delhi Metallo- β- lactamase -1(NDM-1) continues to be the most prevalent carbapenem- resistant determinant in Enterobactericae causing neonatal septicemia.

The gastrointestinal tract is a significant source of infecting organisms, with intestinal colonization being the prelude to bacterial infection. Studies on the neonatal gut have shown that carbapenem resistance is high in neonatal gut isolates. Further, we had also studied the prevalence of the virulent clone of *E.coli*, ST-131 in the neonatal gut and septicemic isolates. This clone of E. coli combines extensive resistance with an extensive virulence gene repertoire and belongs to the highly virulent phylogenetic group B2. The clone also produces the CTX-M-β-lactamase and was isolated from several countries including India and subsequently spread worldwide. The distribution of antibiotic resistance across different phylogroups in the septicemic isolates is shown in Fig. 1. In general, we did not observe significant difference in the antibiotic resistance pattern across most phylogroups. Distribution of ST131 isolates was low in our study both in the gut and septicemic isolates in comparison to the high percentage of CTX-M-15 producers which indirectly indicates that the clonal spread of ST131 did not probably contribute to the high prevalence of CTX-M-15 in our setting.Further studies need to be carried out to understand the extensive spread of CTX-M-15.

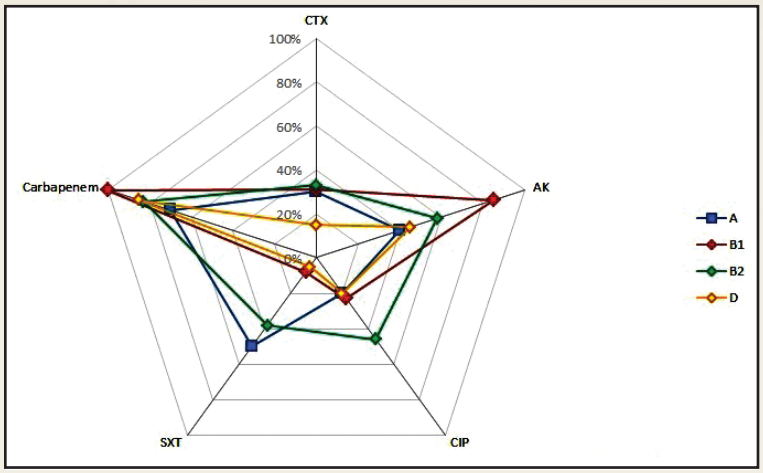


Fig. 1. Percentage of resistance to five different classes of antibiotics presented as a radar chart (CTX, Cefotaxime; AK, Amikacin; CIP, Ciprofloxacin; SXT, trimethoprime/ sulphamethoxazole, MEM, Meropenem) among *Escherichia coli* isolates belonging to four phylogroups.

Th1 cell mediated adaptive immunity and heterologous protection offered by heat killed multi-serotype Shigella immunogens in rabbit model.

Investigators: D. Nag, S. Shinoda, H. Koley

Single serotype of Shigella does not give total protection against all serotypes of shigellae. Recently, we have formulated the heat killed multi serotype *Shigella* (HKMS) immunogen with combination of six Shigella strains, *S. dysenteriae* 1 (NT4907Δstx), *S. flexneri* 2a (B294), *S. flexneri* 3a (C519), *S. flexneri* 6 (C347), *S. sonnei* (IDH00968) and *S. boydii* 4 (BCH612). A complete protection was observed against the six serotypes of wild type shigellae. The short term and long term passive protection offered by the HKMS immunogen was confirmed in neonatal mice model (Fig. 1). After getting successive homologous protective efficacy of HKMS immunogen, we have studied the humoral and adaptive immune response of the immunogen in rabbit model. The humoral immune response was confirmed by antibody in lymphocyte supernatant (ALS) assay. The adaptive immune response was determined by the up-regulation of IL-12p35, IFN-γ and IL-10 mRNA expression in immunized rabbit PBMC. HKMS immunized rabbits were challenged with wild type heterologous strains and complete protection was observed in immunized rabbits compared to the non immunized rabbits. Many antigenic proteins of

heterologous challenge strains were detected in immunoblot assay with the immunized rabbit sera. HKMS immunoegen could be a promising broad spectrum vaccine candidate that confers a long term protection against all *Shigella* serogroups by stimulating both humoral and adaptive immune responses. Indeed, HKMS immunogen has the potential to become an ideal non-living vaccine candidate against human shigellosis in future.

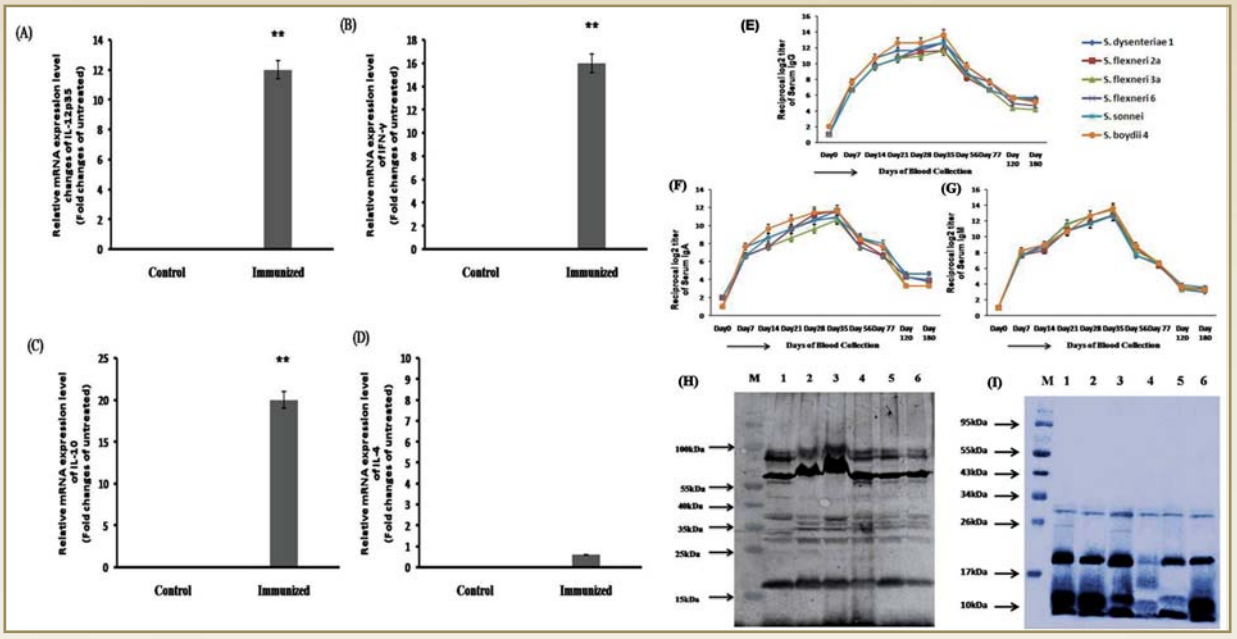


Fig. 1. HKMS immunogen induces humoral responses and also triggers antigen specific memory B-cells production through Th1 mediated pathway in rabbit model. The highly significant rises of fold change of IL-12p35 (A), IFN-γ (B) and IL-10 (C) against untreated can be observed with $p<0.005$ and very little rises of fold change of IL-4 (D) against untreated can be observed with $p>0.05$. Serum IgG (E), IgA (F) and IgM (G) responses were increased and maintained long term against the WCL of heterologous challenge strains. Most of the important antigenic proteins bands were detected against WCL (H) and LPS (I) of Shigella strains with the 35th day's immunized serum of rabbit in immunoblot assay. HKMS immunogen could be a promising broad spectrum vaccine candidate that confers a long term protection by stimulating both humoral and adaptive immune responses. Indeed, HKMS immunogen has the potential to become an ideal non-living vaccine candidate against human shigellosis.

Development of a universal Shigella vaccine based on virulence gene expression.

Investigators: R. Sinha, J. Mitobe, H. Koley

Major RNA binding protein Hfq involves repression of TTSS-encoding genes. The mutant of hfq gene increased potential amount of virulence gene expression and the efficiency of the invasion into cultured cell line was highly increased. The hfq mutants of other pathogens are known to decrease virulence in vivo situation by loss of stress response, which is under the Hfq dependent regulation. Consistently, the hfq mutant of *S. flexneri* 2a induced mild keratocojunctivitis rather than the parental wild-type strain *S. flexneri*, 2457T. These results indicated that the hfq mutant was less virulent for living animal but the expression of TTSS-encoding genes and invasion rate are potentially increased. Considering the antigen presentation in immune system, this property seemed ideal for a live vaccine. Therefore, we have evaluated the protective efficacy and immune response live attenuated shigella in guinea pig model. Construction and preliminary protection work done by our Japanese scientist. After four doses of oral immunization with vaccine strain MF4853 (Δhfq), guinea pigs were challenged by luminally with *Shigella dysenteriae* 1, NT4907 on 28 day. After 24 hrs a quite significant low number of challenge bacteria were recovered from immunized group (Fig 1.A). Animals also were challenged with wild type

Shigella dysenteriae 1 NT4907 on eye. After 72 hrs, in control groups of animals are severely infected whereas no such symptoms were observed in immunized groups (Fig 1.B). The serum anti-bacterial IgG and IgA titters of the immunized group increased during the period of immunization, peaked on the 28th day after the initiation of the immunization and remained at the same level until the 35th day (Fig-1-C & D). These data suggested that MF 4853 could be a useful live vaccine candidate to induce protective humoral immune response against human shigellosis in future.

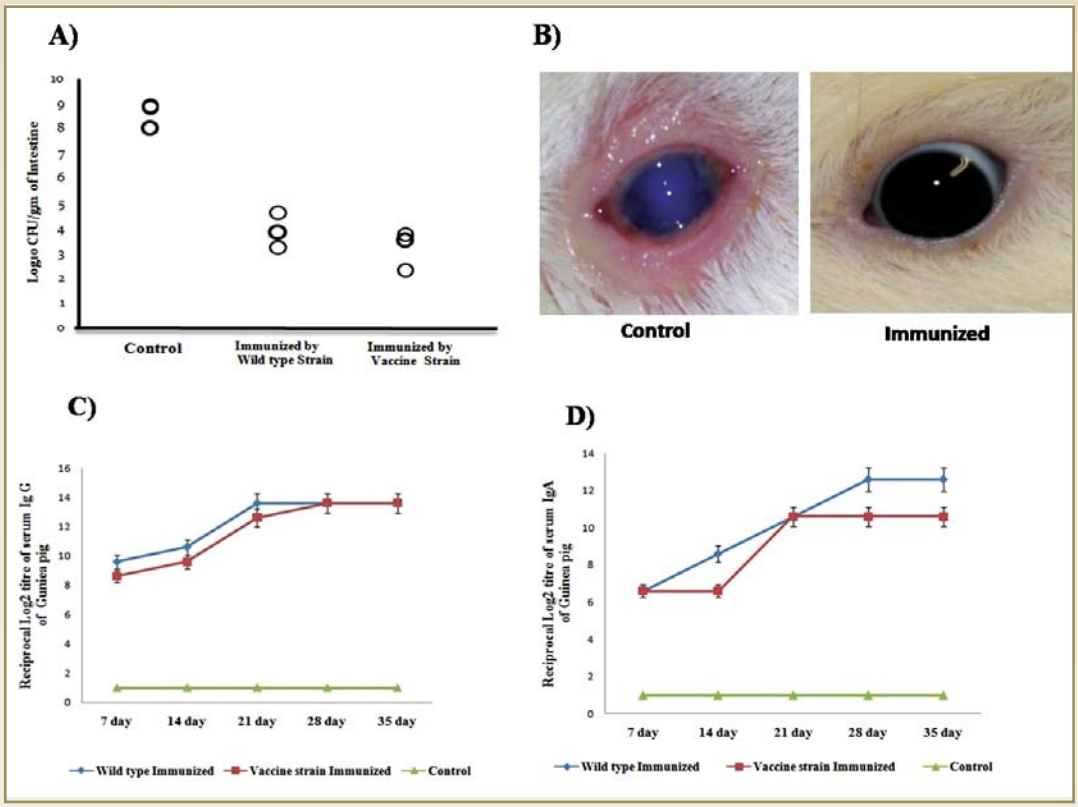


Fig. 1. Representative protective efficacy study after immunizations with vaccine strain, MF4853. A-Recovery of challenged strain *Shigellae dysenteriae* type 1 from distal colon of both immunized and control groups. B-Protective efficacy study by Sereny test after four doses of oral immunization on day 35 , we challenged with wild type *S. dysenteriae* type 1 on eye of both immunized and control animals. Keratoconjunctivitis symptoms was appeared only control group but not to the immunized group of animals. C & D - IgG and IgA reciprocal titters in sera of immunized guinea-pigs against whole bacteria was observed during the period of immunization, the serum antibody titter peaked significantly on the 28th day of 1st immunization episodes. The unimmunized sera levels were below the limit of detection.

Awards and Honors

S. Dutta

- Elected as a Member of the National Academy of Medical Sciences (India) (MNAMS) 2013.
- Invited as an Expert by National Innovation Foundation - India in the project review committee meeting held at NIF, Ahmedabad on 18 Feb 2014 for evaluation of progress of ongoing projects and review of new proposals for scientific validation and value addition in grassroots innovations.
- Invited as an expert in a market research survey on "Understanding the epidemiology and trends of important febrile diseases in India" at NICED on 12 Mar 2014 conducted by IPSOS Healthcare, India, a leading global market research agency.

- Acted as an invited reviewer for following international/National journals
  - Diagnostic Microbiology and Infectious Diseases (DMID)
  - J of Antimicrobial Chemother (JAC)
  - J of Applied Microbiology (JAM)
  - BMC infectious diseases-a BMC series journal,
  - PLoS One
  - International J of infectious Diseases (IJID)
  - Indian Journal of Medical Research (IJMR) etc.
  - Reviewer of STS, ICMR proposals

A. Palit

- Principal Member; Drinking Water sectional Committee, FAD25, Ministry of Consumer affairs, Food and Public distribution, BIS, GOI
- Member; Water Purification system sectional committee, MHD22, Ministry of Consumer affairs, Food and Public distribution, BIS, GOI

S. Basu

- Served as invited reviewer of Infection and Immunity, Antimicrobial Agents and Chemotherapy and Indian Journal of Medical Research.

Conferences/ Seminars/ Workshop/ Training Attended/ organized

T. Ramamurthy

- Coordinated the WHO-CDC sponsored training on "Global Foodborne Infection Network Course for Microbiologist and Epidemiologist" from April 9-12, 2013, Kings Institute, Chennai.
- Coordinated the WHO-CDC sponsored training on "Global Foodborne Infection Network Course for Microbiologist and Epidemiologist" from April 15-18, 2013 Kasturba Medical College International Center, Manipal University, Manipal.
- Coordinated the West Bengal Govt. supported training programme on "Rapid Detection of Cholera" at the National Institute of Cholera and Enteric Diseases, Kolkata from January 27, 2013.
- Coordinated the Okayama University supported training on "Laboratory Microbiology for Surveillance of Acute Diarrheal Diseases" at the National Institute of Cholera and Enteric Diseases, Kolkata from March 18-21, 2014.

S. Dutta

- "Isolation and characterization of a novel bacteriophage with broad spectrum lytic activity against *Shigella* spp."- A poster was presented in the 5th Congress of European Microbiologists-FEMS Microbiology conference held at Leipzig, Germany, July 21-25, 2013.
- Participated in the VIIIth Annual State conference of IAMM (Indian Association of Medical Microbiologists), WB Chapter on September 22, 2013, held at R. G. Kar Medical College, Kolkata.
- Attended 53 rd Annual Conference of National Academy of Medical Sciences held on

25th - 27th October 2013 at All India Institute of Medical Sciences, Jodhpur and the annual convocation of the National Academy of the Medical Sciences held on October 26, 2013 to receive the Membership scroll.

- A POSTER entitled "Growing antimicrobial resistance in *Salmonella* blood isolates from Kolkata-a major therapeutic challenge" was presented in the US-Japan Cooperative Medical Sciences program (CMSP)-16th regional conference on Emerging Infectious Diseases (EID) in the Pacific Rim held on February 9-11, 2014 and US-JAP CMSP Panel meetings on Cholera and other bacterial enteric infections held at ICDDR, B, Dhaka Bangladesh on February 12-13, 2014.
- Participated in the workshop held at NICED, Kolkata on 18-19 sept. 2013 organized by the Intellectual property rights (IPR) unit of Indian Council of Medical Research (ICMR), New Delhi.
- Attended 15th year celebration of patent Information Center jointly organized by Patent Information Center (PIC) West Bengal State Council of Science & Technology (WBSCST), DST- GoWB and Patent facilitating Center (PFC), Technology Information Forecasting & Assessment Council (TIFAC), DST-GoI held at Kolkata, on September 24-26, 2013.

A. Palit

- Specially invited by RMRIMS (ICMR), Patna for emergency basis "water analyses" of diarrhoeal outbreak samples from Muzaffarpur district, Bihar & Nepal and training for "technology transfer" of outbreak water sample analyses in emergency situations, June 25-28, 2013.
- Participated in an ICMR sponsored workshop on "Intellectual property rights in Medical Research" at NICED, Kolkata, on September 18-19, 2013.
- Invited & "CHAIRED" a scientific session in UGC-DRS Sponsored National Seminar on "Bioprospecting of Natural Products", held at Department of Zoology, Burdwan University, India, December 5-6,2013
- Invited & delivered an invitational lecture in UGC-DRS Sponsored national Seminar on "Bioprospecting of Natura Products", held at Department of Zoology, Burdwan University, India, December,5-6, 2013. I contributed as a "Resource person" and on behalf of NICED (ICMR) delivered the talk on "Antibacterial potential of natural products against diarrhoeal pathogens – a pilot approach"
- Invited & delivered an invitational lecture on "Environment and enteropathogenic bacteria" on the occasion of celebration of 70 years of Zoology department of Asutosh College at Asutosh College Training Centre (ACTC) Seminar Hall, Calcutta University on December 16, 2013.
- **Invited** for providing **training** and practical demonstration for **"technology transfer"** of outbreak water sample analyses protocol in emergency situations, 25-28th June, 2013.

B.L. Sarkar

- Invited and participated in Consultation meeting for collaborative project prospective on Diarrhoeal diseases with faculties of Sikkim University, Gangtok, Sikkim on October 5, 2013.
- Invited to attend the NICED-NIID joint bilateral meeting and to deliver a talk entitled

"Retrospective analysis on the evolutionary aspects of *Vibrio cholerae*" at NIID, Japan on from September 25-27, 2013.

- An invited talk entitled "Current scenario of cholera and phages: the disease and treatment" was delivered at the National Seminar organized by DKM College for Women, Vellore on January 10, 2014.
- The Indo- UK joined collaborative project launched a workshop on "Development of bacteriophage based biocontrol technology for the treatment of cholera" held at University of Nottingham, Leicestershire, UK during May 25-31, 2014. Being the PI (Indian side), a seminar lecture entitled "Cholera and cholera bacteriophage: an Indian scenario" was presented on May 29, 2014.

R.K. Nandy

- Presented the laboratory aspect of the project on "Exploration of Biological Basis of Underperformance of Oral Polio and Rota Virus vaccine in India" in Seattle, USA from May 5-7, 2013 at Conference Centre of Bill and Melinda Gates Foundation.
- Delivered a talk titled "Diagnostic methods for diarrheal diseases" under training programme for National Institute of Homeopathy, Kolkata organized by NICED on June 6, 2013 at NICED, Kolkata.
- Delivered a talk titled "An overview of biosafety in laboratory setup" at the Training Programme on Laboratory Safety (Biosafety, Chemical Safety & Radiation Safety) organized by CSIR-Indian Institute of Chemical Biology on September 16, 2013 at IICB, Kolkata.
- Attended workshop on intellectual property rights in medical research organized by Intellectual Property Right Unit, Indian Council of Medical Research on September 18-19, 2013 at NICED, Kolkata.
- Attended Workshop on organized for 15 year celebration of Patent Information Centre with a theme 'Creativity: the next Generation" during September 24-26, 2013 at Kolkata; organized by DST of Govt. of India and Govt. of WB.
- Oral presentation "Rotavirus vaccines: prospects and challenges" at 58th Annual National Conference of Indian Public Health Association at Tirupati during January 21-24, 2014

A.K. Mukhopadhyay

- Presentation of the work entitled "Sequential Genetic Variations of the *Vibrio cholerae* strains from Kolkata, India and its relation with the Haitian strains" in the "Annual Asia-African Research Forum" organized by the Japan Initiative for Global Research Network on Infectious Diseases held in Sendai, Japan during January 20-22, 2014.
- Attended the NICED-NIID joint bilateral meeting regarding the HMSC cleared collaborative project on "Laboratory based collaboration network of infectious diseases in Asia" and presented the progress of his work in the meeting held in NIID, Tokyo, Japan during September 25-27, 2013.
- Presentation of the work entitled "Characteristics of Indian *Vibrio cholerae* strains: Traces of Haitian Variant" in the 5th Congress of European Microbiologists (FEMS) held in Leipzig, Germany during July 21-25, 2013.

- Attended Consultation workshop for Foodborne Infection from February 11-12, 2013 at National Centre for Disease Control, New Delhi.
- Organized WHO-CDC-ICMR supported GFN Course for Microbiologists and Epidemiologists was held from February 14-16, 2013 in NICED, Kolkata.

S. Basu

- 16th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim, Bangladesh, February 9-11, 2014. Plasmids harbouring the New Delhi Metallo-β-lactamase (NDM-1): Transmissibility, Incompatibility groups and Co-resistance" (Poster presentation).

- 48th United States-Japan CMSP Conference on Cholera and Bacterial Enteric Diseases, Bangladesh, February 11-13, 2014 The virulence-associated strain ST-131 among CTX-M-producing Escherichia coli in hospitalized neonates. (Poster presentation).

Biochemistry

The long-term interest of the Division of Biochemistry lies in understanding the molecular basis of pathogenesis of diarrheal diseases with the intension to lay a foundation for translational research. 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 defense 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. The Biochemistry Division focused its research on molecules involved in bacterial pathogens. The key studies undertaken were in defining the role of surface proteins of enterotoxigenic *Escherichia coli* in colonization of the human gut, explaining the structure-function relationship of *Vibrio cholerae* hemolysin with emphasis on the mechanism of membrane penetration by the toxin and interpret the importance of *V. cholerae* chitinase and in their survival. Recently, we have initiated studies on the host signal transduction pathways elicited by bacterial proteins like *V. cholerae* 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:

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

Staff:

R. Naik, Technical Assistant

Pre-Doctoral Fellows:

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

Awards:

- Avishek Ghosh received Ph.D., University of Calcutta;
- Subrata Sabui received Ph.D., University of Calcutta;

A novel carbohydrate-independent role of the β-prism lectin domain of the Vibrio hemolysin in promoting self-assembly of the toxin monomer to the β-barrel heptameric pore.

Investigator : K. K. Banerjee

Vibrio cholerae cytolysin/hemolysin (VCC) is a β-pore-forming toxin (β-PFT) that kills a broad spectrum of eukaryotic cells by destroying the selective permeability of the membrane barrier. The VCC monomer, a water-soluble exotoxin produced by *V. cholerae* O1 El Tor and non-O1 strains, binds to the target cell surface and penetrates the membrane lipid bilayer as a 14-stranded β-barrel channel with two β-strands each from seven monomers. At low toxin concentrations, the cell may respond by undergoing apoptosis or vacuolation. A unique feature of VCC in relation to other β-PFTs is the presence of two carbohydrate-

binding domains at the C-terminus. Previously we showed that the C-terminus jacalin-like β-prism lectin domain is the only functional carbohydrate-binding unit of VCC, which enables the toxin to interact with soluble and cell surface β1-galactosyl-terminated glycoconjugates. Our studies revealed that the β-prism lectin domain regulates interaction of the toxin with the innate immune system of the murine host and interestingly, though directly not involved in self-assembly of the toxin monomer, profoundly affects the shape and symmetry of the assembled β-barrel. In the present work, we worked on a possible alternative mechanism by which the β-prism lectin domain can affect the biological functions of the toxin without contributing to membrane targeting.

Deletion of the β-prism lectin domain or inactivation of its carbohydrate-binding activity by point mutation compromised haemolytic activity toward rabbit erythrocytes by ~800-fold and 16-fold respectively. It is commonly thought that loss of the lectin-like function abrogated binding to the erythrocyte surface; surprisingly, all the three toxin variants moved to the erythrocyte stroma with association constants of the order of $10^7 M^{-1}$. However, loss of the lectin domain severely reduced efficiency of self-assembly of the VCC monomer to the β-barrel heptamer in synthetic lipid bilayer from 83 to a meagre 27%. Notably, inactivation of the sugar-binding activity by Asp617Ala mutation marginally reduced oligomerisation to ~77%. Oligomerization of the 50 kDa truncated variant was temperature-insensitive; by comparison, VCC self-assembly increased with increasing temperature, indicating that oligomerisation was entropy-driven and opposed by enthalpy (ΔH= 115 kJM^{-1}; ΔS= 0.96 $JK^{-1}M^{-1}$). In conclusion, we proposed that the β-prism lectin domain facilitated toxin assembly by producing entropy during relocation in the heptamer. So, thermodynamic analysis of the toxin assembly revealed a novel carbohydrate-independent role for the lectin domain in pore formation.

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

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

Vibrio cholerae normally resides in aquatic environment remains associated with the chitinous exoskeletons of zooplankton and utilizes chitin as the sole source of carbon and nitrogen. 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 have characterized one such factor which is a chitinase ChiA2 and focused on its importance in *V. cholerae* pathogenesis. Purified ChiA2 from a *V. cholerae* pathogenic strain efficiently hydrolyzed mucin as a substrate, and released reducing sugars like GlcNAc and its oligomers as analyzed by HPLC analysis. Deglycosylation of mucin was confirmed by reduced uptake of Alcian blue stain by ChiA2 treated mucin. Hydrolysis of mucin by ChiA2 involved the same catalytic site and same amino acids essential for chitin hydrolysis. Further, we demonstrated that the *V. cholerae* could utilize mucin as a nutrient source. In a *chiA2* deleted mutant strain, the growth was 60-fold less efficient compared to the wild type *V. cholerae* in a mucin-supplemented minimal media. The growth of the mutant strain was also 6-fold less in a human intestinal mucin-secreting cell line (HT29). The effect was reversed when the mutant strain was complemented with Δ*ChiA2*-His plasmid and was able to survive like the wild type strain. Similar results were obtained in animal model experiments. Next, pathogenesis was compared between these strains by analyzing the colonization ability and fluid accumulation in animal models. The *chiA2* mutant caused about 50-fold less fluid accumulation at 18 hours post infection. This was a result of poor proliferation of the mutant strain in the intestine. Results indicated that secreted ChiA2 helped *V. cholerae* to utilize intestinal mucin for their growth and survival in the host and eventually helping in pathogenesis.

Molecular characterization of Enterotoxigenic *Escherichia coli* colonization factors

Investigators : N. S. Chatterjee and T. Ramamurthy

Enterotoxigenic *Escherichia coli* (ETEC) infection is the leading cause of infantile diarrhea in developing countries and an important etiologic agent for traveler's diarrhea. CS6 is a prevalent colonization factor present on approximately 30% of ETEC worldwide. Thus this has become an important vaccine candidate. Our laboratory has been studying different aspects of this colonization factor and aims in developing simple methodologies for detection of CS6 as well as therapeutic strategies to block the host-pathogen interaction. In previous reports, we have reported the characteristics of CS6 and existence of multiple subtypes which is due to point mutations in the CssA and CssB structural genes. Out of multiple subtypes, AIBI and AIIBII were unique; AIBI were mostly associated with diarrhea cases whereas AIIBII were associated with asymptomatic cases. The pathogenicity of AIBI was correlated with their stronger adherence to intestinal cells in comparison to that of AIIBII. Further, we demonstrate the assembly of CS6. We show that in solution, CS6 forms a continuous array of higher order oligomers by formation (CssA-CssB)n complex where 'n' is increases with concentration. The CS6 protomer interacts with each other through their β-sheets during oligomerization. The oligomeric assembly can dissociate if there is any transformation of β-sheet to α-helix in CS6 subunits. The CS6 assembly is concentration-dependent and builds up by non-covalent interaction between CssA-CssB subunits protomer of CS6 through their β-structure which is unique from other CFs assembly.

Conferences/ Seminars/ Workshop/ Training Attended/ organized

N.S. Chatterjee

Presented a talk on 'Role of allelic variants of the colonization factor CS6 of enterotoxigenic *Escherichia coli* in pathogenesis' at the 5th Congress of the European Microbiologists (FEMS 2013) held at Leipzig, Germany, organized by Federation of European Microbiological Societies during July 23-26, 2013.

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. An extramural grant has been received to study the regulation of antimicrobial peptide expression in the intestinal epithelial cells.

Diarrhoeal diseases surveillance system showed that most of the drugs usually use in cholera now-a day is more or less resistant to causative agent of the disease. Recently, few studies showed that Norfloxacin and Azithromycin both are very sensitive to the vibrio Cholerae. Now state health govt. following single dose regime of Azithromycin for treating cholera patients as per our suggestion. Recently we observe during the diarrhoeal outbreak patients who admitted at ID & BG Hospital due to Rotavirus majority rotavirus strain are belong to G9. Hence we are now started evaluation of pentavalent rotavirus vaccine including G9 for preventing the rota virus diarrhoea due to G9 strain. Scientists are involved in investigation of epidemics of diarrhoeal diseases and unknown fever. They are also involved inhuman resource development by providing training to the service providers like doctors and para-medical staff.

Scientist:

Dr. M.K. Bhattacharya, Scientist 'F'
Dr. S. S. Das, Scientist 'D'
Dr. P. Indwar, Scientist 'B'

Staff:

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

Pre-Doctoral Fellow:

Asim Biswas
Atri Ta
Bhupesh Kumar Thakur
Diptaman Nandy
Himanshu Sekhar Das
Nirmalya Dasgupta
Pujarini Dutta
Satyaki Mitra
Sayan Das

SoumendraMaitra
SoumitaMahapatra
TheeyaNagaraja,

Awards:

- 'Young Investigator Award' to Bhupesh Kumar Thakur, PhD Student (SRF) at the International Symposium on 'Probiotics: Microbiome and Gut function; transforming health and well being, 2014' organized by 'Yakult India Microbiota and Probiotic Science Foundation' on 15th-16th February, 2014 at New Delhi, India.
- Best Poster Award to PiuSaha, ICMR Centenary Postdoctoral Fellow at the 'IMMUNOCON, 2013' and the 40th Annual meeting of the Indian Immunology Society at New Delhi, India on 15th to 17th November, 2013.
- Second prize for poster presentation to Rahul ShubhraMondal, Scientist I at the Conference on Recent Advances in Computational Drug Design, held on 16-17th September, 2013 at Indian Institute of Science, Bangalore.

Clinical Laboratory

Clinical, Epidemiological & other Laboratory related projects supported by Clinical Laboratory Division:

This division has already supported different projects with the funding support of ICMR /WHO / different renowned bodies, namely:

1. Measles aerosol vaccine project phase I trial, funded by WHO
2. Killed oral cholera vaccine project (1 vs 2), funded by IVI
3. Reduced ORS Study Project, funded by ICMR
4. Travelers' Diarrhea, funded byGEMS
5. Factors responsible for HIV transmission in married couples : a step towards intervention development (Intramural project, NICED, Kolkata) , funded by ICMR
6. Trial of Candidate Live Oral Cholera vaccine VA 1.4 : A phase I – II study to evaluate the safety and Immunogenicity in Healthy Adults in Kolkata, India, funded by DBT, New Delhi
7. An Open Labeled Controlled Trial to Evaluate the Immune Response of a Boosting Regimen with Shanchol™ , A Killed Whole Cell Oral Cholera Vaccine (wc-ocv), in Previously Immunized Children and Adults in Eastern Kolkata, India, funded by IVI

The laboratory work of the project, named Exploration of Biologic Basis for Underperformance of Oral Polio and Rotavirus Vaccines in India (PROVIDE), funded by IVI, is still going on in this division. Routine biochemical, haematological and microscopic examination of clinical samples (like blood, stool and urine) are performed with the patient samples referred by the I D & B G Hospital, Kolkata, Dr. B.C. Roy Memorial Hospital for Children, Kankurgachi, Kolkata, Dr B.C. Roy Polio Clinic Hospital for Crippled Children, Kolkata, and other State Hospitals on a regular basis.

We are regularly participating in the external quality assurance (EQA) scheme / programme for biochemistry (conducted by Christian Medical College, Vellore) and haematology (conducted by All India Institute of Medical Sciences, New Delhi) to maintain high quality of our testing as well as for smooth running of national and international projects.

Staff:

Mr. C. R. Pal, Technical Officer'A'
Ms. PramitaBhaumik, Technical Assistant

Hospital and Outpatients base surveillance system for diarrhoeal diseases

Investigator: M.K. Bhattacharya

This study was carried out with objectives i) to monitor changes in disease pattern, ii) to create a database on diarrhoeal diseases, and iii) to provide regular reports to the Govt. and other agencies and to improvement in better patients care and preventive measures

From April 2013 to March 2014, a total of 1226 fecal specimens were collected form every 5th patients admitted with acute watery diarrhea at Infectious Diseases Hospital (IDH), Kolkata (during 24 hours a day from 2 randomly selected days per week) for etiological analysis (~5.77% of admitted patients). In case of B.C Roy Post Graduate Institute of Paediatric Sciences (BCRPGIPS), 1254 specimens ware collected (every 5th systematic sample from OPD patients- Monday to Friday) (~20% of total OPD patients). Type of diarrhea at presentation in IDH and BCRPGIPS were watery (77.2% vs. 15.6%), bloody (1.7% vs. 5.3%) and semi-solid (21.1% vs. 79.1%). In ID & BG Hospital 3.8% under five children presented with severe dehydration and 95.9% with some dehydration. But in B. C. Roy Hospital these values are 0% and 2.5% respectively.

In children below 5 years of age, prevalence of rotavirus was found to be common in both the hospitals (~48%). Vibrio cholerae 01 (20%) and Campylobacter spp. (4.3%). Vibrio fluvialis (3.2%) were more in the IDH. In the BCRPGIPS, prevalence of adenovirus (15.3%), C. jejuni (13.8%), enteroaggregative Escherichia coli (5.6%) and Shigella spp. (5%) were high. Vibrios remained susceptible for most of the fluoroquinolones. In both the hospitals, most of the Shigella strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone. NDM-type carbapenemase were detected in 27 strains of V. fluvialis strains isolated from 2011-2013. All these NDM-positive strains were susceptible to azithromycin. Weekly reports sent to Govt. and other agencies for control and improvement for better patients care and suggested treatment regime accordingly drug susceptibility patterns.

Role of commensal Escherichia coli flagellin in the induction of intestinal regulatory responses and protection from experimental colitis.

Investigator: S. Das

T-regulatory (Treg) responses play critical roles in the maintenance of intestinal homeostasis and protect from the development of inflammatory diseases. We have observed that intracolonic administration of commensal E. coli flagellin into BALB/c mice induces regulatory cytokines (TGF-â, IL-10) as well as $CD11c^{+}CD11b^{+}CD103^{+}$ tolerogenic DCs and $CD4^{+}CD25^{+}FoxP3^{+}$ Treg cells. To investigate the functional significance of the above regulatory responses, mice suffering from colitis induced by TNBS were treated with commensal flagellins. The results showed significant amelioration of colitis as evident from macroscopic, histopathological and flowcytometric analysis that correlates with increased numbers of Treg cells in the intestine (Fig 1). The latter protect from colitis upon adoptive transfer to the littermates. In parallel experiments, induction of regulatory responses and protection from colitis by indigenous probiotic Lactobacilli were studied.

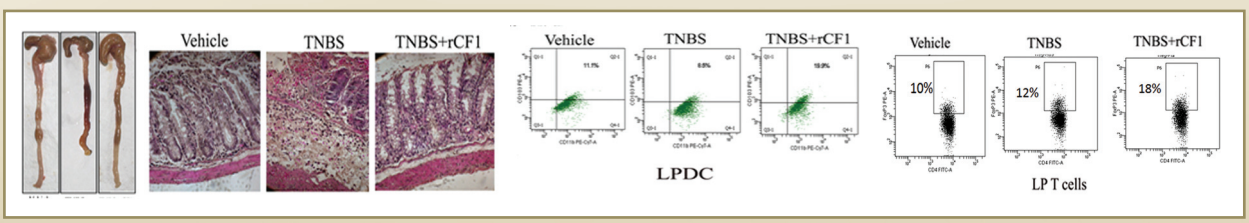


Fig 1. Upper panel. (left). Colon of mice isolated 3 days after intracolonic TNBS administration. (Right). Histopathology of the colonic tissues isolated as above and stained with hematoxylin and eosin. Lower panel. Cells isolated from the colon of mouse treated as above.

Role of eukaryotic-like serine/threonine protein kinases (STPK) in the pathogenesis of Salmonella enterica serovar Typhi (S. Typhi):

Investigator: S. Das

Intracellular pathogens like Salmonella employ multiple mechanisms to subvert host-induced killing within macrophages. Eukaryotic-like Ser/Thr kinases (STPKs) have been shown to contribute to phagosomal survival of bacteria. We found that a putative STPK of Salmonella Typhi Ty2 (T4519) is induced within macrophages and secreted into the cell cytoplasm. T4519 shows ser/thr kinase activities in vitro by autophosphorylation and phosphorylation of the universal substrate myelin basic protein (MBP) and promotes bacterial survival within macrophages. Complementary to this, S. Typhi Ty2Δt4519 strain shows significantly reduced pathogenicity in mice that is reversed by gene or protein complementation of the strain. Further studies revealed that T4519 functions through the activation of NF-kB signaling pathways. This induces cathepsin B, leading to lysosomal membrane permeabilization (Fig 2).

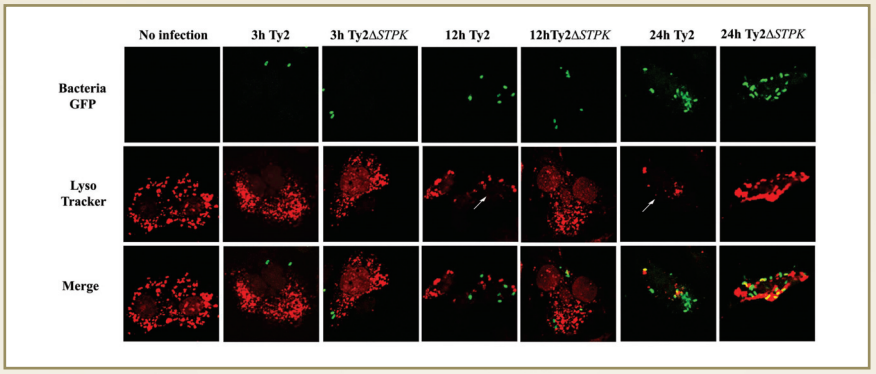


Fig 2. Confocal microscopic images of THP-1 cells-derived macrophages infected with the wild type or mutant S. Typhi for 1 hr, washed and cultured in the presence of gentamicin (50 µg/ml) for the indicated durations. Cells and bacteria were stained with lysotracker (red) and polyclonal Salmonella antisera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

Detection of Rotavirus in CSF in children aged ≤5 yrs hospitalized with acute gastroenteritis with neurological manifestations in Kolkata.

Investigators: P. Indwar, M.K. Bhattacharya, B. Ganesh, M.C. Sarkar, T. Ramamurthy, S. Ganguly, S. Banerjee, S.K. Rout and S. Das

This study was initiated with objectives of i) detection of rotavirus and other diarrheagenic viruses in CSF in children presenting with acute gastroenteritis associated with neurological manifestations and ii) to observe different neurological manifestations and their outcomes. Preliminary results obtained so far, have identified 35 patients who presented with diarrhoea along with neurological symptoms, of them 19 samples were processed in laboratory and out of 19, 8 patients showed positive for Rotavirus in stool. Also Rotavirus RNA was detected in CSF of three patients whose stool sample were positive for rotavirus.

Awards and Honors

S. Das

- Travel Award from Okayama University, Japan Project to make oral presentation at the Annual Asian-African Research Forum held in Sendai, Japan, January 20-22, 2014.

Conferences/ Seminars/ Workshop/ Training Attended/ organized

M.K. Bhattacharya

- As a Special Guest lecturer, delivered lecture on "Health aspects in Disaster Management" on 26th November, 2013 at ATI, West Bengal.
- As a Special Guest lecturer, delivered lecture on "Health & Hygiene Management during and Post Flood Disaster" on 19th February, 2014 at ATI, West Bengal.

S. Das

- Carried out advanced research at the laboratory of Prof. Philip N. Tsichlis, Director, Molecular Oncology Research Institute, Tufts University School of Medicine, Boston, MA, USA from April 10, 2013 to September 9, 2013 under the Fulbright-Nehru Senior Research Fellowship, 2012.
- Attended annual Asian-African Research Forum organized by Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) and Tohoku University Graduate School of Medicine held in Sendai, Japan during January 20-22, 2014.
- Organized workshop entitled "Frequently Used Databases in Biomedical Research" held at NICED on January 31, 2014.
- Organized workshop entitled "Design and Statistical Analysis for Biomedical Research" at NICED on March 25-27, 2014.

P. Indwar

- Capacity Building Workshop on Operational Research & Ethics in HIV/AIDS Research on Targeted Interventions' from June 18-22, 2013 at National Institute of Health & Family Welfare, New Delhi.

Data Management

Biostatistics involves the theory and application of statistical science to address public health problems and to further study on biomedical research. The department's research in statistical methods and interdisciplinary collaborations with other departments provide many opportunities of exploration of research data and its participation. Computational science nowadays needs high-performance infrastructures for scientific processes by providing a paradigm that may encompass all the steps of discovery based on the execution of complex algorithms and analysis of scientific data. For instance, in data-driven discovery processes, knowledge innovation tasks can produce the real experiments and perceptions. In such a way, algorithms, data, services, and other software components are orchestrated in a single simulated structure, which specifies the execution sequence and the more suitable preparation of this collection of resources.

This division primarily focuses on good data management practices and also compliant with Good Clinical practices (GCP) to produce the reliable, complete and accurate data from the various health research projects of this institute. This division has also crucial role for data management and creation of diarrhoea database from ongoing hospital based diarrhoeal diseases surveillance at Infectious Disease Hospital (IDH), and Dr. BC Roy Children Hospital in Kolkata to identify the pattern of diarroeagenic enteric pathogens. The causative organism of diarrhoea and antimicrobial resistant pattern of cholera and Shigella is communicated on weekly basis to IDH and different department of State Government so as to help the physicians for proper patient management of diarrhoeal diseases. The division is also working on climate factor surveillance and diarrhoeal disease which derives the seasonality pattern and association of diarrhoea in West Bengal. It provides the comprehensible vision of basic research of diarrhoeal diseases empowering the epidemiological, clinical and microbiological data envisaging social, environmental and spatial implication by novel statistical model. It has direct access to the data from all concerned division and to provide data management support including data entry/verification to various studies undertaken in this institute with National like the project on National hospital based Rotavirus surveillance network in Eastern zone of India and Integrated Diseases Surveillance Project (IDSP) and International Collaborators like International Vaccine Institute, Korea, and Centre for Vaccine Development, University of Maryland, Baltimore. This division always rendered statistical help for epidemiological, clinical and microbiological research as well as to Ph.D. students for their thesis. There are also future plans to conduct local and country level courses on research methodology, biostatistics use in laboratory science, sample size determination for randomized Clinical trial for health researchers. Final goal is to publish the research findings using modern and appropriate statistical techniques in peer reviewed journals.

Scientists:

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

Generation of a database on cholera outbreaks in India

Investigator: B. Manna

A huge number of diarrhoeal outbreaks have been reported and investigated in different parts of India during last 30 years. All the investigation reports are usually submitted to the respective State Government

as well as Ministry of Health, Govt. of India. But unfortunately, some of the outbreak reports are published in the indexed journal depending on the research interest of the investigators. So, there is a limited scope for any researcher or health policy maker to get the access the information about all outbreaks electronically. Therefore continued monitoring & surveillance of all cholera outbreaks become necessary and there is a need to create database on all cholera outbreaks in India which will facilitate the health planners to make policy for combating future outbreak and to make control strategy based on the evidences gathered from this study.

The published articles on diarrhea outbreak /epidemic have been collected through free medical journals, Medexplorer, Medscape, Medhunt and PubMed. Attempt was made for collection of unpublished data from different sources viz, NCDC (National Centre for Disease Control, Delhi)-annual report, NICED-annual reports, Integrated Disease Surveillance Projects (IDSP) web site. Data collection has been started from different published articles and unpublished documents. So far, about 140 published articles have been collected. Since July, 2009 to May 2014, a total of 2,571 outbreaks have been reported under Integrated Disease Surveillance Project (IDSP) (Fig. 1). It has been observed that maximum number of outbreaks occurred during July – August throughout every year. In 2013, maximum number of outbreaks occurred in West Bengal followed by Karnataka, Orissa and Maharashtra. V. cholerae 01 was the major causative organism (54%) for total outbreaks among published articles. Figure Legends

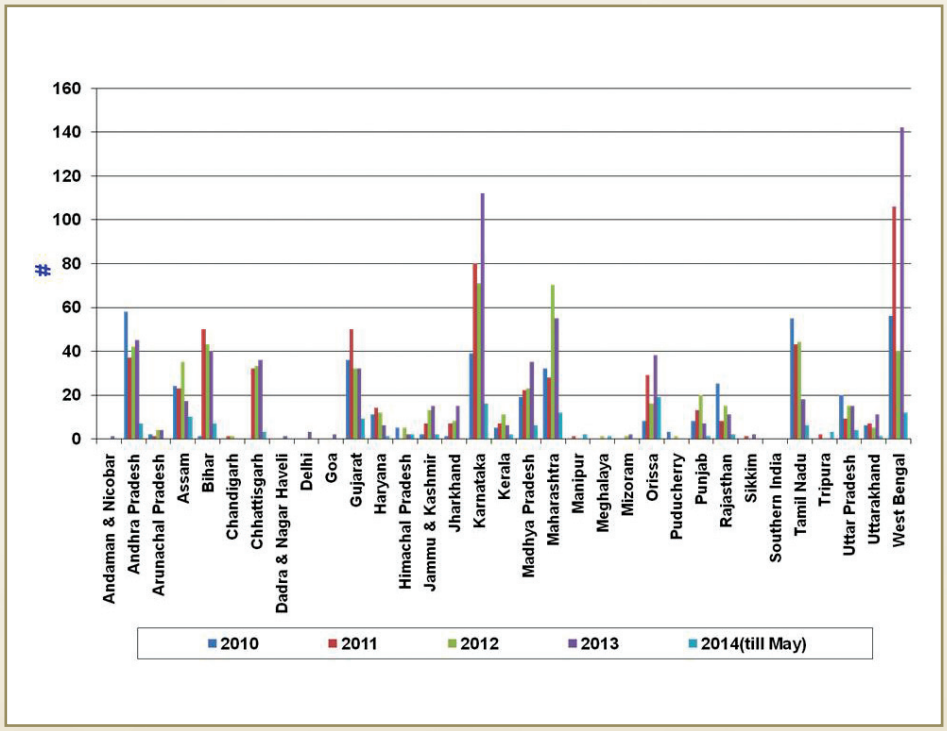


Fig. 1. State wise diarrhoea outbreak reported under IDSP

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

Investigator: K. Rajendran

The objectives of the study is to compare the climatic characteristics such as temperature, relative humidity (RH) and rainfall with observed infection of diarrhoea and cholera in the infectious diseases

hospital, Kolkata and to assess long term changes to develop Time series model and Mathematical Statistical Models. Climatic factors used to explore the relation with V. cholerae infection using i) V. cholerae isolation data from hospital based diarrheal surveillance at infectious diseases hospital and ii) mean monthly and daily rainfall (cm), temperature, relative humidity and sunshine duration data have been procured from Meteorological Department, Alipore, Kolkata.

Climate influences many infectious diseases in the tropical regions, many studies stresses the importance of temperature, precipitation, rainfall, and extreme weather events in the transmission of cholera. This study was then aimed to compare the climatic characteristics with observed infection rate of cholera, to assess long term changes and to develop Time series model. Active surveillance yearly data during 1996-2013 were generated from the Infectious Diseases Hospital (IDH) and the climatic data on relative humidity (RH), temperature and rainfall were collected from meteorological department Kolkata retrospectively. 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 in the parametric model. The mean factors purposefully have been averted to avoid the influence of high variation in the series. Analytical strategies of seasonal decomposition factors were generated for factual finding. Analytical approaches, the Comparative analysis of Rainfall days and No Rainfalls days with V. cholerae infection, Periodic comparison for diarrhoeal pathogens and Seasonal comparisons were adopted to know the progressive correlation over the period between cholera and climate changes. Analytical explorers, the sequential curve to identify pattern, Exponential smoothing model to identify the evidence base existing model, Seasonal Auto Integrative Moving Average (SARIMA), Generalized Linear Model (GLM), Maximum Entropy and least square methods were employed to investigate relative impact of climate changes on cholera (Fig. 2).

Models have identified the changing temporal patterns of enteric pathogens in relation to climatic conditions in Kolkata. Periodicity, seasonality and pattern of V. cholerae infection have been identified (Fig. 3). Rain fall indirectly has stimulated the V. cholerae infection. High relative humidity favours V. cholerae infection, which has direct correlation to it. High temperature does not favour V. cholerae infection. Prevalence of cholera was high during monsoon when the RH was high; in contrast, the rotavirus infection was in peak during winter season when the temperature and RH were at minimum. The sunshine duration was less while cholera was high but the sunshine duration increases when rotavirus infection was high in winter season. Rota viral infection was less influenced by 10-20% RH variation with maximum sunshine duration, no rainfall and low temperature. The parasitic infection was found along with V. cholerae 01 and rotavirus (co-infection), influence of climate on these pathogens remained difficult to determine. The El Niño and La Niña has definite role in determining the prevalence of diarrhea in Kolkata.

The study is addressing that the pathogens had quick climate adaptation even though high variability which may have chance for mutation of organism. Climate significantly fluctuated and the consistency was not similar during the study periods. Climatic factors are the real challenges for diarrhea diseases which have to be sensed in time for welfare of next generation.

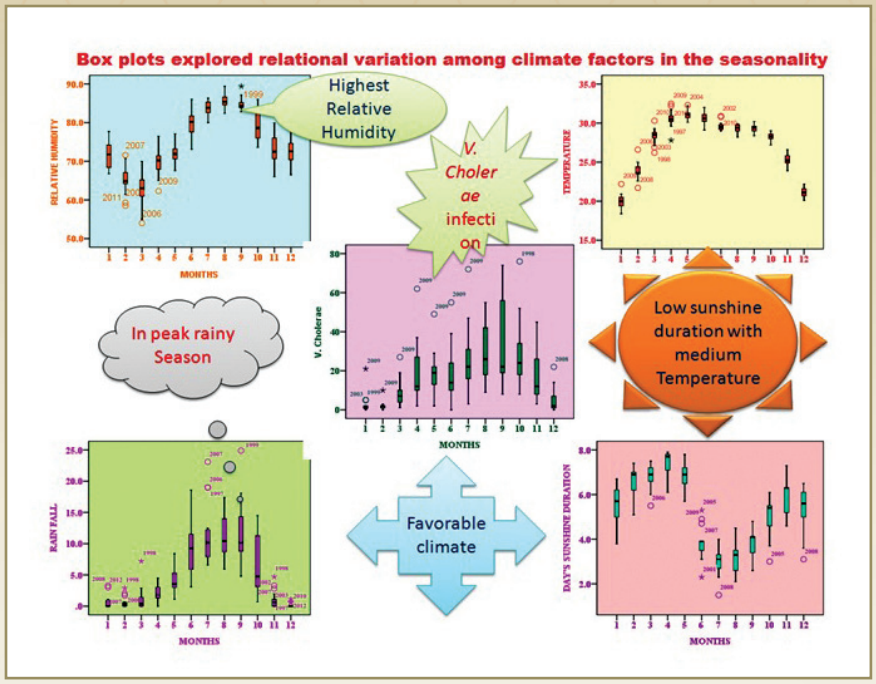


Fig. 2. Derived time series model articulate message beside cholera dissemination in favourable climate.

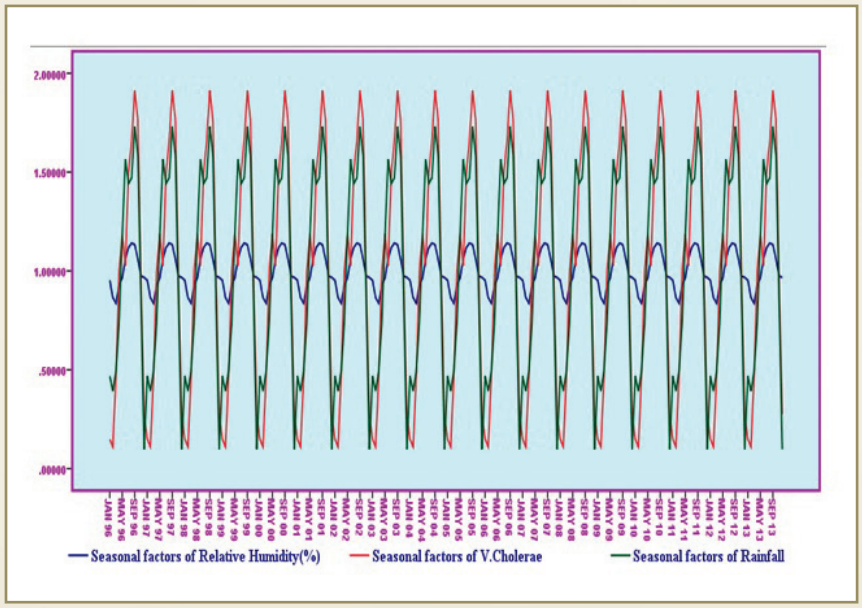


Fig. 3. Appears to be strong co-relation with relative humidity and rainfall associated cholera in Kolkata

Conferences/ Seminars/ Workshop/ Training Attended/ organized

B. Manna

- Attended and presented data management update in the Annual Meeting for the NICED-IVI collaborative project on PROVIDE Study during May 5-7, 2013 at Seattle, USA.
- Attended Research Methodology Workshop for Field Units of ICMR during September

26-28, 2013 at Govt. Medical College, Srinagar, J&K and delivered lectures for under graduate, postgraduate medical students and faculties on "Statistical Measure in Research and Sample Size for various types of Research Studies".

- Attend the conference "Vaccines for Enteric Diseases (VED)" for poster presentation entitled "Comparison between protective efficacy of whole cell killed oral cholera vaccine and vibriocidal response: evidence from a study in Kolkata, India" during November 6-8, 2013 at Bangkok, Thailand.
- Attended 58th Annual National Conference of Indian Public Health Association from January 22-24, 2014 at Sri Venkateswara Medical College, Tirupati, AP and presented a paper on "Determinants of Health Care Seeking for Childhood Diarrhoeal Illness in Urban Slum of Kolkata, India".

K. Rajendran

- Conducted a one day academic one day workshop entitled "Research Methodology" at the 17th annual conference 2014 of the association of chest Physicians, West Bengal at RG KAR Medical College, Kolkata.
- Conducted an investigators workshop on "National Rotavirus Surveillance Network –Eastern Region" during September 3-5, 2013 at the National Institute of Cholera and Enteric Diseases, Kolkata.
- Participated in the ICMR sponsored Workshop entitled "Intellectual Property Rights in Medical Research" at National Institute of cholera and Enteric Diseases, Kolkata during September 18-19, 2013.
- Participated in the ISI Kolkata sponsored Workshop entitled "Modern trend in Soft Computing & Security Issues" at National Institute of Science and Technology, Berhampur, Odisha during September 23-28, 2013.
- Participated in EL Niña and La Niña thirty years persuade cholera in Kolkata, India: Time series model: at 16th International conference on Emerging Infectious Diseases in the Pacific Rim held at International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) during February 09-11, 2014 at Dhaka, Bangladesh.
- Participated in Climate factors role in cholera prevalence in Kolkata, India: Time series models at the 48th Joint Meeting and Conference of the US-Japan Panel on Cholera and Other Bacterial Enteric Infections held at International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) during February 11-13, 2014 at Dhaka, Bangladesh.

Electron Microscopy

The Division of Electron Microscopy is engaged in research and diagnosis in the field of diarrhoeal diseases. These include 3-dimensional image reconstruction using cryo-electron microscopy, negative stain analysis, ultrastructural and histopathological studies. The division also organized one workshop on electron microscopy which was attended by a large number of participants from different research institutes, universities and hospitals. During the reported period, two virulent enteroaggregative strains (EAEC) were isolated from our culture collection and were studied histologically in animal model by light microscopy. Enteropathogens like *H. Pylori, V. cholerae* and *A. hydrophilae* related to different projects were studied both by light and electron microscopy. Haemagglutinating activity (HA) and colonization ability of *Shigella* were studied in suckling mice model. Pathogenic bacteria usually develop various surface structures known as capsule, slime, glycocalyx or fimbriae whose primary function is to interact with receptors on the membranes of target cells. These surface structures of the bacteria have been studied ultrastructurally. Outer membrane vesicles (OMVs) of *Shigella* as a candidate vaccine was studied in animal model ultrastructurally. Gut microflora in neonatal sepsis with special reference to gm -ve bacteria were studied in animal model histologically.

Scientist:

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

Staff:

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

Arsenic-resistant bacteria from contaminated water-bodies in West Bengal, India

Investigators: S. Majumder, D.R. Saha, Dr. R. Goswami

Bengal Basin is known for severe arsenic contamination. In the present study, we have isolated six bacteria from the arsenic contaminated surface water of Bengal Basin. 16S rDNA sequence analysis identified them as *Microbacterium oleivorans, Acinetobacter soli, Acinetobacter venetianus, Acinetobacter junii, Acinetobacter baumannii, Acinetobacter calcoaceticus*. All the isolates possess arsenic accumulation potential and high molecular weight plasmid (>10 kb). PCR amplification indicated the presence of arsenic-resistance genes (*arsB* and *aoxB*) either in the genome or plasmid or in both in the isolated bacteria (except in *Acinetobacter venetianus*).Transmission electron microscopy showed arsenic exposure affected bacterial growth and induced alteration in cytoplasmic membrane integrity. Despite being resistant to arsenic, this metalloid induced ultra-structural changes to varying extent in all the isolates. It is important to note that the ultra-structural changes observed in the present study are akin to lead-induced changes reported in *A. hydrophila* suggesting these as general response subsequent to metal-stress in bacteria. Bio-remediation is an effective tool to reduce contaminant toxicity to levels that are innocuous to human health and ecosystem & bacteria have their own mechanisms for metal accumulation and helps in bio-remediation. The advantages of using microbes for bio-remediation include natural occurrence, cheap production and easy availability to treat large volumes of waste

water. Time and concentration dependent accumulation of arsenic in the isolates was observed suggesting the usage of the isolates for bio-remediation in arsenic-prone areas. This study provides valuable information of microbial species in surface waters of arsenic affected areas which could be probably responsible for natural arsenic speciation. It adds basic inputs regarding the possible mechanisms of arsenic tolerance in these bacteria and provides a platform for future study of arsenic-remediation through microbial route in endemic areas.

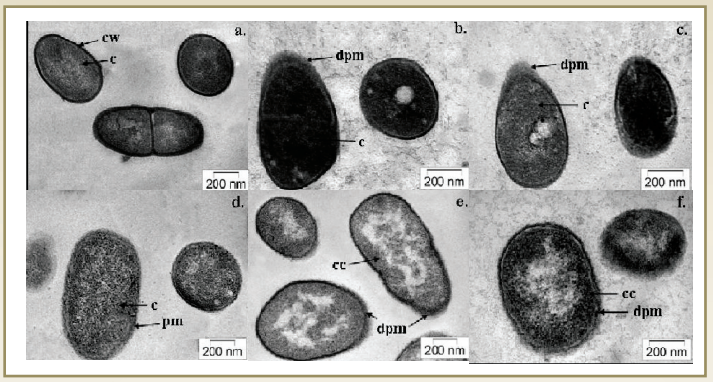


Fig. 1. Arsenic-induced ultra-structural changes. Bacteria were exposed to different concentrations of arsenite (0.1 and 1 mg L-1) and structural alterations studied. a) unexposed Ransu-1; b) 0.1 mg L-1 exposed Ransu-1; c) 1 mg L-1 exposed Ransu-1; d) unexposed IBL-1; e) 0.1 mg L-1 exposed IBL-1; f) 1 mg L-1 exposed IBL-1

Conferences/ Seminars/ Workshop/ Training Attended/ organized

A. N. Ghosh

- Delivered invited talk "Pore-forming toxin of *Vibrio cholerae* and its interaction with erythrocyte stroma: a cryoEM study" at the International Conference on Electron Microscopy and XXXIV Annual Meeting of Electron Microscope Society of India held at Kolkata during July3-5, 2013.
- Convened a workshop entitled "Cryo-electron Microscopy in Life Sciences" held at the National Institute of Cholera and Enteric Diseases, Kolkata, during July 1-2, 2013.

D.R. Saha

- Participated & presented a paper on 'Electron microscopic study of *Helicobacter pylori* and associated gastroduodenal diseases in Indian population' in an 'International Conference on Electron Microscopy and XXX1V Annual meeting of the Electron Microscope Society of India (EMSI), Kolkata India' July3-5, 2013.
- Attended a work shop on 'Cryo Electron Microscopy in Life Sciences' organized by National Institute of Cholera & Enteric Diseases, Kolkata& Electron Microscope Society of India, East Zone Chapter and Saha Institute of Nuclear Physics, Kolkata on 1st and 2nd July, 2013.
- Attended a workshop on **'Intellectual Property Rights in Medical Research'** organized by: Intellectual property Right Unit, Indian Council of Medical Research Department of Health Research, New Delhi. September 18-19, 2013 held at NICED, Kolkata.

Epidemiology

The Epidemiology Division continued its works, ranging from surveys and surveillance to development and/or testing of several public health interventions, especially in the area of diarrheal diseases, HIV and other related public health problems prevailing in the state. Highlights of some of the major activities are summarized below:

Development and testing of public health interventions:

A training module was developed and tested to improve diarrhea-related knowledge and practices of non-qualified practitioners in the urban slums of Kolkata. This work followed another institutionally funded study where these practitioners demonstrated very poor knowledge and practice. The main public health concernsbehind these studies included incomplete/inadequate treatmentof diarrhea leading to antibiotic resistance, complications and uncontrolled spread. If the intervention succeeds, it may be implemented at a larger scale. Based on a 3-year childhood diarrhea surveillance study that revealed important knowledge and practice gaps arising out of inadequate / inappropriate health information dissemination by the existing health services, a study has been taken up using a participatory intervention development approach aiming to develop and implement an improved health communication technique, especially for the rural populations.

Vaccine trials:

The vaccine-related works encompass a DBT-funded study that establishedthe safety and immunogenicity of anindigenously developed live recombinant oral cholera vaccine (VA1.4) among the adults through a double-blind, randomized placebo controlled trial. The study demonstrated that VA 1.4 at a single dose of $1.9X10^9$ CFU is safe and immunogenic in adults in a cholera endemic region. This study now paved the way for studies in the younger age groups and large scale field trials. The division is also working on a project to understand the biological basis of underperformance of oral vaccines, especially Polio and rotavirus vaccines. Another salient work is the establishment of different dosing schedules (4 weeks vs. two weeks) of the already licensed and marketed killed oral cholera vaccine, which showed no significant difference in immune responses of the two schedules. This study has got immense public health implication consideringits feasibility of administering along with EPI schedules. A multisite placebo controlled randomized trial to see the efficacy of an indigenously produced pentavalent recombinant live oral rotavirus vaccine was also undertaken.

Health surveys and operations research:

Apart from intervention studies, the division also carried out operational research like District Level Health Surveys, where more than 74,000 populationsfrom all districts ofWest Bengal were sampled for estimation of Hb%, blood sugar and dietary iodine in salt, as well as anthropometric measurements. Recently, the scientists carried out a community-based cross-sectional study among 6000 rural and 4000 urban families in Malda, one of the most backward districts with high infant & maternal morbidly and mortality. The primary objectives were to assess the perceived health care needs of the community along with availability and utilization of various health services. The study also explored other important health problems like diabetes, hypertension, childhood malnutrition and arsenic contaminated drinking water.

HIV-related studies:

An intramural project titled 'Care needs assessment of children living with or affected by HIV in selected districts of West Bengal' is currently being undertaken from the Division of Epidemiology of NICED. Field work has generated a registry in the district of PaschimMedinipur containing details of 362 children in which girls and boys are present in almost equal proportion. Of 362 children, 104 are living with HIV and the rest are not. The registry development has recently been initiated in the district of PurboMedinipur as well and has recorded details of 68 children. Less than one third of these children in Purbo Medinipurare living with HIV and the rest are HIV affected. Dr Samiran Panda is the Principal Investigator of this project. Health related 'Quality of Life' (QOL)of children living with or affected by HIV (where a child is free from HIV infection but one or both parents of the child are living with HIV) will also be explored under this project. At the first phase of this component, a socio-culturally appropriate tool to measure HIV specific QOL is being developed through formative research. Apart from the research studies, scientists of the division also conducted / coordinated a number of workshops for the graduate and post-graduate medical students and research communities.

Scientist:

Dr. Kamalesh Sarkar, Scientist 'E'
Dr. Samiran Panda, Scientist 'E'
Dr. Alok Kumar Deb, Scientist 'D'
Dr. Suman Kanungo, Scientist 'C'
Dr. FalguniDebnath, Scientist 'B'

Staff:

Dhiren Das Technical Officer 'A'
Swapna Manna, Technical Officer 'A'
RatanLalSaha, Technical Officer 'A'
SubratoSil, Technical Officer 'A'
ChandanMondal, Technical Assistant
Abhijit Chakraborty, Technician 'C'
B. Roy, Technician 'B'

Pre-Doctoral Fellow:

TanmayMahapatra
Aritra Das

District Level Household and Facility (DLHS) – 4 Survey in West Bengal

Investigators: K. Sarkar, and S. Kanungo

DLHS-4 was a nation-wide cross-sectional survey to collect the necessary information for planning on health related issues. It has two components – (1) household survey and (2) facility survey. DLHS-4 was attempted to have a number of Clinical, Anthropometric and Biochemical (CAB) information/tests to produce district level estimates for nutritional status and prevalence of certain life style disorders (Diabetes, Hypertension, Anemia & Iodization of household salt) not only among on women of reproductive ages and their children below the age of 6 yr., but also among all other members of households. NICED, Kolkata, was responsible for the CAB component of the DLHS-4 survey in all districts of West Bengal. Major constituents in the proposed CAB components are measuring height and weight, blood pressure,

estimation of hemoglobin, and plasma glucose along with testing of salt used by households for iodine component. NICED-DLHS-4 laboratory was set up to receive on an average 500 dried blood spot (DBS) samples per day collecting from different districts from the second week of January 2013 and last samples were received on first week of August 2013. A total of 74,305 blood samples were collected & tested on the same or next day with sending test results to NIHFW on every Monday. NICED was also responsible for training & supervision of field works, monitoring quality assurance of field equipments and internal & external quality control of laboratory test results.

Assessment of perceived illness and available health care facilities of Malda district

Investigators: K. Sarkar, and S. Kanungo

A community-based cross-sectional study involving 10,000 families (6000-rural & 4000-urban) was conducted in Malda District, which is known to be as one of the most backward north-Bengal districts with high infant & maternal morbidly and mortality. The primary objectives were to assess the perceived health needs of the community that require medical assistance along with assessing the problems of Diabetes, Hypertension among >18 yr. in the said district. The other objective was to assess the problem of regular exposure to arsenic contaminated drinking water by primary school students in vulnerable blocks of Malda District. The field work was initiated on 16 November 2013 and finished on Mid-June 2014. A total of 300 water samples were collected from 300 block primary schools' tube wells and all samples were transported at Regional Occupation Health Center (ICMR), Kolkata, for testing the arsenic content of them. Additionally, data on morbidity/mortality from hospital admitted cases was collected as per protocol. Water sample analysis showed that out of 300 water samples tested, 45.6 % (n=137) showed the presence of high arsenic content of >10 microgram/Litre as per WHO criteria of maximum permissible limit of arsenic for drinking water. Following figure shows the number of schools with level of arsenic content in their drinking water.

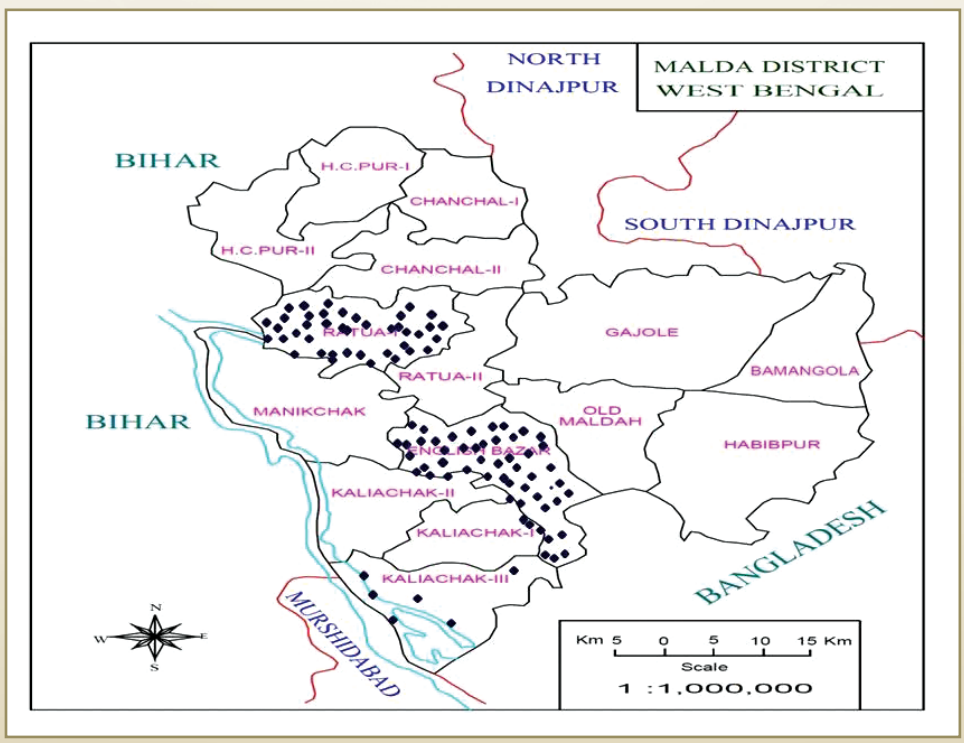


Fig 1 : Map showing with red spots indicating water sample collection blocks of Malda District

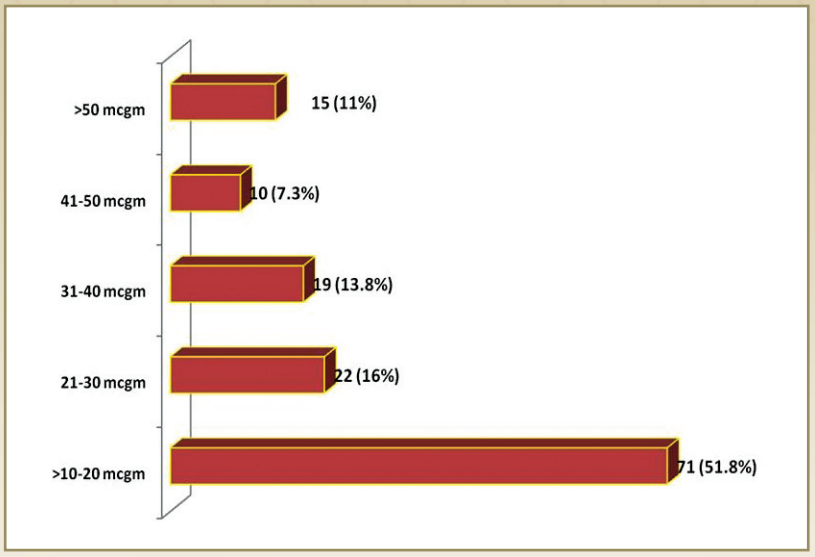


Figure-1. Number of schools with levels of arsenic content in their drinking water

Fig 2 : Water sample was being collected from a school

Interim analysis of socio economic data showed that more than one - fourth (26.5 percent) of households had either "No toilet facilities" "or "used open space / field / Jungle" for defaecation.

Out of the households using any kind of toilets (73.5 percent) the first three ranked types of toilets facilities used were:

I. Flush to septic tank – 31.7%

II. Flush to pit latrine – 25.4%

III. Pit latrine with slabs - 12.9 %

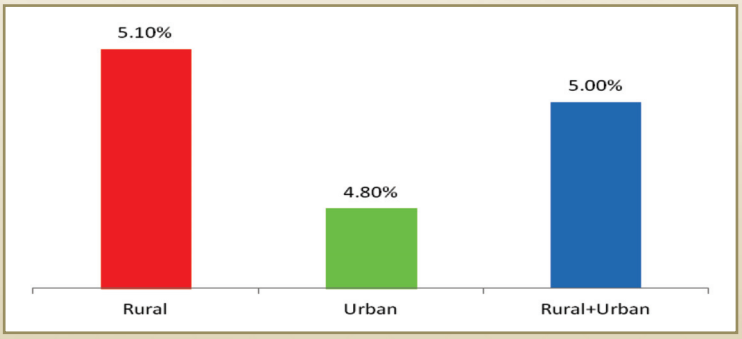


Fig 4 : Prevalence of Diabetes in rural & urban sector of Malda District

Assessment of nutritional status among primary and upper-primary school students in all districts of West Bengal

As a result of request from the Chief Secretary, Govt. of West Bengal, above-said project was designed and initiated to evaluate the impact of Mid-Day Meal services provided to the students of Government-sponsored/aided schools in West Bengal. Necessary initiates including obtaining funding, approval from SAC & IEC and recruitment of staff were taken to start the project w.e.f. April 2014.

Care Needs Assessment of Children Living with or Affected by HIV/AIDS in Selected Districts of West Bengal

Investigator: S. Panda

This work is currently being undertaken in the districts of Paschim Medinipur, Purbo Medinipur and Kolkata. As part of this on-going research, qualitative and quantitative investigation techniques are being employed. NICED collaborated with the civil society organization named 'Society for Positive Atmosphere and Related Support to HIV/AIDS (SPARSHA) for gaining access to study population. Issues around 'disclosure of HIV status to children' were one of the areas of investigation under this project. In order to generate understanding in this area, in-depth interviews with the informal care providers of 8 to 15 years old children living with HIV (CLH) were conducted. While some of the care givers were biological parents of the children in concern, relatives and neighbors involved in care were interviewed in case of orphans. The broader themes emerging from content analysis of the interview transcripts were: a) perceived difficulties around disclosure process, b) rationale for disclosure, d) reluctance to disclose, d) individuals best suited to disclose HIV status to children, and e) approaches to employ for the disclosure process. No difference between perspectives of biological parents and non-parents was found. While health care providers were identified as the preferred person by most of the primary care givers of CLH for HIV-disclosure, some expressed their willingness to take up the responsibility and a few wanted to leave the onus on the children. Assistance to care-givers to deal with disclosure and 'CLH reaching out to other such children' were two suggested approaches. The current research is the first of its kind in India to qualitatively explore caregivers' perception about diagnosis disclosure to CLH, and, thus, may inform policy making in this regard. Further information having programmatic and intervention implications will emerge from this on-going research.

Improving maternal and child health communications in rural areas of West Bengal - an innovative participatory intervention development approach

Investigators: A.K. Deb, S. Panda and A. Sinha

Following a childhood diarrhea surveillance in a rural community of one of the coastal districts of West Bengal, where insufficient knowledge and inappropriate practices of caregivers led to childhood diarrhea and resulted from inadequate access to health information, the current study was undertaken to explore the feasibility of development and implementation of an improved health communication technique. This qualitative study will look into the possibilities both from the health care user as well as provider perspectives. As part of the study procedures, information on existing / tested methods of newer health communication efforts from the country and elsewhere, especially those related to maternal and child health, are also being collected and compiled.

Awards and Honors

S. Panda

- Has been elected as the FELLOW of the ROYAL SOCIETY of PUBLIC HEALTH, UK

Conferences/ Seminars/ Workshop/ Training Attended/ organized

K. Sarkar

- Meeting of NNMB steering committee on April 2013 at National Institute of Nutrition, Hyderabad
- Meetings to review Mid-Day Meal situations in West Bengal on a number of occasions with Chief Secretary & Secretary, Dept. of School Education, Govt. of West Bengal at `Writers Building' & `Nabanna' – West Bengal State Secretariat.
- Organised a 3 days workshop to train field workers and supervisors to assess the perceived health needs, Diabetes, Hypertension & Arsenicosis (3-5 Nov. 2013).

S. Panda

- A Workshop titled 'Conducting Medical Research: Tools & Techniques' was organized at NICED at the behest of the Indian Medical Students' Association (IMSA) on 12th April, 2013.
- On 18th & 19th of September, 2013, 'Intellectual Property Rights (IPR) Workshop' was coordinated at NICED under the aegis of the Department of Health Research, Ministry of Health & Family Welfare, Government of India for ICMR intra and extramural scientists.
- Scientist from NICED acted as focal person & coordinated 'Continued Medical Education (CME) on various aspects of HIV' at NICED on 20th September, 2013 immediately preceding and linked with 8th Indian Association of Medical Microbiologists (IAMM) State Conference 2013.
- Attended as a faculty in the "Investigators Workshop on National Rotavirus Surveillance Network -Eastern Region" held at NICED, Kolkata during September 3-5, 2013.

A. Deb

- Attended and presented a poster titled COXBOX: An Affordable Medicine Dispenser for Overcoming Drug Non-adherence at the "International Knowledge Millennium Conference, (IKMC) 2013: Global Innovation Exchange" organized by IKP Knowledge Park and DBT during October 25-26, 2013 in Hyderabad.
- Attended South Asia Symposium on Pneumococcal Disease titled "Pneumococcal Disease in South Asia: The Promise of Vaccines" organized by the International Vaccine Access Center at Johns Hopkins Bloomberg School of Public Health and INCLEN Trust in Hyderabad on March 9th, 2014.
- Attended as a faculty in the "Investigators Workshop on National Rotavirus Surveillance Network -Eastern Region" held at NICED, Kolkata during September 3-5, 2013.
- Attended the Intellectual Property Rights (IPR) Workshop organized by the IPR Unit of ICMR at NICED, Kolkata on September 25, 2013.
- Attended as a member of the working group to plan and formulate study design and other issues related to the topic "Pneumococcal Conjugate Vaccine - Impact Evaluation in India " organized in New Delhi by the Johns Hopkins University, USA on November 14, 2013.

- Attended as a mentor the National Data Analysis Plan (NDAP) under the National AIDS Control Programme (NACP) - Phase IV to address the programme needs with respect to evidence and research and make the best use of available data. Organized by the Data Analysis and Dissemination Unit of Department of AIDS Control (DAC), MoHFW, supported by the CDC and FHI360 in JIPMER, Puducherry during January 16-18, 2014.
- Attended the National ToT for the IBBS organized by the Department of AIDS Control, Govt. of India, in New Delhi during March 18-23, 2014.

S. Kanungo

- Expert in the meeting organized by Dept. of Biotechnology, Govt of India, THSTI, Govt. of India and BIRAC, Govt. of India on " Cholera, Typhoid and Polio Vaccine-From Products to Policy to Practice", on 4th April 2013 at New Delhi, India.
- Presented Clinical Update at the Annual Meeting for NICED-IVI collaborative "PROVIDE" Study on May 5th - 7th, 2013 at Seattle, USA.
- Represented the institute and organized NICED activities and participated 17th National Exhibition on the theme "India Advancing towards the World Power" at Belur Math, Howrah during 21st through 25th September, 2013
- Oral Presentation on the topic entitled "A randomized controlled trial to evaluate the immunogenicity of two doses of the modified killed whole cell oral cholera vaccine under two alternate vaccination schedule" at 7th International Conference on vaccines for Enteric Diseases (VED), held on 6-8 Nov, 2013 at Bangkok, Thailand.
- Oral Presentation on the topic titled "Potential biological basis for poor performance of oral vaccines in developing countries: current understandings" at the 58th Annual Conference of Indian Public Health Association at the Shri Venkataswara Medical College at Tirupati, Andhra Pradesh from 22nd-24th Jan 2014.
- Participant in the workshop "Population based clinical Studies" as a part of Clinical Investigator Development Program, organized by PATH-OWH and CDSA, in collaboration with Kings Edward Memorial Hospital and Research Centre (KEM HRC, Pune) are organizing the training workshop from June 17 to June 20, 2013 at KEMHRC.

Immunology

Porin is the major outer membrane protein present in Gram-negative bacteria. Patients recovering from shigellosis produce convalescent antibodies to porin. The protein is primarily a TLR2-ligand that attracts attention because of its capacity to bridge innate signaling with adaptive immunity. Mucosal effector sites including intestinal lamina propria house B1 cells which provide first line of defense against pathogenic microorganisms. B-1a cells are predominantly found in the mucosal surfaces of peritoneal cavity and are the major B cell (~30%) subset that respond to foreign invaders. B-1a cells originate from precursor cells as a separate lineage than that of B2 cell population. Unlike conventional B2 cells, B-1a and B-1b are two sister populations which can operate independent of T cell participation. These cells produce 'natural' IgM and secrete dimeric and polymeric forms of IgA antibodies without any stimulation, indicating its preparedness to respond to pathogens. By virtue of their ability to migrate to gut-associated lymphatic tissues, B1 cells of peritoneal cavity reconstitute 50% of intestinal IgA plasma cells. Infective *Shigella spp.* breaches the intestinal homeostasis where B-1a cells are the key players of mucosal immunity. At present, the work of this laboratory focus on B-1a cell in orchestrating the adjuvant action of porin, which is antigenically related among the four *Shigella spp.*

Scientist:

Dr. T. Biswas, Scientist 'F'

Staff:

Swapan Kumar Shaw, Technician 'B'
Narayan Chandra Mondal, Attendant Services

Research Scientist:

Dr. R. Biswas

Pre-Doctoral Fellows:

Subhadeep Mukherjee
Debolina Sinha
Amlan Kanti Ghosh

Cytokine Regulation of Porin Stimulated B-1a Cell

Investigator : T. Biswas

Peritoneal B-1a cells of C57BL/6 mice were sorted by FACSAria II using fluorescence conjugated CD19, CD5 and CD11b antibodies. Total B cells from peritoneal wash were gated as $CD19^+$ cells, among which $CD5^+CD11b^+$ double-positive fraction yielded the desired B-1a population. The percentage of B-1a cells in the peritoneal cavity was analyzed to be 53%. The cells were of 98% purity. Porin treatment of B-1a cells up-regulated TLR2 indicating its role in the downstream signaling of the pathogen-associated molecule.

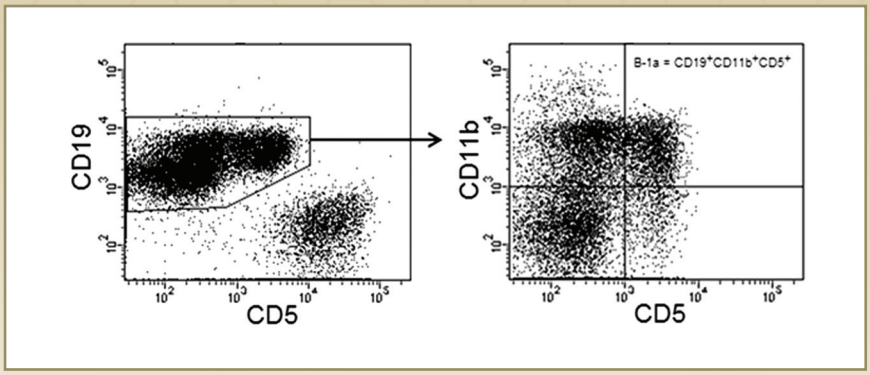


Fig 1. Purification of B-1a cells from peritoneal cavity of mice. The peritoneal cells were stained with PerCP-conjugated anti-mouse CD19, PE-conjugated anti-mouse CD11b and APC-conjugated anti-mouse CD5 mAbs. The stained cells were gated on $CD19^+$ cells, and further separated as $CD19^+CD11b^+CD5^+$ B-1a cells on a FACSAria II using FACSDiva software.

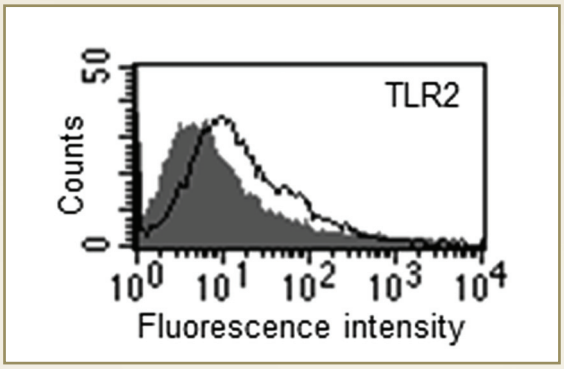


Fig 2. Porin-induced up-regulation of TLR2 on B-1a cells. B-1a cells were cultured with and without porin for 24 h and analyzed by flow cytometry for the expression of TLR2 as indicated by untreated (shaded) and treated (black line) cells. The data shown are representative of three independent experiments.

Awards and Honours

T. Biswas

- Served as invited reviewer of Infection and Immunity; World Journal of Gastroenterology.
- Served as member of the task force on Infectious Disease Biology, DBT, New Delhi (2012-2014).
- Editorial board member: World Journal of Immunology (2011-2015).

Conferences/ Seminars/ Workshop/ Training Attended/ organized

T. Biswas

- Seventh meeting of the task force on Infectious Disease Biology held on May 22-23, 2013 at National Institute of Immunology, New Delhi.
- Eighth meeting of the task force on Infectious Disease Biology held on September 24-25, 2013 at International Centre for Genetic Engineering and Biotechnology, New Delhi.
- Presented a lecture "Innate and adaptive immunity" in Continuing Medical Education Programme in Immunology at Rajendra Memorial Research Institute of Medical Sciences, Patna, October 18-19, 2013.
- Lecture and hands-on training on flow cytometry to Department of Regenerative Medicine and Translational Science, Calcutta School of Tropical Medicine at National Institute of Cholera and Enteric Diseases, January 15-17, 2014.

Parasitology

The division of Parasitology integrates the research into understanding the biology of major diarrhea causing parasites at cellular and molecular level along with the molecular epidemiological studies. Increasing understanding of human parasitic diseases like Giardiasis, Amoebaisis and Cryptosporidiosis can serve as the basic foundation for further development in screening, diagnosis and therapeutics research. The major areas of our interest are understanding the effects of oxidative stress on microaerophilic Giardia at its cellular, genomic, trancriptomic and metabolomic level and its relation with basic signaling pathway in response to oxidative stress, to understand the extent of genetic diversity among the clinical isolates of Giardia and Entamoeba and its correlation with the disease outcome. Along with this major study we also engaged with extensive surveillance study of enteric parasites.

During the calendar year 2013-14 high extent of genetic diversity among the Giardia isolated form diarrhoegenic population of Kolkata were identified along with identification of mixed or recombinant genotypes. We have also addressed the possibility of Zoonotic transmission among Giardia and Cryptosporidium between human to other mammals as well as environmental transmission through contamination of drinking water source were evident. High resolution genotyping of *Entamoebahistolytica* isolates using tRNA linked short tandem repeat as the molecular marker showed presence of new pattern of repeat orientation which is unique to Indian origin. Significant relation between genotypes and disease outcome in amoebiasis could be addressed and we could also prove that genotypes found in asymptomatic isolates is evolutionary much closer or have significant association with the isolates found in Liver abscess cases as per our genotyping results. It has been observed for the first time that Giardia trophozoites at high oxygen environment produces higher reactive oxygen species (ROS) in a time dependent manner. It is also evident that mtochondrialremnant proteins are not the key proteins from stress regulation, rather a cascade of other biochemical pathways and proteins are involved in stress relief. Surveillance study revealed that Giardia still remained as the major parasite in the diarrhoegenic population of Kolkata. A new method of KatoKatz screening was successfully used in estimating the soil transmitted helminth burden in the state of western India. The survey estimated the prevalence of Ascaris around 20% in the study region.

Scientist:

Dr. Sandipan Ganguly, Scientist 'D'

Staff:

Mr. S. L. P. Singh, Sr. Laboratory Attendant

Pre Doc Students:

AvikKumar Mukherjee
Koushik Das
Dibyendu Raj
Sumallya Karmakar

Award:

Arjun Ghosh received Ph.D. degree, Jadavpur University.

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

Investigator: S. Ganguly

The study was formulated to assess the extent of genetic diversity or similarity among the clinical *Entamoeba histolytica* isolates with differential disease outcome. Three tRNA linked loci and one protein coding regions were analysed to see the genetic variations among the *E. histolytica* isolates of asymptomatic, diarrheal and amoebic liver abscess cases. Most of repeat patterns were newly assigned and possess specific relation with diseases outcome groups. No repeat patterns were found in case of tRNA linked STR loci which were present in all three disease groups (Fig. 1). So, it could be inferred that the STR loci may have comparatively high degree of resolution for determining the relationship between parasite genotypes and outcome of amoebic infection. Statistical analysis (Table 1) revealed that repeat patterns which showed strong positive association with LA (liver abscess), also showed strong positive association with AS (asymptomatic) or vice versa. Whereas, repeat patterns which showed positive association with D (diarrhoea), showed strong negative association with and AS group. So, isolates derived from liver abscess and asymptomatic cases may be genetically closer or may have originated from similar lineage rather than, isolates from diarrheal outcome. The finding was well supported by the clustal analysis where STR patterns exclusive from asymptomatic (AS) and liver abscess (LA) group forms a single cluster in DA-H and NK-2 tree, whereas STR patterns exclusive for diarrheal (D) always forms a separate cluster in all three trees (Fig. 2).

Table 1. Repeat patterns of target loci showing significant association with disease outcomes.

Loci	Repeat pattern	Liver abscess (LA)	Diarrheal (D)	Asymptomatic(AS)
D-AH	6DA	Co-eff[a]: 0.507 p[b]= 0.0026	Co-eff[a]: -0.385 pb = 0.029	X[c]
	14DA	Co-eff[a]: 0.403 p[b] = 0.0096	Co-eff[a]: -0.684 p[b] = 0.000004	Co-eff[a]: 0.281 p[b] = 0.0026
N-K2	18NK	Co-eff[a]: -0.404 p[b] = 0.0116	Co-eff[a]: 0.532 p[b] = 0.0008	X[c]
R-R	IND11RR	X[c]	Co-eff[a]: -0.619 p[b] = 0.0004	Co-eff[a]: 0.286 p[b] = 0.0082
Chitinase (CHI)	NIH:200	Co-eff[a]: -0.429 p[b] = 0.0014	Co-eff[a]: 0.577 p[b] = 0.00001	X[c]
	HM1:IMSS	X[c]	Co-eff[a]: -0.456 p[b] = 0.0173	X[c]

[a]Correlation co-efficient value of the particular association indicate whether the association is positive or negative.

[b]Probability value of the particular association.

[c]Does not have any significant association with disease outcomes.

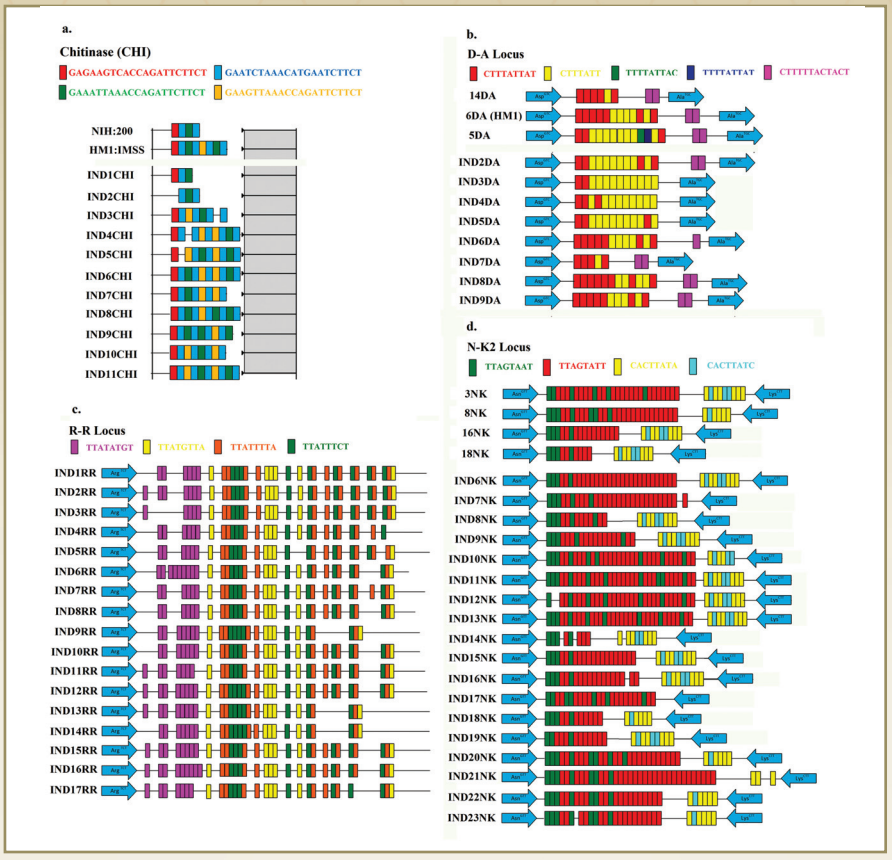


Fig. 1. Schematic representation of tandem repeat types within target gene loci where the repeat units are depicted in rectangles with specific colour coding along with the repeat sequences while non-repeat regions are shown in lines.a: Repeat pattern within chitinase gene; b: Repeat pattern within DA-H loci; c: Repeat pattern within R-R loci; d: Repeat pattern within NK-2 loc.

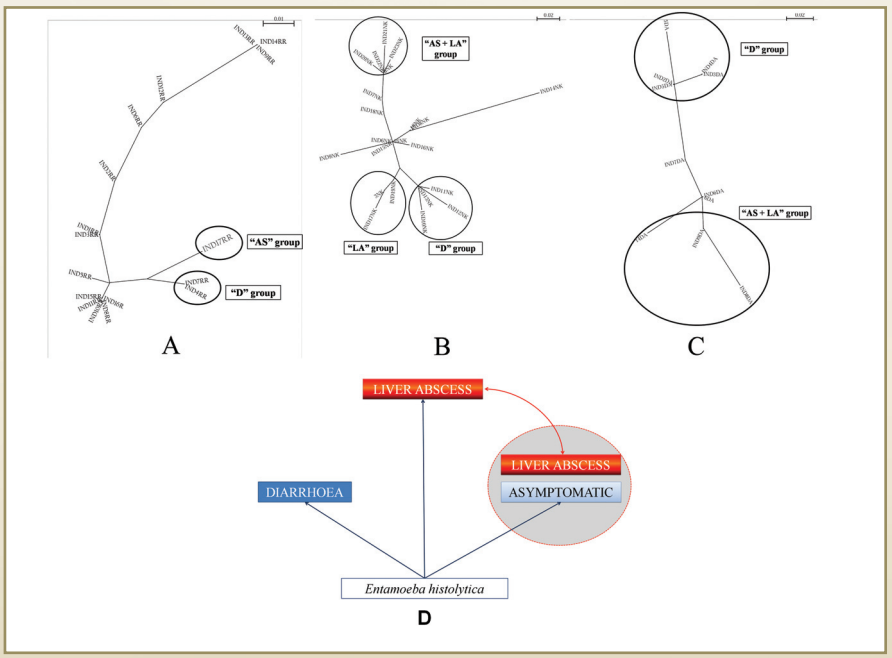


Fig. 2. The phylogenetic analysis showing cluster patters of different repeat type in three selected loci "RR (A)", "NK (B)" and "DA (C)". Schematic representation based on the phylogenetic analysis showing genotype of asymptomatic cases more closely related to that of liver abscess cases and have possibly originated from same lineage (D).

Studies on burden of parasitic infections among different communities in Western part of India to support health impact evaluation of Total Sanitation Campaign.

Investigator: S. Ganguly

This study was performed from samples from different parts of semi urban and rural western India. Recent technological advances of parasites and helminthes have improved our understanding of host specificity, clinical manifestations and transmission mechanisms, although no previous information is available in this part of India in different communities about their incidence, prevalence and disease burden. The overall objective of this study is to gather basic epidemiological data on the prevalence, disease burden and transmission of different helminthes and parasites in children below 12 years of age. Modified Kato katz method was applied for helminth detection and PCR was adapted for other diarrheagenic parasites. The principal aim of this impact evaluation is to estimate the impact of the methods/tools (interventions) such as Community Led Total Sanitation (CLTS) implemented under the auspice of Government of India's Total Sanitation Campaign (TSC) on the health and welfare of the rural poor.

The prevalence of Ascaris, Trichuris, and Hookworm is presented in the Table with estimated 95% confidence interval. The sample was designed for state level representativeness and precision of 3% so that it is expected that the 95% CI is wider at district and block levels. However, the survey has estimates the prevalence at state level with a very high precision. Given the average rate of Ascaris isolation was around 20% so annual deworming was recommended for the study region. Table 2. Prevalence of Ascaris lumbricoides, Trichuris trichiura and Hookworm (mean [95% ci])

Total Sample	Ascaris	Trichuris	Hookworm
2167	21.87% [20.13% to 23.61%]	3.69% [2.9% to 4.49%]	4.66% [3.77% to 5.55%]

Differential pathogenesis of Giardia: Role of Giardia Virus.

Investigator: S. Ganguly

Giardia genotyping result showed presence of mixed assemblages among the Kolkata isolates (Fig. 3) but the exact reason behind this was not known. It is supposed that the GLV may play an important role in transferring genetic material from one Giardia cell to another. And may be GLV can also act as a Transposable Genetic Element facilitating the transfer and recombination of genetic material. So our goal was to identify the GLV infected Giardia strain in the local population. This would be followed by genetic screening of GLV depending on target markers for understanding the possible recombination event. Giardia positive stool samples were taken randomly from the surveillance program and were subjected to viral RNA isolation using Viral RNA Minikit, Qiagen. cDNA preparation and PCR amplification was performed by using Superscript-III One Step RT-PCR kit, Invitrogen. Various amounts (1–2 ng) of eluted RNA were subjected to RT-PCR using the SuperScriptIII One-Step RT-PCR kit and primers directed to the GLV-capsid protein sequence (GenBank L13218). RT-PCR products were analysed by polyacrylamide gel electrophoresis followed by ethidium bromide staining (Fig. 4). The desired bands were subjected to DNA sequencing. But the sequence obtained showed no significant similarity with the caqpsid protein of GLV inspite of the use of specific primers. Rather it showed more similarity with the Giardia house-keeping genes. Our next phase would be to try to isolate the GLV particles and to develop a positive control which would act as a major resource for conducting this study.

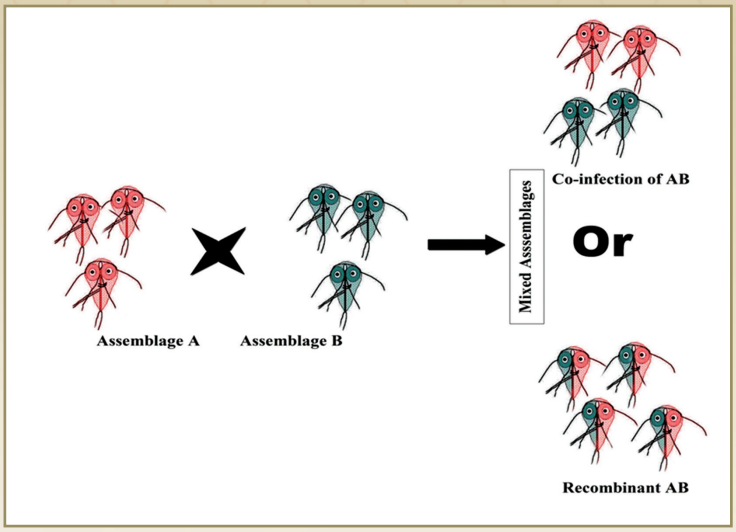


Fig. 3. Possible insights of Giardia genotypes in an endemic region like Kolkata

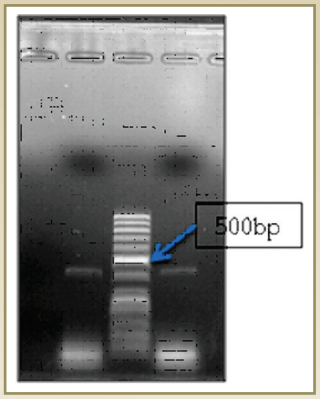


Fig. 4. cDNA and PCR amplification using Superscript III one step RT PCR kit, Invitrogen using Ref primer for GLV capsid protein.

Awards and Honours

S. Ganguly
Nominated committee member,
board of studies of Department of Microbiology,
St. Xavier's College, Kolkata.

Conferences/ Seminars/ Workshop/ Training Attended/ organized

- "NICED-NIID Joint Forum on collaborative projects" held in Tokyo, Japan from September 25-27, 2013 and delivered oral presentation entitled "Differential Pathogenessis of Giardia: Role of Giardiavirus.
- "Annual Asian-African Research Forum" held in Sendai, Japan during January 20-22, 2014. Organized by the Japan Initiative for Global Research Network on Infectious Diseases. Presentation on "Role of pyruvate in oxidative stress relief in Giardia".
- Participated and presented a research paper in Okayama University Forum for Emerging Enteric Diseases in Okayama, Japan during January 24-25, 2014
- Invited participation as a resource person for training in the workshop of the participants in 2nd Kolkata Annual Research and Medical (and Dental) International Congress, KARMIC 2013 at NICED, India on 12th April, 2013.
- Participation in "National Workshop on Good Laboratory Practice" held at National Institute of Occupational Health, Ahmedabad, ICMR, organized jointly with NIOH and ICMR from 9-10th Sep, 2013.

Pathophysiology

The Division of Pathophysiology, NICED is actively undertaking research on various project related to different diarrhogenic bacteria. This division also aims to develop an effective and cheap candidate vaccine against shigellosis, super ORS and use of proper antibiotics against diarrhoea. Study on vaccine development against shigellosis showed that oral administration of heat killed *Shigella flexneri* 2a could give 100% protection against homologous challenge in rabbit model of shigellosis. The 34 kDa outer membrane protein (OmpA) of Shigella flexneri 2a was identified as a major protective antigen. This protein possesses the essential characteristics of a potential vaccine antigen like cross reactivity, surface exposed epitope and conservation among strains and can be developed as an ideal subunit vaccine against Shigellosis.

Our Division also works on microbial proteases from *V. cholerae*: their role in pathogenesis and their transport through outer membrane vesicle. The most well characterized protease in cholera is hemagglutinin protease. Almost all results of earlier studies suggest an indirect pathogenic role of HAP, we showed the direct role of HAP in pathogenesis of *V. cholerae*. We also reported the presence of a novel 59-kDa serine protease in a Δ*hapA*Δ*prtV V. cholerae* O1 strain and its role in hemorrhagic response in RIL model The major protease in *V. cholerae* hemagglutinin protease (HAP) and 59 kDa serine protease (VesC) are secreted by type II secretion system. Our data suggests HAP and VesC are tightly associated with outer membrane vesicles suggesting such vesicles play a role in secretion of these proteases. Vesicle containing these proteases could exert the effect known for this protease in tissue culture and animal models showing that the proteases are transferred by vesicles in active form. Outer membrane containing VesC showed hemorrhagic response in mice ileal loop and IL8 response in Int407 cells which was abolished in VesC knockout strains. Our results strongly suggest that outer membrane vesicles could be a means for *V. cholerae* to deliver proteases to the infected tissue. The other research interests include Outer membrane vesicles mediated transport of biologically active *Vibrio cholerae cytolysin* (VCC) from *V. cholerae* strains.

Scientist:

Dr. M.K. Chakrabarti, Scientist 'G'
Dr. A. Pal, Scientist 'E'

Staff:

B. Roy, Technician 'B'

DBT Ramalingaswami Fellow:

Dr. M. H. Kazi

Post-doctoral fellow:

Dr. T. Ray

Pre-Doctoral Fellow:

R. Tapader
Sk. Irshad Ali
R. Bhowmick
A. Mondal
P. Sarkar
J. Aoun
T. Saha

Awards:

- PoulomeeKarmakar received Ph.D. award from University of Calcutta, Kolkata in 2013.

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

Investigator: M. K. Chakrabarti

Previously we have shown that oral immunization with heat-killed whole cell *S. flexneri* 2a gives protection against challenge with virulent *S. flexneri* 2a both in rabbits and in guinea pigs. Moreover, we have also shown that 34 kDa outer membrane protein OmpA of *Shigella flexneri* 2a is cross-reactive, antigenically conserved among *Shigella* spp., and the epitope is surface exposed on the intact bacterium, established itself as highly immunogenic. 34 kDa protein has been found to up regulate the combinatorial expression of TLR2 and TLR6 on peritoneal macrophages of BALB/c mice. In addition to TLRs, 34 kDa OMP enhances the mRNA expression of adaptor protein MyD88, p38 MAP kinase, NF-κB, 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. We have cloned and overexpressed the 34 kDa protein from *S. flexneri* 2a (N.Y-962/92) genomic DNA. MALDI-TOF MS analysis of the purified 34 kDa OMP of *S. flexneri* 2a shows considerable sequence homology (Identity 65%) with the OmpA of *S. flexneri* 2a. We have found that OmpA significantly induces low levels of NO production by mouse macrophages in a time-dependent manner via phosphorylation of PKCα and consequent up-regulation of NF- κB activation. Knockdown of PKCα with siRNA significantly inhibits the OmpA-induced luciferase reporter activity and the release of NO.Our findings also indicate that silencing of TLR2 activation with siRNA results in the reduction of OmpA-mediated PKCα phosphorylation or activation, NF-κB luciferase activity, and production of NO, demonstrating that TLR2 activation facilitate NO-dependent OmpA processing via up-regulation of NO production. These results clearly exhibit an important role of PKCα in the TLR2-mediated release of NO from murine macrophages by OmpA with the involvement of NF-κB. We have also found OmpA of *S. flexneri* 2a trigger B cell immune response which regulates the surface expression of MHC II and CD86.

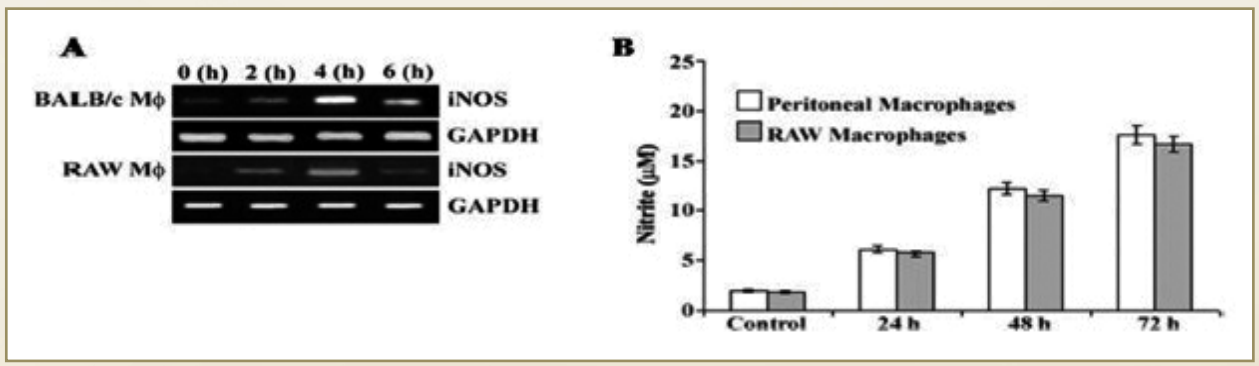


Fig 1: OmpA induces iNOS expression and NO production in peritoneal macrophages and RAW264.7 macrophages. Peritoneal macrophages and RAW264.7 macrophages were stimulated with OmpA (5 μg/ml), and mRNA was extracted at different time points. A, expression of iNOS was evaluated by RT-PCR. PCR products were quantified and expressed as the ratio of each product to GAPDH band density. The data are representative of three independent experiments. B, cell-free supernatants were collected at 24, 48, and 72 h after incubation of peritoneal macrophages and RAW264.7 macrophages with the antigen, and NO production in the supernatant was determined using a Griess assay. Results shown are representative of three independent experiments. Data are mean ± S.E., <p 0.005.

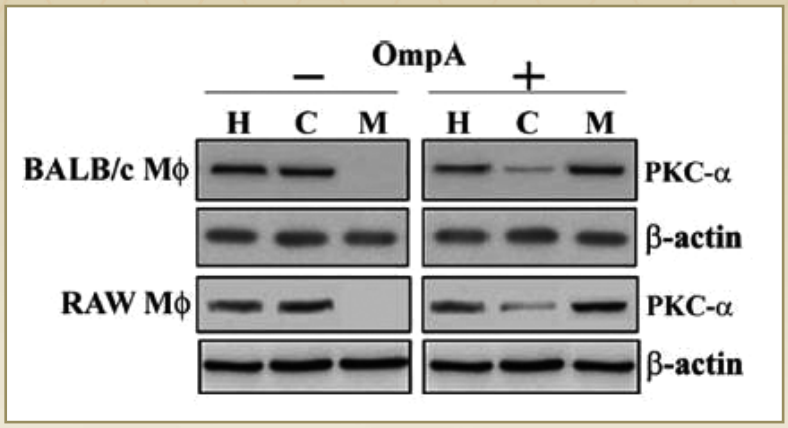


Fig 2: OmpA stimulated translocation of PKCα to the plasma membrane. Both peritoneal macrophages and RAW264.7 macrophages were stimulated with OmpA for 30 min. Total protein from cell homogenates (H) as well as the cytosolic (C) and membrane(M) fractions was electrophoresed and immunodetected by Western blotting. β-actin was used as an internal control. Results are the mean ±S.E.for three experiments.

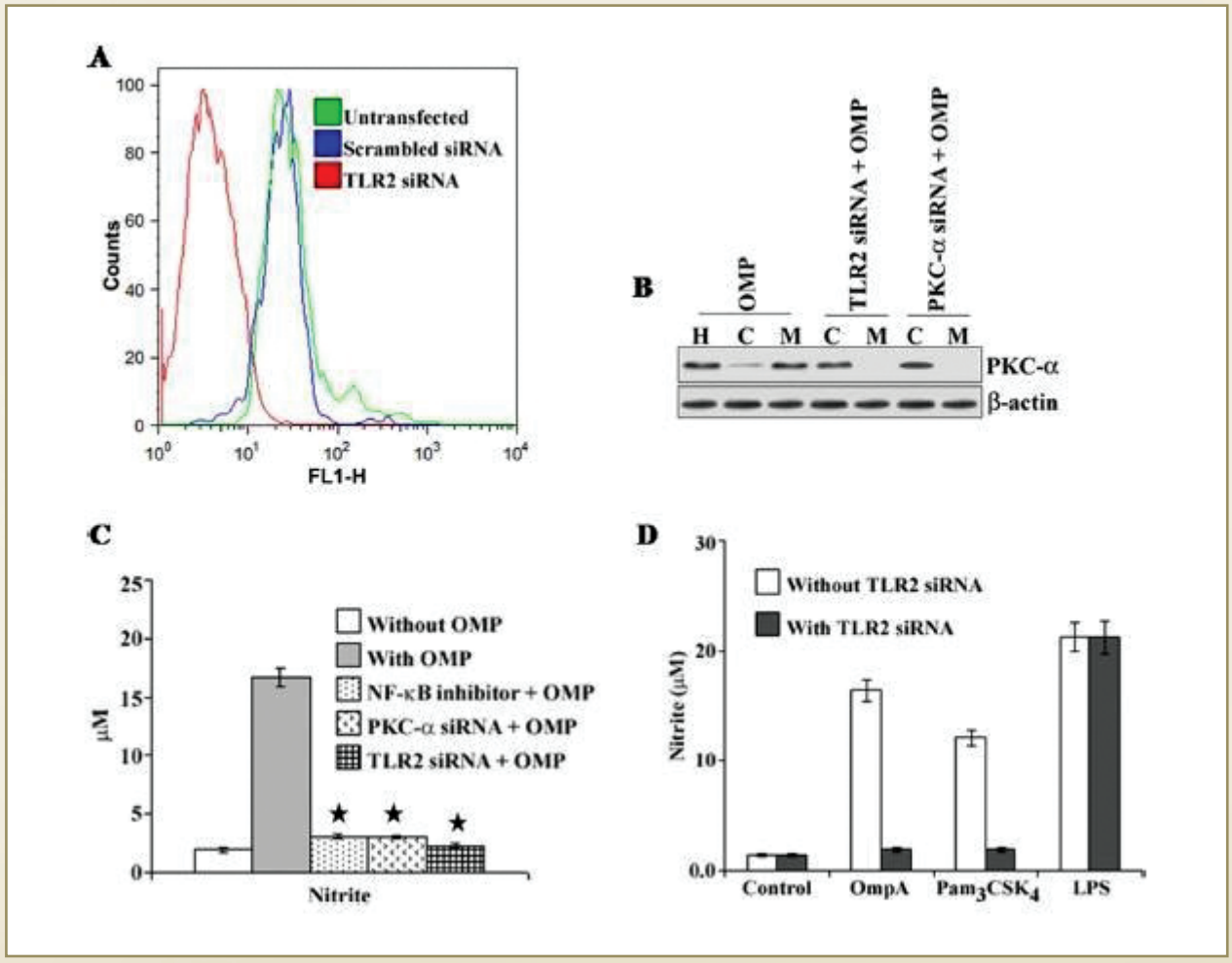


Fig 3: A, TLR2 knockdown with siRNA is detectable in mouse monocyte-derived macrophages using flow cytometry. RAW264.7 macrophages either remained untransfected or were transfected with siRNA against TLR2 or scrambled siRNA. After 48 h, cells were stained with FITC-conjugated TLR2 antibody. Cells transfected with TLR2 siRNA (red-lined histogram) had less TLR2 staining than the untransfected and scrambled siRNA-transfected cells (green-lined and blue-lined histograms, respectively). The data are representative of three independent experiments. B, OmpA induced PKCα activation depends on TLR2 expression. RAW264.7 macrophages were transiently transfected with either TLR2 siRNA or PKCα siRNA for 24 h separately followed by stimulation without or with

OmpA for 30 min. Total protein from cell homogenates (H) as well as the cytosolic (C) and membrane (M) fractions were electrophoresed. Activation of PKCα was then checked by immunoblotting with anti-PKCαmAb. The figures are representative of three independent experiments having similar results. C, OmpA induced NO production depends on TLR2, PKCα, and NF-κB. RAW264.7 cells were incubated in the absence and presence of OmpA or preincubated with either NF-κB inhibitor for 1 h or transiently transfected with TLR2 siRNA and PKCsiRNA for 48 h separately prior to addition of OmpA. The cell-free supernatants were assayed forNO accumulation after 72 h of incubation with the Griess reaction. Data represent mean ±S.E. of three independent experiments. *, p 0.001, relative to the OmpA-stimulated group in the absence of NF-κB inhibitor, PKCα siRNA, or TLR2 siRNA. D, NO induction by TLR2 ligands is diminished in RAW264.7 cells with the siRNA targeted against TLR2. RAW macrophages were transfected with TLR2 siRNA or remained untransfected and 48 h posttransfection, cells were stimulated with medium,OmpA(5μg/ml), Pam3CSK4 (1000 ng/ml), or LPS (1000 ng/ml). Nitrite production in the cell supernatants was measured at 72 h by the Griess reagent system. Data represent mean ±S.E. of three independent experiments, p <0.01.

Studies on Serine Protease Autotransporters of Enterobacteriacae (SPATEs) from clinical isolates of *Escherichia coli* causing neonatal septicemia

Investigators: A. Pal, and S. Basu

Serine protease autotransporters of Enterobacteriaceae (SPATEs) are secreted proteins demonstrating diverse virulence functions. The distribution of SPATEs is studied among diarrheagenic and extraintestinal pathogenic *Escherichia coli*. However, the contribution of SPATEs to the virulence of neonatal septicemic *Escherichia coli* (NSEC) has not yet been elucidated. This study was undertaken to evaluate the prevalence and phylogenetic distribution of different subtypes of SPATEs among NSEC. The presence of virulence factors and subtypes of SPATEs among different *E. coli* isolates was determined by polymerase chain reaction (PCR). *E. coli* phylogrouping was done by triplex PCR. Clonality of the isolates was assessed by pulsed-field gel electrophoresis (PFGE). The presence of SPATEs was significantly higher among the septicemic isolates (89 %) than the fecal (7.5 %) and environmental isolates (2.5 %) (Fig1). Vat (vacuolating autotransporter toxin) and Sat (secreted autotransporter toxin) were found to be the two most predominant SPATEs. The incidence of SPATEs was high in septicemic isolates of phylogroups A and B1 (87 %), lacking other virulence factors (Fig2). The high prevalence of SPATEs in the non-B2 phylogroups of septicemic isolates in comparison with fecal and environmental isolates indicates an association of SPATEs with NSEC. The NSEC isolates were found to be clonally distinct, suggesting that the high prevalence of SPATEs was not due to clonal relatedness of the isolates. This study is the first to show the association of SPATEs with NSEC. The presence of SPATEs in the septicemic/NSEC isolates may be considered as the most discriminatory trait studied here

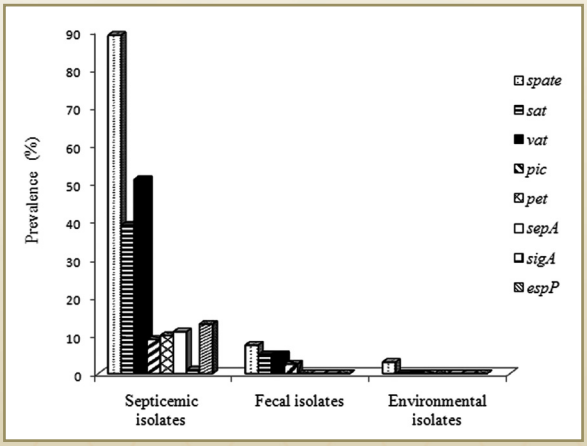


Fig. 1 Comparison of the prevalence of subtypes of SPATEs among three groups of isolates. Prevalence of the gene for SPATE sandits subtypes was significantly higher among the septicemic isolates compared to the fecal and environmental isolates. No isolate was found to be positive for genes *hbp/tsh, eatA* and *espC*

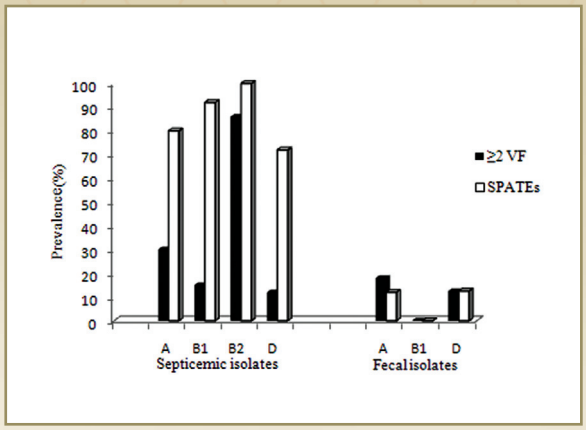


Fig. 2 Comparison of the occurrence of SPATEs and virulence factors between septicemic and fecal isolates. Among septicemic isolates of phylogroups A, B1, and D, prevalence of SPATEs was higher than the tested virulence factors. For fecal isolates the presence of SPATEs and VFs was almost negligible

Studies on proteases of *Vibrio cholerae*

Investigator: A. Pal

Hemagglutinin protease (HA/P) produced by *Vibrio cholerae* is the most well characterized protease in pathogenic Vibrio. The matured 45 kDa and 35 kDa processed forms of HAP were purified from a *ctx* gene negative *Vibrio cholerae* 01 strain. The 35 kDa HAP showed hemorrhagic fluid response in a dose dependent manner in the rabbit ileal loop assay. Histopthological examination of purified protease treated rabbit ileum showed the presence of erythrocytes and neutrophils in the upper part of the villous lamina propria, gross damage of the villous epithelium with inflammation, hemorrhage and necrosis. The 35 kDa form of HAP, when added to the luminal surface of the rat ileum loaded in an Ussing chamber, showed a decrease in the intestinal short-circuit current and a cell rounding effect on HeLa cells. Almost all results of earlier studies suggest an indirect pathogenic role of HAP but this study showed the possibility of a direct role of HAP in pathogenesis (Infect & Immunity 2006). The other well characterized protease secreted by *Vibrio cholerae* is PrtV a metalloprotease which has a role in the protection from predator grazing in natural aquatic environments and has also has a role in human pathogenicity. PrtV also modulated hemolysin which plays a role in inflammatory response in human epithelial cells. We have identified a novel 59 kDa serine protease from a ΔhapAΔprtV *Vibrio cholerae* 01 strain. The serine protease has been shown to cause hemorrhagic fluid response in RIL assay (PloS ONE, 2010). Proteases secreted in *Vibrio cholerae* play a role in its pathogenesis. We have also shown that HAP and serine protease is transported through outer membrane vesicles and play a role in its pathogenesis.

Awards and Honors

M.K. Chakrabarti

- Elected as Fellow, National Academy of Science, Allahabad, 2013
- Served as the permanent Council Member of Indian Science Congress Association, 2013-2014.
- As elected Council Member of the West Bengal Academy of Science and Technology. The Scientist has been actively involved in different scientific activities of the Academy.
- As Vice-President of The Physiological Society of India, 2010-2014 the scientist has been actively involved in the scientific activities of the Society.
- Member of the Editorial Board of Indian Journal of Physiology and Allied Sciences, Asian Journal of Experimental Sciences and Al Ameen Journal of Medical Sciences and Area Editor (Medical Sciences including Physiology) of "Everyman's Science".

Conferences/ Seminars/ Workshop/ Training Attended/ organized

M.K. Chakrabarti

- M.K. Chakrabarti Delivered a Plenary lecture on "Enteric Vaccines" at a seminar at Ramnagar College, Midnapore, West Bengal on 05.04.13
- M.K. Chakrabarti attended and delivered lectures at the Regional Science Congress organised by Kanpur, Chennai, Manipur and Port Blair Chapter of Indian Science Congress Association on 22-24th November 2013, 12-13th December 2013, 29th December 2013 to 1st January 2014 and 22-26th January 2014 respectively.
- M.K. Chakrabarti delivered an Invited lecture at the Animal and Veterinary Science Section of Indian Science Congress Association on "Outer Membrane Protein A of Shigella flexneri 2a promotes TLR2 dependent Immune Response" on 3-7th Feb, 2014.
- M.K. Chakrabarti delivered a popular lecture on "Innovation in Science & Technology for Inclusive development" at a seminar at Sagar Mahavidyalya on 07.03.2014.

A. Pal

- Attended the International Conference on Molecular Biology and its applications held at Jadavpur University, Kolkata on 14-15th February, 2014 and delivered a lecture on "Proteases and their role in pathogenesis in *Vibrio cholerae*"

Virology

Diagnosis, molecular characterization and functional aspects of diarrhoeagenic viruses

The researchers and staff of Division of Virology have been involved actively in the surveillance studies undertaken by the National Institute of Cholera and Enteric Diseases to understand the etiological role and disease burden of different diarrhoeagenic viruses in and around Kolkata. Molecular phylogenetic analysis of the circulating enteric viruses in and around Kolkata is being carried out with focus on Rotaviruses, Caliciviruses viz. Norovirus and Sapovirus, Astroviruses, Picobirnaviruses and Adenoviruses to study their genetic diversity and monitor the emergence of new strains and variants in stringent manner. The basic research activities cater towards understanding functional aspects of host pathogen interaction through analysis of the signaling mechanisms during Rotavirus- host cell interactions with special reference to study of host cellular proteins required for viral replication and pathogenesis

Other viruses of national importance

The Division has also extended its activites to include studies on influenza viruses and has organized a routine surveillance program in collaboration with World Health Organization and Centers for Disease Control and Prevention, Atlanta, USA for close monitoring of genetic diversity among circulating strains. The Division also maintains necessary laboratory facilities to carry out investigations during sudden outbreaks to diagnose the strain of influenza virus. The Virology Division of NICED has also made notable contribution to understand the transmission of HIV in North eastern states and conducted invaluable research to unravel the molecular aspects of HIV strains among infected individuals and their partners.

Human resource development and collaborations

The Division also serves to impart training to graduate and doctoral students and staff so as to improve the human resources capable of studying viral diseases of national importance across the country. The research programs include intramural projects and extramural projects with national and international funding and collaborating scientists. The current programs are associated with DBT, ICMR, NACO, WBSAP & CS, WHO, CDC Atlanta and Okayama University, Japan.

Scientist:

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

Staff:

Dr. S. C. Bhunia, Technical Officer A
S. Omesh, Technical Officer A
Dr. S. K. Sadhukhan, Technical Officer A
M. Mullick, Technical Officer A

K. Sen, Technician C
P. De, Technician B
MD M. Hossain, Technician B
C. Das, Attendant Services

Pre-Doctoral Fellow:

S. Nandi
R. Bhowmick
S. Chanda
S. Mullick Bagchi
P. Mandal
U. Patra
A. Mukherjee

Awards:

Shiladitya Chattopadhayay
received Ph.D. award from University of Calcutta, Kolkata

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

Investigator: T. Krishnan

Human astroviruses (HAstVs) were associated with acute gastroenteritis (AGE) among infants, younger children (up to 6 years), older children and adolescents (>6-17 years) and adults (18 years and above). Molecular characterization enabled monitoring of human astrovirus strains circulating among hospitalized diarrhea cases in Kolkata, India. Sole or mixed infections were detected among all age groups including adults; mixed infections included other enteric viral, bacterial and parasitic pathogens such as Rotavirus, Vibrio cholerae, Cryptosporidium spp and Giardia lamblia. Further, novel recombinations have been detected through sequencing of the highly conserved ORF1b (RdRp) region of seven human astrovirus strains in Kolkata, India. Primers were designed and the ORF1b region was amplified by RT-PCR and sequenced. Partition-wise phylogenetic analyses of the IDH1300 Kolkata strain did not show close homology to the reference strains. Further phylogenetic analyses of full length ORF1b region of the seven human astrovirus strains showed that they formed a close cluster with each other and displayed a separate lineage in comparison to reference human astrovirus strains worldwide. This study shows the emergence of novel recombinant human astrovirus strains in Kolkata, India, warranting stringent surveillance to monitor the genetic diversity of human astrovirus strains infecting different age groups.

Performance and diagnostic usefulness of ELISA and Rapid kits for detection of HIV in India.

Investigator: M.K. Saha

HIV poses a major public health problem throughout the world. India harbors the 3rd highest HIV infected population globally. The magnitude of the HIV detection challenge is enormous. Detection of infection HIV markers is a major challenge for testing laboratories in a resource poor setting. ELISA is the most commonly used screening technique for HIV. There is always an acute need for good quality ELISA kit. However, the quality evaluation data on Indian kits are very limited in comparison with internationally recognized kits. As blood transfusion is an important activity saving millions of live every year, it also carries a risk of transfusion transmissible infections caused by this fatal blood borne pathogens if the quality of testing is compromised. Conventional ELISA is regarded as the mostly used screening technique but due to limitations like high cost, unavailability in many blood banks and testing

sites, involvement of costly instruments, time taking nature and requirement of highly skilled personnel for interpretation, rapid tests are gaining more importance and warrants comparison of performance.

The ELISA kits evaluated using an in-house characterized 100 member sera panel revealed 100% sensitivity for all the batches. However, batch to batch variation in terms of specificity, positive predictive value (PPV) and efficiency, although not statistically significant ($p>0.05$), was observed. For specificity, the 3rd generation kits (mean 99.6% to 99.3%) were comparatively better than the 4th generation assays (97.2% to 96.9%). But the 4th generation kits performed far better in the ability for early detection post HIV infection in the 25 member sero-conversion panel with a margin of at least 22 days and as high as 35 days than the 3rd generation assays.

The commercial ELISA kits with 100% sensitivity seem appropriate for HIV screening. The ability of early detection post HIV infection favors use of 4th generation kits for ensuring HIV free blood for transfusion. Lot to lot variations, especially kits having the specificity level <98.0 %, indicate the need for a regular mechanism of kit evaluation for each batch for procuring kits appropriate for intended use. Rapid kits were more efficient in specificity with synthetic antigens along with high PPV than ELISA mostly. Rapid kits, though having high degree of specificity are not 100 % sensitive.

Surveillance and molecular characterization of Group A Rotavirus among children reporting with acute gastroenteritis

Investigators: M. Chawla-Sarkar, T. Ramamurthy, M.K. Bhattacharya, K. Rajendran

A total number of 353 and 390 stool samples (n=743) from hospitalized and OPD diarrhoeal patients (<5 yrs old) were screened for rotavirus during 2013-2014. The stool samples were screened for rotavirus using VIKIA Rota-Adeno kit detecting the VP6 antigen. Among 743 total samples, 338 samples (165 from hospital; 173 from OPD) were detected as rotavirus positive (45.49%). A large variety of genotypes were detected {G1P[8], G1P[6], G2P[4], G2P[6], G9P[4], G9P[8], G12P[6] AND G12P[8]} during this study. G9 (40%) followed by G2 (37%) were the most common types among hospitalized children. In OPD cases, G2 was most common (41%) followed by G9 (23%) and G1 (22%) genotypes. Unusual zoonotic strains were detected at low frequency (<3%).

Analysis of rotaviruses and their interactions with the host: A viral proteomics approach

Investigators: M. Chawla-Sarkar, N.S. Chatterjee

In cellular environment, viruses constantly adapt and modulate to survive and replicate while the host cell also responds to combat the situation and this results in the differential regulation of a large number of cellular proteins. To identify the virus induced differential expression of proteins, 2D-DIGE based proteomics was used. For this, HT-29 cells were infected with Rotavirus strain SA11 for 0 hours, 3 hours and 9 hours post infection (hpi) and differentially expressed spots were excised from the gel and identified using MALDI-TOF/TOF mass spectrometry. 2D-DIGE based proteomics study identified 32 differentially modulated proteins, of which 22 were unique. Calmodulin (CAM), a calcium regulating protein was induced by RV during early infection (3-4hpi). Co-immunoprecipitation experiment confirmed that Calmodulin directly interacted with RV-VP6 protein in absence of other viral proteins. CaM-VP6 interaction was also Ca^{2+} dependent as reduced interaction was observed in presence of Ca2+ chelators. W-7, a Ca^{2+}/CaM antagonist had no effect on CaM-VP6 interaction but was able to inhibit rotavirus infection by downregulating expression of viral protein. This is the first report where cellular proteins which are necessary for RV infection have been identified (Fig. 1). Inhibitors against these proteins are being analyzed for developing future antiviral therapies.

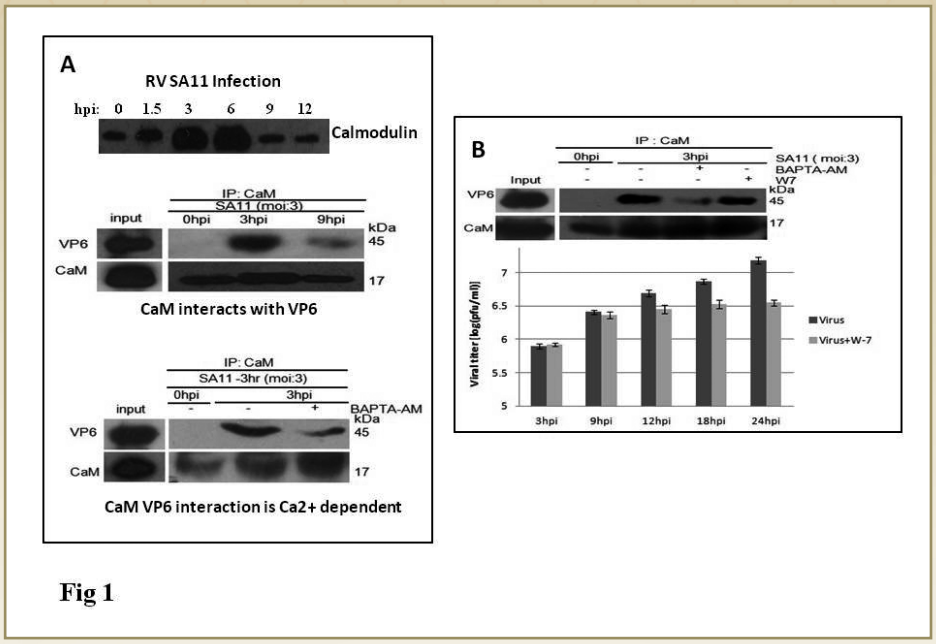


Fig 1

Fig 1. (A) During rotavirus (SA11) infection, Calmodulin (CaM) is upregulated during 1.5 to 6 hpi. CaM interacts with rotavirus structural protein VP6 in Ca2+ dependent manner during infection as in presence of Ca2+ chelator CaM-VP6 interaction is reduced. (B) CaM antogonist (W7) does not inhibit CaM-VP6 interaction but inhibits rotavirus replication

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

Investigators: M. Chawla-Sarkar, S. Kanungo

Nasal or throat swabs were collected from symptomatic patients (fever >37.5, running nose, cough/sore throat, body ache etc) from three hospitals in Kolkata (BC Roy Children's hospital, Nilratan Sarkar hospital and National Medical College and hospital) after obtaining informed consent form from the guardian/parent. A total of 962 samples were screened during this period of which 66 (6.5%) were positive for Inf A/B and one was positive for both A+ B. Of 66 samples 47 were typed as Inf A and 19 as Inf B . Of 66 real time PCR samples, 60 were inoculated in MDCK cells for virus culture. Of 60, 35 isolates were obtained. Of 35 isolates 21 were H3N2, and 14 were Inf B. One sample had both Inf A and B. Majority of samples were from paediatric population (0-5 yrs) and no correlation with gender was observed. The virus positivity correlated positively with rainfall as shown in previous years. Kolkata does not have cold winter and no secondary peak is observed.

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

Investigators: B. Ganesh, T. Krishnan, M. Chawla-Sarkar, M.K. Bhattacharaya, U. Mitra

This study was carried out with objectives i) Detection of human picobirnaviruses causing acute watery diarrhoea in children, ii) molecular characterization of human Picobirnaviruses (PBVs) detected among diarrhoeic children in Kolkata, and iii) single primer amplification, cloning and sequencing of complete nucleotide sequences of PBVs. Picobirnavirus infection in hospitalized adult patients with acute gastroenteritis in Kolkata has been identified and reported for the first time. In addition, picobirnavirus infection in porcine hosts has also been identified. Characterization virus genome of human and porcine origin has been made which suggested zoonotic transmission. The objectives were fulfilled and the project was allowed to get concluded as per SAC-2013.

Genomic characterization of human adenovirus (AdV) strains circulating in Kolkata and to analyze the cellular-signaling pathways activated during AdV infection.

Investigators: B. Ganesh, M. Chawla-Sarkar

This study was carried out with objectives i) Genomic characterization of capsid protein of human adenovirus strains (ADV40 & ADV41) identified in Kolkata, ii) cloning and expression of the fiber, hexon gene and penton base in intestinal cell lines to analyze the host-virus interaction, and iii) to study the signaling pathways activated by Adenovirus in cells. From August 2013 to May 2014, a total of 2159 diarrheic stool/ rectal swab samples were collected as part of our Hospital based diarrheal diseases surveillance study from the ID & BG Hospital, Kolkata and Dr. BC Roy Memorial Children's hospital, Kolkata. From this only 1229 neat diarrheic stool samples were taken and processed for adenovirus detection using the Vikia® Rota-Adeno kit (bioMérieux). A total of 84 adenovirus positives were detected, in which 83 were positive in children aged up to 5 years of age and one was positive in above 5 years category.

Awards and Honors

M.K. Saha

- Award of Excellence for outstanding performance by Division of Global HIV/AIDS, Centre for Disease Control (CDC), USA, in 2013 for qualitative HIV 1 early infant diagnostic testing using dried blood spots.
- Member for Immuno-Biological Diagnostic Kits Sectional Committee of Bureau of Indian Standards, Govt of India.
- Invited reviewer for PG Diploma course, Public Health Labs Modules in Applied Epidemiology (PGDAE) Program for Institutional and Technical Support Project, European Union – Govt of India Sector Policy Support Program, NIHFW, New Delhi.
- Extension of accreditation of the National HIV Ref lab as per ISO: 15189 by NABL.
- Invited as external examiner for Ph.D. thesis.
- Supervising the activities for National AIDS Control Program:
  - National HIV Reference Laboratory (External Quality Assurance Program for the states of A&N, Assam, Jharkhand, Meghalaya and Orissa)
  - Regional HIV Reference Lab (Early infant diagnosis using dry blood samples for all the 14 states of East and North-East India)
  - Consortium of National Ref Labs (Quality assessment of HIV, HBV & HCV test kits)
  - Regional Institute for HIV surveillance (HIV sentinel surveillance and Integrated Biological and Behavioral Surveillance –IBBS for WB, A&N, Sikkim, Assam, Meghalaya, Nagaland and Chhattisgarh)
  - Integrated Counseling and Testing Centre for HIV at NICED.

M. Chawla-Sarkar

- Awarded Fellowship (FNASc) of National Academy of Science in India (NASI) in 2013.

Conferences/ Seminars/ Workshop/ Training Attended/ organized

M.K. Saha

- Delivered lecture on "NABL Accreditation – the process involved & Quality control issues" in Continued Medical Education (CME) linked with 8th Indian Association of Medical

Microbiologists State Conference, at NICED on September 20, 2013 organized by Department of Microbiology, R G Kar Medical Collage, Kolkata .

- As invited speaker delivered lecture titled "Vaccine development: Opportunities and Challenges" in National Seminar on Strategies to Combat Diseases Threatening the Nation's Health at Gupta Collage of Technical Sciences, Asansol, on September 5, 2013, organized by Association of Pharmaceutical Teachers of India, Bengal Branch.
- Attended the workshop on Intellectual Property Rights in Medical Research organized by ICMR at NICED on September 18-19 2013.
- Attended the Training of National Trainers for Pre Surveillance Assessment, Bio-Behavioral Surveillance, conducted by NACO at NIHFW, New Delhi on May 1 to June 1, 2013.
- Attended the Training of National Trainers for Bio-Behavioral Surveillance conducted by NACO at NCDC, New Delhi on March 18-24, 2014.
- Organized EQAS workshop for HIV testing for the State Reference Labs of A&N, Assam, Meghalaya, Orissa and Jharkhand at NICED on September 6, 2014 and on January 23, 2014.
- Organized Pre-Surveillance Assessment: IBBS at NICED Kolkata during June 11-15, 2013.
- Organized Training on Pre testing questionnaires, Computer Assisted Personal Interview (CAPI) and Sample Frame Designing (SFD) for IBBS at NICED, Kolkata, March 10-11, 2014.

M. Chawla-Sarkar

- 5th Congress of European Microbiologists (FEMS), Leipzig, Germany during July 21-25, 2013. "Dual role of Matrix 1 protein in modulation of apoptosis during Influenza virus infection". (**Poster Presentation**).
- 5th European Rotavirus biology Meeting Valencia, Spain during 6-9 October 2013, "Multiple Strategies adopted by Rotavirus encoded Non-structural protein (NSP1) for evasion of cellular responses". (**Oral Presentation**).
- 5th European Rotavirus biology Meeting Valencia, Spain during October 6-9, 2013. "Community based case-control rotavirus surveillance study among Children (< 5 yrs old) revealed coexistence of large number of Genotypes and increased prevalence of G9 strains in Kolkata. (**Poster Presentation**)
- Asian-African Research Forum on Emerging and Reemerging Infections, Sendai, Japan during January 20-22, 2014. "Multiple functions of Rotavirus encoded Nonstructural proteins (NSPs) for Combating host Responses during Infection"(**Oral Presentation**).
- Organized Workshop and Training on "Surveillance and Laboratory Techniques for Indian National Rotavirus Surveillance Network: Eastern Region" funded by NICED, ICMR during September 3-5, 2013.
- Faculty in Training on "Conducting Medical Research: tools and Techniques" for Medical students attending IMSA organized by Kolkata Annual Research and Medical International Congress (Karmic 2013) on April 12, 2013 at NICED, Kolkata.

B. Ganesh

- Participated in a Two-day Workshop on "Intellectual Property Rights in Medical Research" organized by ICMR at NICED, Kolkata on September 18- 19, 2013.
- Participated in the 3-day Workshop on "Design and Statistical Analysis for Biomedical Research" organized jointly by BIC-NICED-ICMR, on March 25 - 27, 2014.

SERVICES

Services

Participation of NICED scientists in National Task Force (NTF) on Laboratory Containment of Wild Polioviruses.

- Drs. Sekhar Chakrabarti, Ranjan K Nandy, Mamta Chawla Sarkar and Suman Kanungo joined the programme of National Task Force (NTF) on Laboratory Containment of Wild Polioviruses. Under this programme, awareness visits to different Institutes/ Universities were made. These visits were also included verification of information submitted by the respective Institutes to NTF and to assign nodal person for each of the Institute who may be contacted for all future communications related to wild Poliovirus containment programme, as a part of national importance.

T. Ramamurthy

- NICED is assisting West Bengal State Govt. in the identification of cholera cases. During the current year NICED has screened 109 samples of which 32 were positive for *Vibrio cholerae* 01.

S. Dutta

- Confirmed the identification of *Salmonella* spp. and determined the serotypes of *Salmonella* isolates sent to NICED from various Medical Colleges of India. Timely feedbacks were sent to the concerned organizations.

M.K. Bhattacharya

- Actively in involved in routine, teaching of internees from different medical colleges of Kolkata at diarrhea treatment unit located at ID & BG Hospital, Kolkata and also the trainee who come from abroad. My special assignment included to work as a course facilitator in a number of workshops carried out at NICED, Kolkata.
- Coordinating submission of weekly report on diarrhoeal disease surveillance to ID & BG Hospital and B. C. Roy Hospital, Health commissioner of Kolkata Municipal Corporation and Health dept. of Govt. of West Bengal for keeping them aware on the status of the isolation of the *Vibrio cholerae* so as to enable them to take necessary action to prevent the outbreaks and to provide the better management of the diarrhea patient.

A. Palit

- During the epidemic outbreaks (2013-14) of diarrhea spreading across different southern districts of West Bengal, microbial analysis and examination of samples of potable water sources, from different parts of West Bengal and reporting of results to the Govt. agencies, has been a routine activity of the "environmental laboratory".
- Water samples had been received from different PHCs of Howrah, N. 24 Pargana, Nadia and Hooghly as well as from endemic and epidemic affected Municipal wards under the Kolkata Municipal Corporation and its adjoining areas. Results have been conveyed to the respective agencies with a copy of the same to State Health secretariat, Govt. of West Bengal. During the period under report, 66 samples had been received from various sources of which 28 had been found to be positive for culture positive and 9 for presence of PCR positive *V. cholerae* 01 and 28 samples for *E. coli* (Table 1).

Table - 1

Sl. No.	District	No. of samples received	Sources					Culture Positive V. cholerae	PCR positive V.cholerae	E. coli
			Tap	Tube well	Pond	Unknown	Stored			
1.	Howrah	14	10	-	-	-	-	5	2	8
2.	North 24 Parganas	11	11	-	-	-	4	8	3	4
3.	Nadia	2	2	2	-	-	-	1	-	1
4.	Hooghly	39	-	23	7	3	4	14	4	15
	Total	66	23	25	7	3	8	28	9	28

- Specially invited by RMRIMS (ICMR), Patna for "potable water sample analyses" from outbreak foci of Muzaffarpur district, Bihar & Nepal, June 25-28, 2013. Demonstrated technical aspects of know-how, handling and usage of "field kits" in emergency "outbreak" situations.
- Invited for display and demonstration for "technology transfer" of outbreak water sample analyses in emergency situations and rapid detection methods of water samples of endemic foci.

K. Sarkar

- Looking after National Nutrition Monitoring Bureau, West Bengal Unit as Officer-in charge since April 2009 till date. This unit is conducting a study on life style diseases (Problem of Diabetes, Hypertension, Dyslipidaemia, Obesity etc.) of 6-urban cities of West Bengal. The staff are being supervised both technically as well as administratively in co-ordination with National Institute of Nutrition, Hyderabad.
- Worked as Steering Committee Member, National Nutrition Monitoring Bureau.
- Worked as Expert Committee Member of Mid-Day Meal Programme, Govt. of West Bengal.
- Reviewed Mid-Day Programme situation in Schools of Uttar Dinajpur District.
- Prepared a Training Manual on Nutrition Assessment for School Children.

A. Deb

- Served as External Examiner for thesis and viva-voce for the Master of Medical Science & Technology course of Indian Institute of Technology (IIT), Kharagpur.
- Reviewer of research proposals and project works of undergraduate medical students for Short Term Studentships (STS) under the Indian Council of Medical Research.
- Associate Member, Drinks and Drinking Water Sectional Committee, FAD 14, Bureau of Indian Standards, Govt. of India.
- Chief Trainer, Eastern Region, Integrated Biological & Behavioral Surveillance (IBBS), National AIDS Control Program – IV, Dept. of AIDS Control, Govt. of India.

S. Das

- Assisted in computational analysis of genomes and proteins and statistical analysis to the scientists and research scholars from NICED, other research institutes, regional medical colleges and universities.

S. Ganguly

- Training, support and collaborative work on confocal microscopy to students and scientists of other divisions of this institute and from other Research Institutes like RMRI, Patna, Bose Institute Kolkata etc.
- Training and support on parasite detection and isolation.

- Field studies have been performed during last fiscal year from this division, in Chakdah, Nadia, West Bengal for investigation of presence of different enteric parasites by improper hand wash. And in Indore, MP for identification of different parasites among rural populations.
- QC and QA support facility in eastern India for parasitic detection under Indo-US joint program.

M. Chawla-Sarkar

- The virology lab provides laboratory diagnosis for Influenza A viruses (H1N1/ H3N2/ H5N1) and Group A Rotavirus for referred cases from Hospitals in Kolkata for effective patient management.

M.K. Saha

- National HIV Reference Laboratory, National Institute of Cholera and Enteric Diseases (ICMR) has been Assessed and Accredited in accordance with the International Standard ISO-15189:2007,Medical Laboratories - Particular Requirements for Quality and Competence in the field of Medical Testing (Microbiology and Serology) for the duration from08-09-2011 to 07-09-2013 and further renewed from 06-02-2014 to 05-02-2016 with the scope of HIV Testing employing Rapid Immunoassay, ELISA and Western Blot.
- NICED Lab as a member of "Consortium of National Reference Laboratories for Kit Quality" evaluated HIV, HBV and HCV kits for quality assurance for all the National procurements for AIDS Control Program (NACP III and NACP IV).
- NICED Lab has been implementing HIV testing Quality Assurance program for the State Reference Laboratories of Andaman & Nicobar Islands, Assam, Jharkhand, Meghalaya and Odisha.
- Regional Institute (East), NICED, implemented HIV Sentinel Surveillance (HSS) for the states of Andaman & Nicobar Islands, Meghalaya, Nagaland, Sikkim and West Bengal with the objectives to monitor the (i) trends in prevalence of HIV infection, (ii) distribution and spread of HIV prevalence in different population subgroups and in different geographical areas and (iii) to identify emerging pockets of HIV epidemic in the country.
- The role of NICED as Regional Institute (East) is also to focus on technical aspects of Integrated Biological & Behavioral Surveillance (IBBS) implementation starting from Pre surveillance Assessment, Pre testing of tools for IBBS, regional level & field level trainings, coordination meetings with NACO, SACS & FRA, monitoring & supervision of rapid field assessment, sampling frame development and field survey in assigned domain of 5 States respectively Assam, Meghalaya, Nagaland, Sikkim & West Bengal. RI (E) also has an important role in web based data management of IBBS and valuable inputs regarding the necessary modification of web based system (Integrated Information Management System) and tablet based application.
- Molecular diagnosis of HIV among babies (upto 18 months) born to HIV infected mothers employing Dry Blood Spot (DBS) samples, for the first time in the country in such a massive scale covering all the 14 eastern and north-eastern states of India being done at NICED Lab to address the monumental challenge of implementing this Nationwide program.
- The NICED lab received Certificate of Excellence from Molecular Monitoring Team, Division of Global AIDS, Centre for Global Health, CDC&P (Centre of Diseases Control and Prevention), USA, for excellence in lab performance in Molecular Diagnosis employing DBS sample.
- Manpower development for all the eastern and northern states of India through numerous hands on training conducted at NICED as well as at different remote places in the respective states to ensure quality in HIV testing, HIV Sentinel Surveillance and Molecular Diagnosis of HIV employing DBS.

Activities:

Quality Assurance for HIV Testing

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

- EQAS and Panel Sera preparation for State Reference Labs.
- Quality Assurance for HSS Lab result (Retesting of all positive and 5% negative).
- Testing laboratory for HIV Sentinel Surveillance.
- Referral for confirmation of HIV testing results of the samples received from different SRLs.
- Training for Medical Officers, Lab/Program Supervisors and Medical Lab Technologists for HIV testing as and when requested by different organizations.
- Testing of Dry Blood Spot and serum samples for HIV Sentinel Surveillance.

External Quality Assurance and referral service for SRLs under NRL and other, NICED, Kolkata

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

HIV Sentinel Surveillance 2012-13 (ANC): Quality Assurance for SRLs under NACO NRL, NICED, Kolkata (sample received from April 2013 to March 2014).

Sl. No	Name of SRL/Testing Centre	Samples sent by SRL		Samples rejected by NRL	Confirmed Result at NRL		
		HIV -ve	HIV +ve		HIV -ve	HIV +ve	
1.	Rajendra Institute of Medical Science, Ranchi, Jharkhand	05	00	00	05	00	Nil
2.	Patuliputra Medical College, Dhanbad, Jharkhand	41	02	00	41	02	Nil
3.	SCB Medical College, Cuttack, Orissa	94	06	00	94	06	Nil
4.	VSS Medical College, Burla, Orissa	51	03	00	51	03	Nil
5.	MKCG Medical College, Beharampur, Orissa	100	07	00	100	07	Nil
6.	Assam Medical College, Dibrugarh, Assam	160	04	03 positive & 08 negative	152	01	Nil
7.	Gauhati Medical College, Guwahati, Assam	272	05	24 negative	248	05	Nil
8.	Silchar Medical College, Silchar, Assam	80	07	00	80	07	Nil
9.	Pasteur Institute, Shillong, Meghalaya	81	07	03 negative	79	06	01
10.	Tura Civil Hospital, Meghalaya	77	01	00	75	01	Nil
11.	Rajendra Institute of Medical Sciences, Imphal	141	23	00	141	23	Nil
12.	G. B. Pant Hospital, Port Blair, A & N Islands	93	00	00	93	00	Nil

Referral Services

National Reference Lab, NICED has been entrusted with the responsibility of verifying results for all samples sent by several Hospitals. The samples tested, result communicated within the turnaround time (TAT) of 7 working days, analyzed the root cause of discordance and trained the referring lab for improvement and technical capacity building. Most of the samples are positive for HIV antibody indicating great improvement of quality of the referring labs.

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

Sl. No.	Source of Samples	No. of sample Tested	No. of sample Positive
1.	Command Hospital, Kolkata	47	45

HIV Sentinel Surveillance 2012-13 (ANC): Testing Center data (Tested during 2013-14), NACO-NRL, NICED, Kolkata.

District	Site Name	No. of Sample received	No. of Sample rejected	No. of Sample tested
Medinipur (East)	Egra Sub-Divisional Hospital	400	00	400
Medinipur (West)	Kharagpur Sub-Divisional Hospital	408	08	400
Hooghly	Khanakul Rural Hospital	421	21	400
Howrah	Uluberia Sub-Divisional Hospital	400	00	400
Kolkata	Abinash Dutta Maternity Home	404	04	400
Kolkata	Vidyasagar State General Hospital	400	00	400
Nadia	Nabadwip State General Hospital	402	02	400
Nadia	Aranghata BPHC	203	03	200
Nadia	Ranaghat Sub-Divisional Hospital	200	00	200
24 Parganas (North)	Madhyamgram Rural Hospital	206	06	200
24 Parganas (North)	Barasat District Hospital	205	05	200
24 Parganas (South)	Baruipur Sub-Divisional Hospital	400	00	400
Murshidabad	Jangipur Sub-Divisional Hospital	402	02	400
			TOTAL SAMPLE TESTED =	4400

Kit Evaluation done by NICED Consortium Lab during April 2013 to March 2014.

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

Diagnostic Kits Evaluated by NICED Lab

Type of Kit	No of Batch/ Lot evaluated
HIV ELISA	05
HIV Rapid	25
HBsAg ELISA	01
HBsAg Rapid	04
HCV ELISA	06
HCV Rapid	05
Total	46

Counseling and Testing for HIV

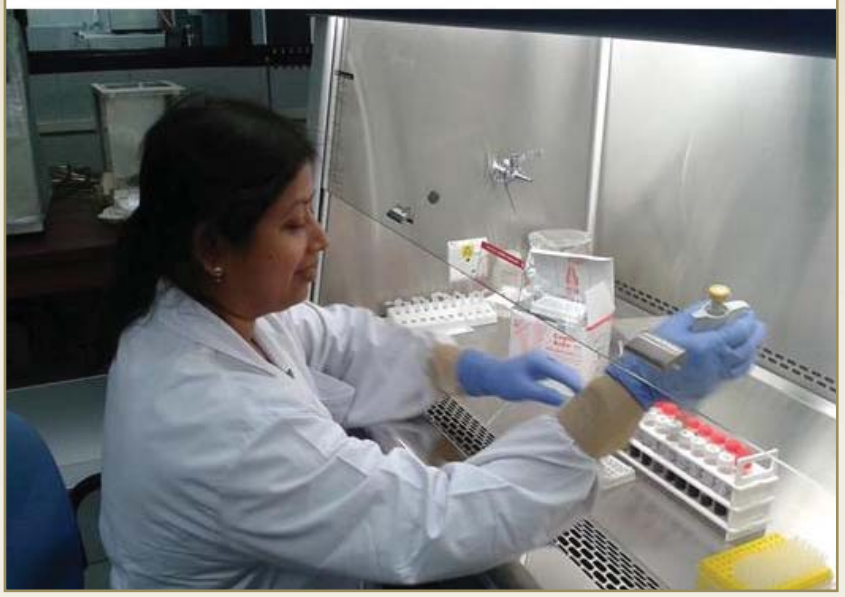
Integrated Counseling &Testing Center

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.

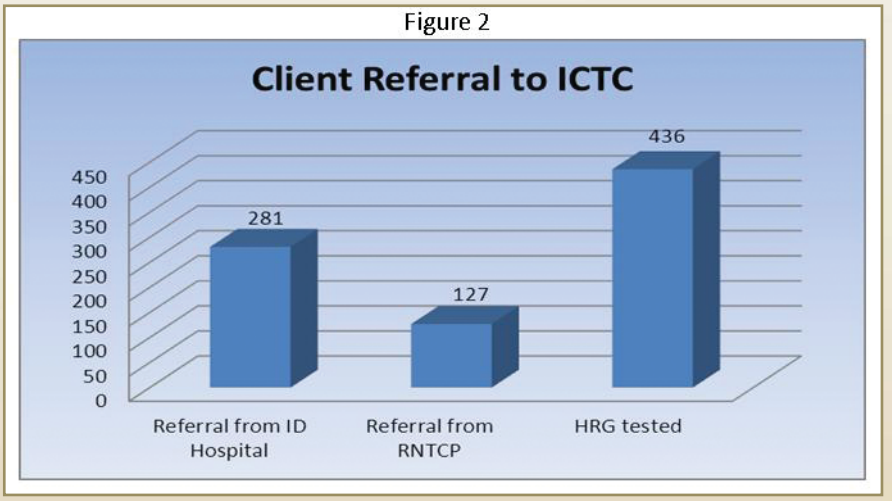
Figure 1. Testing at ICTC Lab



ICTC Data from April 2013 - March 2014

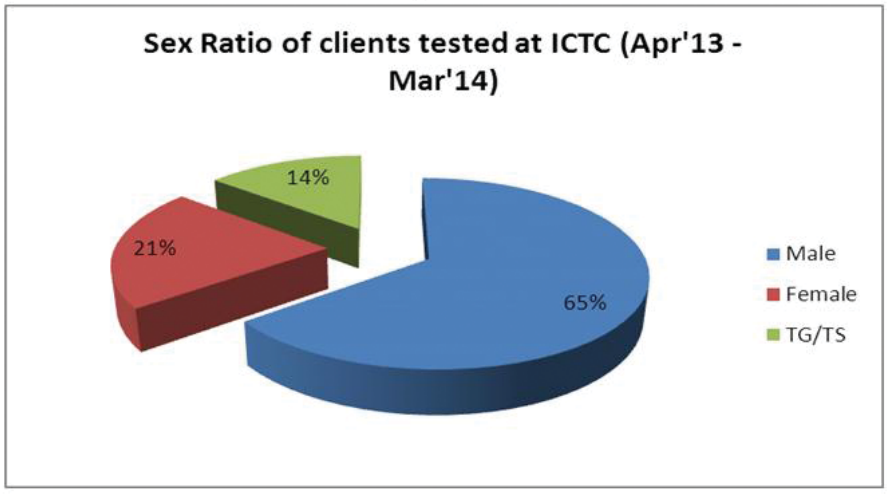
Total Tested	Positive	Positivity	Referred from ID Hospital	Referred from RNTCP	HRG tested (from TI/Non TI)
1081	26	2.40%	281	127	436

Figure 2



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

Figure 3

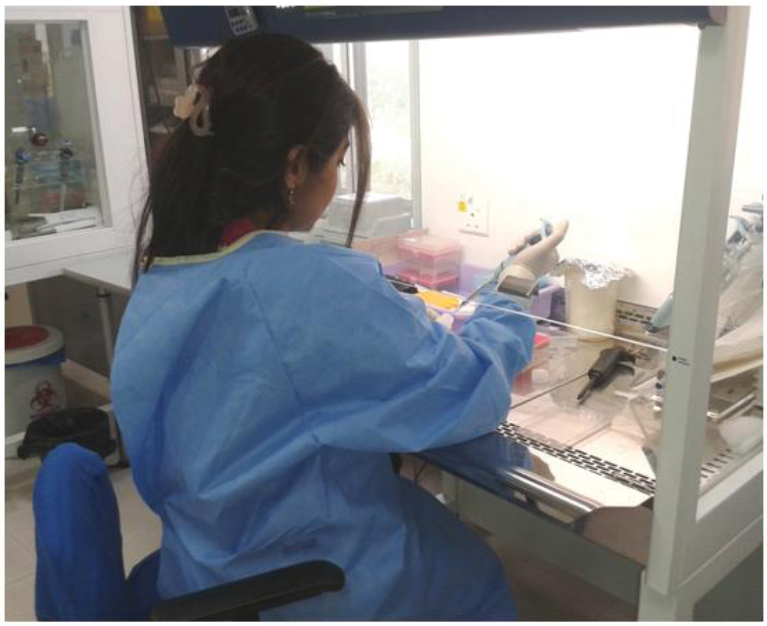


Early Infant Diagnosis

NACO conducted EID Program 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.

National Institute of Cholera and Enteric Diseases (NICED) is one of the 7 Regional Reference Laboratories (RRL) under NACO performing HIV-1 DNA PCR from Dried Blood Spot (DBS) and Whole Blood Samples. In NICED, EID program has been started from August, 2010 initially with three states, West Bengal, Orissa and Chhattisgarh. With gradual success of the program, the North Eastern states (Jharkhand, Bihar, Assam, Manipur, Mizoram, Nagaland, Meghalaya, Arunachal Pradesh, Sikkim, Tripura, and Andaman & Nicobar Islands) were also included under NICED-RRL.

Figure 4: Testing at EID Lab



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

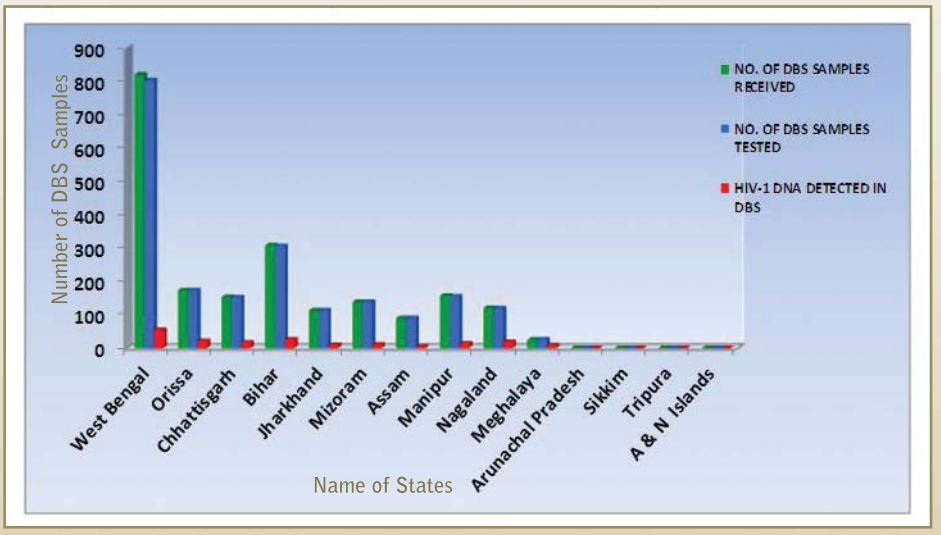
A total of 2096 DBS and 95 Whole Blood Samples received at NICED-RRL for the period of 01.04.2013 to 31.03.2014 and their status is depicted in Table 1 and Fig. 1 & 2.

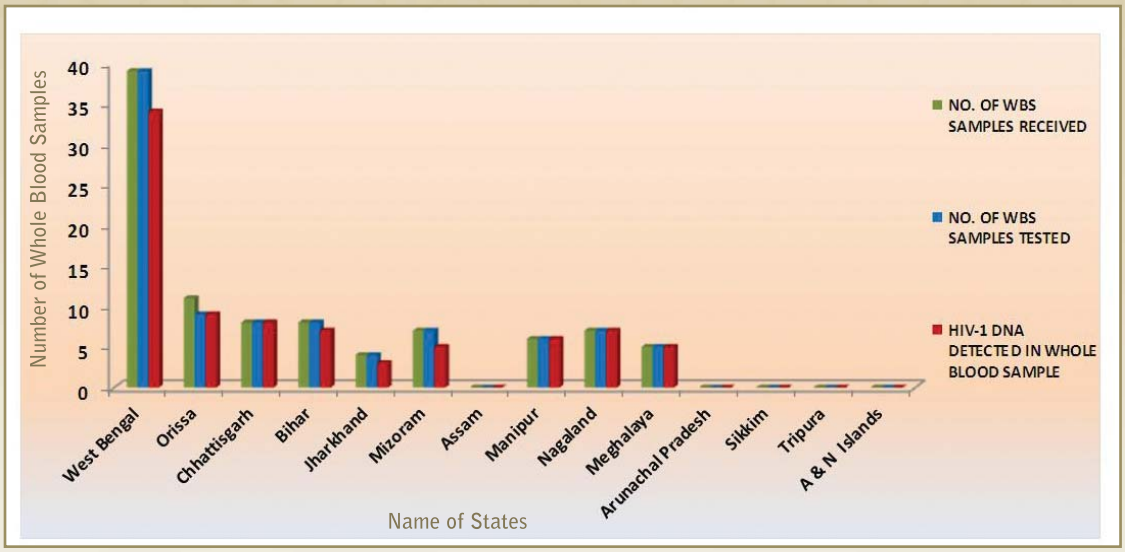
All DBS DNA PCR reactive specimens are further confirmed by 2nd HIV-1 DNA PCR test performed with Whole Blood samples. Status of Whole Blood HIV-1 DNA PCR reactive specimens are presented in Fig. 2.

Status of DBS and Whole Blood Samples received at NICED from April' 2013 to March' 2014.

Name of States	No. of DBS samples received	No. of DBS samples tested	HIV-1 DNA detected in DBS	No. of Whole Blood samples received	No. of Whole Blood samples tested	HIV-1 DNA detected in whole blood
West Bengal	819	802	54	39	39	34
Orissa	173	173	21	11	09	09
Chhattisgarh	153	152	16	08	08	08
Bihar	308	306	26	08	08	07
Jharkhand	113	113	09	04	04	03
Mizoram	138	138	10	07	07	05
Assam	90	90	04	00	00	00
Manipur	157	155	14	06	06	06
Nagaland	120	119	19	07	07	07
Meghalaya	25	25	08	05	05	05
Arunachal Pradesh	-	-	-	-	-	-
Sikkim	-	-	-	-	-	-
Tripura	-	-	-	-	-	-
A & N Islands	00	00	00	00	00	00
TOTAL	2096	2073	181	95	93	84

Status of DBS samples received at NICED from April'2013 to March'2014.





Samples received at NICED from April'2013 to March'2014

Figure 7

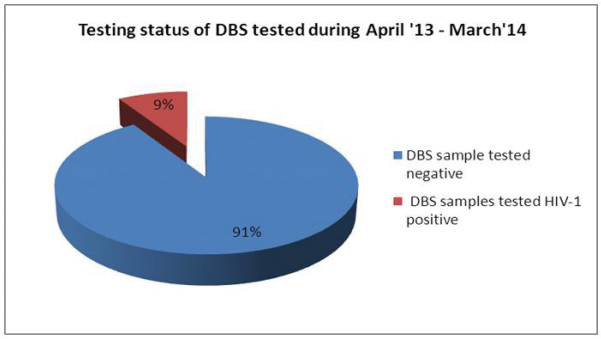
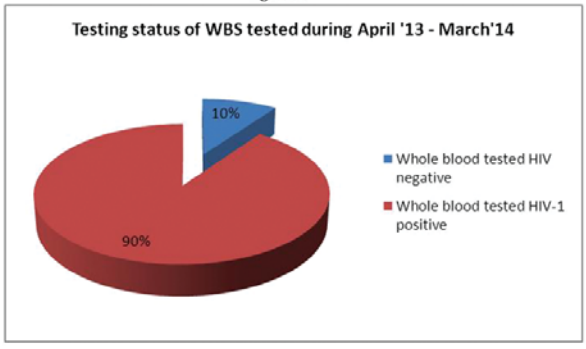


Figure 8



Estimation of HIV as Regional Institutefor HIV Sentinel Surveillance

The Regional Institute (RI) has been functioning at NICED since its inception in early September-October 2008, when National AIDS Control Organization (NACO) decided that NICED would be given the responsibility of functioning as the sixth RI engaged for the purpose of HIV Sentinel Surveillance (HSS) for eastern region of the country.

Further to this, an RI team comprising of the Focal person Dr Malay Kumar Saha, Scientist 'D', NICED (also in charge of NACO NRL, NICED); Epidemiologist & RI member Dr. Alok Kumar Deb, Scientist 'D'; NICED; Subrata Biswas, Project Coordinator; Dr. Sandipta Chakraborty, Research Officer; Pankaj Kumar Khan, Data Manager and others.

Spectrum of Activity

- 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 Strategic Information Management System (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.
- Conduction and supervision of trainings at different levels for National IBBS.
- Technical support, guidance & supervision to SACS & Field Research Agency (FRA) in overall implementation of IBBS
- Monitoring and supervision for IBBS field recruitment at FRA, field training and submission of feedback through IIMS.

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

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

HIV Sentinel Surveillance Sites for ANC Round

	ANC	STD	TOTAL
Andaman & Nicobar Islands	4	1	5
Chhattisgarh	18	0	18
Meghalaya	8	0	8
Nagaland	13	0	13
Sikkim	4	0	4
West Bengal	22	0	22
Site Type Totals	69	1	70

About IBBS

Given the low level and concentrated nature of the HIV epidemics in country, National Integrated Biological and Behavioral Surveillance (IBBS) is being implemented as a strategic focus to strengthen surveillance among high risk groups and migrant population to generate evidence on prevalence and risk behaviors, support planning and prioritization of programme efforts at district, state and national levels.

The broad IBBS activities include Planning and Pre-Surveillance Assessment at the first phase, followed by Sampling Frame Development and Community Preparation at the second phase as preparation for

field work; and this will be followed by third phase which will include all activities pertaining to Behavioral & Biological Data Collection; and finally the fourth phase which includes Data Management, Analysis and Dissemination of top line findings.

Objectives of National IBBS

- To analyze and understand HIV related behaviors and HIV prevalence among key risk groups in different regions, by linking behaviors with biological findings.
- To measure and estimate the change in HIV-related risk behaviors and HIV prevalence among key risk groups, between baseline and end line for NACP-IV.

IBBS Implementation Structure

Department of AIDS Control (DAC) under the Ministry of Health and Family Welfare (MoHFW) is the nodal agency for policy, strategy and planning at national level. In order to steer the whole process of planning, coordination, implementation and monitoring of the survey, and to advise in decision making in technical and operational areas, it is proposed to constitute a **Technical Advisory Group** (TAG), **National Working Group** (NWG) and **Project Management Unit** (PMU). They are being supported by representatives from Regional Institutes (RIs), State AIDS Control Society (SACS) and Field Research Agencies (FRAs) on various aspects of IBBS implementation.

Regional Institutes (RIs)

Two national institutes and six regional institutes including NICED currently involved in implementing HIV Sentinel Surveillance (HSS) has been identified as nodal institutes for implementation of National IBBS focusing on technical aspects of IBBS implementation especially guideline preparation, training and monitoring.

Nodal Institute	States of Responsibility
NIMS, New Delhi	Madhya Pradesh, Chhattisgarh, Orissa
NIHFW, New Delhi	Delhi, Rajasthan
AIIMS, New Delhi	Uttar Pradesh, Uttarakhand, Bihar, Jharkhand
PGIMER, Chandigarh	Punjab, Haryana, Himachal Pradesh, Jammu & Kashmir, Chandigarh
NARI, Pune	Maharashtra, Gujarat, Karnataka, Goa
NIE, Chennai	Tamil Nadu, Kerala, Andhra Pradesh, Puducherry
NICED, Kolkata	West Bengal, Assam, Meghalaya, Nagaland, Sikkim
RIMS, Imphal	Manipur, Mizoram, Arunachal Pradesh, Tripura

IBBS Domains

A 'Domain' is defined as a geographical unit for which the bio-behavioral estimates will be generated for a specific risk group. Generally, a single district is the basic domain in National IBBS, which are called *Independent domains*. However, if a single district does not have adequate number of HRGs to meet the sample size, neighboring districts are grouped to form a Domain. These are called *Composite domains.*

IBBS Target Groups

The high risk groups and bridge population that National IBBS covers across the country are Female Sex Workers (FSW), Men who have Sex with Men (MSM), Trans-genders (TG), Injecting Drug Users (IDU), Migrants (MIG) and Currently Married Women (CMW).

Target Group and Sample Size

Risk Group	Number of Domains	Sample size/ domain	Total Sample Size
Female Sex Workers (FSW)	81	400	32,400
Men who have Sex with Men (MSM)	69	400	27,600
Injecting Drug Users (IDU)	60	400	24,000
Trans-genders (TG)	15	400	6,000
Male Migrants (MIG)	35	1,200	42,000
Currently Married Women (CMW)	16	1,200	19,200
Total	276	-	1,51,200

States to be monitored by RI (E) for IBBS:

- Assam – FSW (3 domain), MSM (3 domain), IDU (3 domain)
- Meghalaya - FSW (1 domain)
- Nagaland - FSW (1 domain), MSM (1 domain), IDU (3 domain)
- Sikkim - IDU (1 domain)
- West Bengal - FSW (3 domain), MSM (3 domain), IDU (2 domain), TG (2 Domain), MIG (2 Domain), CMW (2 Domain)

Training/Workshops:

National Training of Trainers (ToT) on Pre-Surveillance Assessment

PSA ToT held at New Delhi during 30th May - 1st June 2013. Dr. Alok Deband MrSubrata Biswas from Regional Institute (East).attended the 5-day training programme.

PSA Regional Training of Trainers

PSA Regional ToT was held at NICED, Kolkata on 11th – 15th June 2013. Total no. of participants was 106 researchers from IMRB, RI (E) staffs, representatives from CGSACS, NSACS, NERO, Sikkim SACS, OSACS, Meghalaya SACS, WBSACS and SST members. Resource persons were from CDC, NACO, NICED RI(E) and FHI 360.

Pre-Testing Training

Held at NICED, Kolkata on 10th – 11th March 2014. Total number participants – 60. Participants

were from RI(E), Assam SACS, Meghalaya SACS, NSACS, Sikkim SACS, WBSACS, GFK MODE and IICT Mahiti.

National ToT for IBBS

Held at New Delhi during 18th -23rd March 2014. Dr. M. K. Saha, Dr. Alok Deband MrSubrata Biswas attended the training from Regional Institute (East).

IT Coordinator Meeting

Held at Bangalore during 25th -27th March 2014. Mr. Pankaj Kumar Khan and Mr. Rajesh Das from RI (E) attended the program.

Regional ToT for IBBS

Held at NICED, Kolkata during 31st March 2014 – 6th April 2014. Total number of participants was 66 including 15 SST members of this region.

EXTRAMURAL PROJECTS

Ongoing Extramural Projects

1. Title: : Global Enteric Multicenter Study. 2007-2014
   PI : **Dr. T. Ramamurthy**
   Funding Agency : University of Maryland, Baltimore, USA.
   Duration : 2007- 2014

2. Title: : Studies on Emerging and Reemerging Infectious Diseases (Phase-II).
   PI : **Dr. T. Ramamurthy**
   Funding Agency : Okayama University, Okayama, Japan.
   Duration : 2012-2015

3. Title: : Hospital based surveillance system for diarrhoeal diseases.
   PI : **Dr. M.K. Bhattacharya**
   Funding Agency : Okayama University, Japan.
   Duration : 2012-2015

4. Title: : Outpatients based surveillance of Diarrhoeal Diseases at Dr. B. C. Roy Institute of Pediatric Sciences, Kolkata.
   PI : **Dr. M.K. Bhattacharya**
   Funding Agency : Okayama University, Japan.
   Duration : 2012- 2015

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

6. Title: : District Level Household Survey – 4 in West Bengal.;
   PI : **Dr. K. Sarkar**
   Funding Agency : National Institute of Health & Family Welfare, New Delhi, And International Institute of Population Sciences, Mumbai
   Duration : 2012 - 2013

7. Title: : Assessment of perceived health needs and available health care facilities of Malda District'
   PI : **Dr. K. Sarkar**
   Funding Agency : ICMR, Got. of India;
   Duration : 2013 - 2014

8. Title: : Assessment of nutritional status among primary and upper-primary school students in all districts of West Bengal.
   PI : **Dr. K. Sarkar**
   Funding Agency : Dept. of School Education, West Bengal
   Duration : 2013 -2015

9. Title: : Retrospective analysis on the evolutionary aspects of *Vibrio cholerae*
   PI : **Dr. B.L. Sarkar**
   Funding Agency : National Institute of Infectious Diseases, Japan.
   Duration : 2011-2015

10. Title: : Development of a bacteriophage-based biocontrol technology for the treatment of cholera.
    PI : **Dr. B.L. Sarkar**
    Funding Agency : Indo-UK, DST, Govt. of India
    Duration : 2014-2016

11. Title: : Gastro Intestinal Tract Pathogen Repository, GTPR
    PI : **Dr. B.L. Sarkar**
    Funding Agency : ICMR, Govt. of India
    Duration : 2011- 2016

12. Title: : Host intestinal response induced by *Vibrio Cholerae* chitin-binding proteinGbpA and the subsequent effect on the pathogen.
PI : **Dr. N. S. Chatterjee**
Funding Agency : CSIR, Govt. of India
Duration : 2010-2013

13. Title: : COXBOX: Designing An Affordable Medicine Dispenser for Overcoming Non-adherence.
PI : **Dr. A. Deb**
Funding Agency : Gates Foundation
Duration : July-Sep., 2013

14. Title: : Exploration of the Biological Basis of Underperformance of Oral Polioand Rota Virus Vaccines in India.
PI : **Drs. R.K. Nandy and S. Kanungo**
Funding Agency : International Vaccine Institute, Korea.
Duration : 2012-2014

15. Title: : Comparative analysis of the *Helicobacter pylori* strains isolated from North East India with otherparts of India in causing gastro-duodenal diseases.
PI : **Dr. A.K. Mukhopadhyay**
Funding Agency : DBT, Govt. of India
Duration : 2012-2014.

16. Title: : Evolution of CTX prophages of *V. cholerae* 01 and 0139 strains in Asia and Africa.
PI : **Dr. A.K. Mukhopadhyay**
Funding Agency : Okayama University, Japan
Duration : 2010- 2015

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

18. Title: : Development and pre-clinical studies on safety and immunogenicity of novel candidate vaccines against *Salmonellaentericaserovar* Typhi and Paratyphi.
PI : **Dr. S. Das**
Funding Agency : DBT, Govt. of India
Duration : 2012 - 2015

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

20. Title: : 2nd Phase of the Task Force Project Biomedical Informatics Center of ICMR.
PI : **Dr. S. Das**
Funding Agency : ICMR, Govt. of India
Duration : 2013- 2018

21. Title: : Studies on the Regulation of Antimicrobial Peptide Expression and Their Role in Mixed and Opportunistic Infections of the Gut.
PI : **Dr. S. Das**
Funding Agency : Okayama University, Japan.
Duration : 2010-2015

22. Title: : Study the prevalence and genetic characterization of *Entamoebahistolytica* reference strains from Kolkata, India
PI : **Dr. S. Ganguly**
Funding Agency : Japan Health Science Foundation, Japan
Duration : 2011-2014

23. Title: : Studies on burden of parasitic infections among different communities in Western part of India to support health impact evaluation of Total Sanitation Campign.
PI : **Dr. S. Ganguly**
Funding Agency : GFK Mode
Duration : 2012 - 2015

24. Title: : Analysis of rotaviruses and their interactions with the host: A Viral Proteomics Approach.
PI : **Dr. M. Chawla-Sarkar**
Funding Agency : Okayama University, Japan.
Duration : 2010-2015

25. Title: : National Rotavirus Surveillance Network - Referral Lab Eastern India.
PI : **Dr. M. Chawla-Sarkar**
Funding Agency : ICMR, Govt. of India
Duration : 2013-2017

26. Title: : Multisite Monitoring of Influenza Virus Strains in India Phase II.
PI : **Dr. M. Chawla-Sarkar**
Funding Agency : ICMR and DHHS USA.
Duration : 2009-2014

27. Title: : Development and evaluation of a heat killed multi-serotype oral Shigella vaccine.
PI : **Dr. H. Koley**
Funding Agency : Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), Japan.
Duration : 2010- 2015

28. Title: : Development of *Shigella* vaccine based on the virulence gene expression.
PI : **Dr. H. Koley**
Funding Agency : National Institute of Infectious Diseases, Japan.
Duration : 2010-2015

29. Title: : External Quality Assurance for HIV testing.
PI : **Dr. M. K. Saha**
Funding Agency : National AIDS Control Organization
Duration : 2012-17

30. Title: : HIV Sentinel Surveillance
PI : **Dr. M. K. Saha**
Funding Agency : National AIDS Control Organization
Duration : 2012-17

31. Title: : Evaluation of diagnostic kits for HIV, HBS and HCV
PI : **Dr. M. K. Saha**
Funding Agency : National AIDS Control Organization & Self Sustaining.
Duration : 2010-15

32. Title: : Molecular detection of HIV in infants & children under 18 months
PI : **Dr. M. K. Saha**
Funding Agency : National AIDS Control Organization
Duration : 2012-17

33. Title: : Counseling and Testing for HIV, Blood borne Infections and STIs.
PI : **Dr. M. K. Saha**
Funding Agency : WBSAP&CS
Duration : 2008-17

PUBLICATIONS

Publications

1. Ali M, Sur D, You YA, Kanungo S, Sah B, Manna B, Puri M, Wierzba TF, Donner A, Nair GB, Bhattacharya SK, Dhingra MS, Deen JL, Lopez AL, Clemens J. Herd protection by a bivalent killed whole-cell oral cholera vaccine in the slums of Kolkata, India. Clin Infect Dis. 2013 Apr; 56(8): 1123-31.
2. Bagchi P, Bhowmick R, Nandi S, Kant Nayak M, Chawla-Sarkar M. Rotavirus NSP1 inhibits interferon induced non-canonical NFêB activation by interacting with TNF receptor associated factor 2. Virology. 2013 Sep; 444(1-2):41-4.
3. Barman S, Koley H, Ramamurthy T, Chakrabarti MK, Shinoda S, Nair GB, Takeda Y. Protective immunity by oral immunization with heat-killed Shigella strains in a guinea pig colitis model. Microbiol Immunol. 2013 Nov; 57(11):762-71.
4. Batabyal P, Einsporn MH, Mookerjee S, Palit A, Neogi SB, Nair GB, Lara RJ. Influence of hydrologic and anthropogenic factors on the abundance variability of enteropathogens in the Ganges estuary, a cholera endemic region. Sci Total Environ. 2014 Feb; 472:154-61.
5. Batabyal P, Mookerjee S, Sur D, Palit A. Diarrheogenic *Escherechia coli* in potable water sources of West Bengal, India. Acta Trop. 2013 Sep; 127(3): 153-7.
6. Bhattacharya MK, Maitra S, Ganguly A, Bhattacharya A, Sinha A. Dengue: a growing menace a snapshot of recent facts, figures & remedies. Int J Biomed Sci. 2013 Jun; 9(2):61-7.
7. Bhattacharya SK, Sur D ,Ali M, Kanungo S, You YA, Manna B ,Binod S, Niyogi SK ,Park JK, Sarkar BL, Puri MK ,Kim DR, Deen JL ,Holmgren J, Carbis R, Dhingra MS, Donner A ,Nair GB, Lopez AL, Wierzb TF , Clemens JD.5 year efficacy of a bivalent killed whole-cell oral cholera vaccine in Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2013 Dec; 13(12): 1050-6.
8. Bhattacharya SK, Sur D, Dutta S, Kanungo S, Ochiai RL, Kim DR, Anstey NM, von Seidlein L, Deen J. Vivax malaria and bacteraemia : a prospective study in Kolkata. Malar J. 2013 May; 12:176.
9. Bhattacherjee A, Mandal RS, Das S, Kundu S. Sequence and 3D structure based analysis of TNT degrading proteins in *Arabidopsis thaliana*. J Mol Model. 2014 Mar; 20(3):2174.
10. Bhowmick R, Halder UC, Chattopadhyay S, Nayak MK, Chawla-Sarkar M .Rotavirus encoded non-structural protein 1 modulates cellular apoptotic machinery by targeting tumour suppressor protein p53. J Virol. 2013 Jun; 87(12):6840-50.
11. Biswas, S, Sen KK, Roy D, Saha MK. Chitosan based particulate system for oral vaccine delivery: A review. Int J Pharm.2014; 4(1): 226-36.
12. Biswas S, Sen KK, Saha MK, Roy D. Development and characterization of o-methylated free n, n, n-trimethylated chitosan nanoparticles as new carriers for oral vaccine delivery in mice. Int J Pharm Bio Sci. 2014(Jan-Mar); 4(1):1-13.
13. Chowdhury G, Ghosh S, Pazhani GP, Paul BK, Maji D, Mukhopadhyay AK, Ramamurthy T. Isolation and characterization of pandemic and non-pandemic strains of *Vibrio parahaemolyticus* from an outbreak of diarrhea in North 24 Parganas, West Bengal, India. Foodborne Pathogens and Dis. 2013 Apr;10(4):338-42.
14. Chowdhury G, Sarkar A, Pazhani GP, Mukhopadhyay AK, Bhattacharya MK, Ramamurthy T. An outbreak of foodborne gastroenteritis caused by dual pathogens, *Salmonella enterica* serovar Weltevreden

and *Vibrio fluvialis* in Kolkata, India. Foodborne Pathog Dis. 2013 Oct; 10(10): 904-6.

15. Das P, Singh AK, Mukherjee S, Rajendran K, Saha DR, Koley H, Basu S. Composition of *Escherichia coli* population in the neonatal gut phylogroups and virulence determinants. J Med Microbiol. 2013 Nov; 62(Pt 11): 1680-7.
16. Datta S, Mitra S, Viswanathan R, Saha A, Basu S. Characterization of novel plasmid-mediated β-lactamases (SHV-167 and ACT-16) associated with New Delhi metallo-β-lactamase-1 harbouring isolates from neonates in India.J Med Microbiol. 2014 Mar; 63(Pt 3): 480-2.
17. Dutta D, Chowdhury G, Pazhani GP, Guin S, Dutta S, Ghosh S, Rajendran K, Nandy RK, Mukhopadhyay AK, Bhattacharya MK, Mitra U, Takeda Y, Nair GB, Ramamurthy T. *Vibrio cholerae* non-01, non-0139 serogroups and cholera-like diarrhea, Kolkata, India. Emerg Infect Dis. 2013 Mar; 19(3):464-7
18. Dutta S. Fitness gains hamper efforts to tackle drug resistance. eLife. 2013 Dec; 2:e01809.
19. Dutta S, Guin S, Ghosh S, Pazhani GP, Rajendran K, Bhattacharya MK, Takeda Y, Nair GB, Ramamurthy T. Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. PLoS One. 2013; 8(2): e56068.
20. Ganguly S, Mukherjee A, Mazumdar B, Ghosh AN, Banerjee KK.The β-prism lectin domain of *Vibrio cholerae* hemolysin promotes self-assembly of the β-pore-forming toxin by a carbohydrate-independent mechanism. J Biol Chem. 2014 Feb; 289(7):4001-8.
21. Ghosal A, Chatterjee NS, Chou T, Said HM. Enterotoxigenic *Escherichia coli* infection and intestinal thiamin uptake: studies with intestinal epithelial Caco-2 monolayers. Am J Physiol Cell Physiol. 2013 Dec; 305(11):C1185-91.
22. Ghosh P, Naha A, Basak S, Ghosh S, Ramamurthy T, Koley H, Nandy RK, Shinoda S, Watanabe H, Mukhopadhyay AK. Haitian variant tcpA in *Vibrio cholerae* 01 El Tor strains in Kolkata, India J Clin Microbiol. 2014 Mar; 52(3):1020-1.
23. Ghosh S, Taniguchi K, Aida S, Ganesh B, Kobayashi N. Whole genomic analyses of equine group A rotaviruses from Japan: evidence for bovine-to-equine interspecies transmission and reassortment events. Vet Microbiol. 2013 Oct; 166(3-4):474-85.
24. Ghosh S, Urushibara N, Chawla-Sarkar M, Krishnan T, Kobayashi N. Whole genomic analyses of asymptomatic human G1P[6], G2P[6] and G3P[6] rotavirus strains reveal intergenogroup reassortment events and genome segments of artiodactyl origin. Infect Genet Evol. 2013 Jun; 16:165-73.
25. Ghosh, A, Karmakar S, Mukherjee A K, Raj D, Das K, Sarkar S, Nozaki T, Ganguly S. The spliceosomal protein SnRNP F binds to both U3 and U14 class of snoRNA in *Giardia lamblia*. GJBAHS. 2013(July – September); 2(3):178-84.
26. Halder UC, Bhowmick R, Roy Mukherjee T, Nayak MK, Chawla-Sarkar M. Phosphorylation drives an apoptotic protein to activate antiapoptotic genes: paradigm of influenza A matrix 1 protein function. J Boils Chem. 2013 May; 288(20):14554-68.
27. Kang G, Desai R, Arora R, Chitamabar S, Naik TN, Krishnan T, Deshpande J, Gupte MD, Venkatasubramaniam S, Gentsch JR, Parashar UD; Indian Rotavirus Strain Surveillance Network, Mathew A, Anita Sr, Ramani S, Sowmynarayanan TV, Moses PD, Agarwal I, Simon A, Bose A, Arora R, Chhabra P, Fadnis P, Bhatt J, Shetty SJ, Saxena VK, Mathur M, Jadhav A, Roy S, Mukherjee A, Singh NB. Diversity of circulating rotavirus strains in children hospitalized with diarrhea in India, 2005–2009. Vaccine. 2013 Jun; 31(27):2879-83.

28. Kanungo S, Chakrabarti MK. Enteric disease and prevention: where we stand? Everyman's Sci. 2013 (June–July); XLVIII (2):87-8.

29. Kanungo S, Mahapatra T, Bhaduri B, Mahapatra S, Chakraborty ND, Manna B, Sur D. Diarrhoea-related knowledge and practice of physicians in urban slums of Kolkata, India. Epidemiol Infect. Epidemiol Infect. 2014 Feb; 142(2):314-26.

30. Karmakar S, Raj D, Ganguly S. Identification of the N-terminal glycine-arginine rich (GAR) domain in *Giardia lamblia* fibrillarin and evidence of its essentiality for snoRNA binding. Int J Trop Dis Health. 2013 Sep; 3(4): 318-27.

31. Koley H, Ray N, Chowdhury , Barman S, Mitra S, Ramamurthy T, Mukhopadhyay AK, Sarkar BL, Katyal R, Das P, Panda S, Ghosh S. Outbreak of cholera caused by *Vibrio cholerae* 01 El Tor variant strain in Bihar, India. Jpn J Infect Dis. 2014; 67(3):221-6.

32. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, WuY, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, TambouraB, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R,Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, AntonioM, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K,O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013 Jul; 382(9888):209-22.

33. Labala RK, Das S, Basak S. ASRDb: A comprehensive resource for archaeal stress response genes. Bioinformation. 2013 Jul; 9(12): 650-5.

34. Malik YS, Kumar N, Sharma K, Sharma AK, Sircar S, Jeena LM, Singh NK, Mondal A, Joardar SN, Balasubramanian G. Molecular characterization of a genetically diverse bubaline picobirnavirus strain, India. Thai J Vet Med. 2013 Dec; 43(4): 609-13.

35. Malik YS, Sharma AK, Kumar N, Sharma K, Ganesh B, Kobayashi N. Identification and characterisation of a novel genogroup II picobirnavirus in a calf in India. Vet Rec. 2014 Mar 15; 174(11): 278.

36. Malik YS, Sharma K, Kumar N, Shivachandra SB, Rawat V, Rakholia R, Ranjan R, Ganesh B, Parida M. Rapid detection of human rotavirus using NSP4 gene specific reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay. Indian J Virol. 2013 Sep; 24(2):265-71.

37. Manna B, Nasrin D, Kanungo S, Roy S, Ramamurthy T, Kotloff KL, Levine MM, Sur D. Determinants of health care seeking for diarrheal illness in young children in urban slums of Kolkata, India. Am J Trop Med Hyg. 2013 Jul; 89(Suppl1): 56-61.

38. Majumder PP, Sarkar-Roy N, Staats H, Ramamurthy T, Maiti S, Chowdhury G, Whisnant CC, Narayanasamy K, Wagener DK. Genomic correlates of variability in immune response to an oral cholera vaccine. Eur J Hum Genet. 2013 Sep; 21(9):1000-6.

39. Mitra S, Chakrabarti MK, Koley H. Multi-serotype outer membrane vesicles of Shigellae confer passive protection to the neonatal mice against shigellosis. Vaccine. 2013 Jun; 31(31):3163-73.

40. Mukherjee A, Mullick S, Deb AK, Panda S, Chawla-Sarkar M. First report of human rotavirus G8P[4] gastroenteritis in India: evidence of ruminants-to-human zoonotic transmission. J Med Virol. 2013 Mar; 85(3):537-45.

41. Mukherjee AK, Chowdhury P, Das K, Raj D, Karmakar S, Ganguly S. Helminth burden among

school going children of southern Bengal, India: a survey report. GJBAHS. 2013(Jul – Sep); 2(3):189-91.

42. Mukherjee AK, Chowdhury P, Rajendran K, Nozaki T, Ganguly S. Association between *Giardia duodenalis* and coinfection with other diarrhea-causing pathogens in India. Biomed Res Int. 2014; 2014:786480.
43. Mukherjee H, Ojha D, Bharitkar YP, Ghosh S, Mondal S, Kaity S, Dutta S, Samanta A, Chatterjee TK, Chakrabarti S, Mondal B, Chattopadhyay D. Evaluation of the wound healing activity of *Shorea robusta*, an Indian ethnomedicine, and its isolated constituent(s) in topical formulation. J Ethnopharmacol. 2013 Aug; 149(1):335-43.
44. Mukherjee P, Ramamurthy T, Bhattacharya MK, Rajendran K, Mukhopadhyay AK. *Campylobacter jejuni* in hospitalized patients with diarrhea, Kolkata, India. Emerg Infect Dis. 2013 Jul; 19(7):1155-6.
45. Mukherjee S, Biswas R, Biswas T. Alternative TLRs are stimulated by bacterial ligand to induce TLR2-unresponsive colon cell response. Cell Signal. 2013 Aug; 25(8):1678-88.
46. Nandi S, Chanda S, Bagchi P, Nayak MK, Bhowmick R, Chawla-Sarkar M. MAVS protein is attenuated by rotavirus nonstructural protein 1.PLoS One. 2014 Mar; 9(3):e92126.
47. Panda S, Roy T, Pahari S, Mehraa J, Sharma N, Singh G, Singh J, Joseph F, Singh S, Sharma NM. Alarming epidemics of human immunodeficiency virus and hepatitis C virus among injection drug users in the northwestern bordering state of Punjab, India: prevalence and correlates. Int J STD AIDS. 2013 Dec; 25(8):596-606.
48. Papp H, László B, Jakab F, Ganesh B, De Grazia S, Matthijnssens J, Ciarlet M, Martella V, Bányai K. Review of group A rotavirus strains reported in swine and cattle. Vet Microbiol. 2013 Aug; 165(3-4):190-9.
49. Pativada M, Bhattacharya R, Krishnan T. Novel human astrovirus strains showing multiple recombinations within highly conserved ORF1b detected from hospitalized acute watery diarrhea cases in Kolkata, India. Infect Genet Evol.2013 Dec; 20:284-91.
50. Pore D, Chakrabarti MK. Outer membrane protein A (OmpA) from *Shigella flexneri* 2a: a promising subunit vaccine candidate. Vaccine. 2013 Aug; 31(36):3644-50.
51. Raj D, Ghosh E, Mukherjee AK, Nozaki T, Ganguly S. Differential gene expression in *Giardia lamblia* under oxidative stress: significance in drug designing and eukaryotic evolution. Gene. 2014 Feb; 535(2):131-9.
52. Ramamurthy D, Parham GP, Sarkar A, Nanty RK, Rajendran K, Sur D, Manna B, Ramamurthy T. Case-Control study on the role of enterotoxigenic *Bacteroides fragilis* as a cause of diarrhea among children in Kolkata, India. PLoS One. 2013Apr; 8(4):e60622.
53. Ramamurthy T, Chowdhury G, Pashing GP, Shinoda S. 2014.*Vibrio fluvialis*: an emerging pathogen. Front. Microbiol. 2014 March; 5: 91.
54. Ribeiro Silva R, Bezerra DA, Kaiano JH, Oliveira Dde S, Silvestre RV, Gabbay YB, Ganesh B, Mascarenhas JD. Genogroup I avian picobirnavirus detected in Brazilian broiler chickens:a molecular epidemiology study. J Gen Virol. 2014 Jan; 95(Pt 1):117-22.
55. Roy Choudhury S, Mandal A, Ghosh M, Basu S, Chakravorty D, Goswami A. Investigation of antimicrobial physiology of orthorhombic and monoclinic nanoallotropes of sulfur at the interface

of transcriptome and metabolome. Appl Microbiol Biotechnol. 2013 Jul; 97(13):5965-78.

56. Roy Mukherjee T, Chanda S, Mullick S, De P, Dey-Sarkar M, Chawla-Sarkar M. Spectrum of respiratory viruses circulating in eastern India: prospective surveillance among patients with influenza-like illness during 2010-2011. J Med Virol. 2013 Aug; 85(8):1459-65.
57. Roy Mukherjee T, Mukherjee A, Mullick S, Chawla-Sarkar M. Full genome analysis and characterization of influenza C virus identified in eastern India. Infect Genet Evol. 2013 Jun; 16: 419-25.
58. Roy S, Gaind R, Chellani H, Mohanty S, Datta S, Singh AK, Basu S. Neonatal septicaemia caused by diverse clones of *Klebsiella pneumoniae* & *Escherichia coli* harbouring blaCTX-M-15. Indian J Med Res. 2013 Apr; 137(4):791-9.
59. Roy S, Krishnan R, Mukherjee S, Schneiders T, Niyogi SK, Basu S. Prevalence of ST131 virulence-associated strains among CTX-M-producing *Escherichia coli* in the gut of hospitalized neonates in India. Diagn Microbiol Infect Dis. 2013 Oct; 77(2):158-9.
60. Saha DR, Guin S, Krishnan R, Nag D, Koley H, Shinoda S, Ramamurthy T. Inflammatory diarrhea due to enteroaggregative *Escherichia coli*: evidence from clinical and mice model studies. Gut Pathog. 2013 Dec; 5(1):36.
61. Sarkar BL, Rao UA. Cholera–A disease revisited. Indian J App Microb. 2013; 16(1):1-12.
62. Sergeev G, Roy S, Jarek M, Zapolskii V, Kaufmann DE, Nandy RK, Tegge W. High-throughput screening and whole genome sequencing identifies an antimicrobially active inhibitor of *Vibrio cholerae*. BMC Microbiol. 2014 Feb; 14:49-62.
63. Sheikh IA, Koley H, Chakrabarti MK, Hoque KM. The Epac1 signaling pathway regulates Cl- secretion via modulation of apical KCNN4c channels in diarrhea. J Biol Chem. 2013 Jul; 288(28):20404-15.
64. Singh M, Singh NRK, Singh NH, Chakrabati LAS, Sarkar K, Singh B. Safe sex among married male IDUs in Manipur: a cross-sectional study. Int J Med Appl Sci. 2013; 2(Suppl1):155-65.
65. Sumi A, Rajendran K, Ramamurthy T, Krishnan T, Nair GB, Harigane K, Kobayashi N. Effect of temperature, relative humidity and rainfall on rotavirus infections in Kolkata, India. Epidemiol Infect. 2013 Aug; 141(8):1652-61.
66. Viswanathan R, Singh AK, Basu S, Chatterjee S, Roy S, Isaacs D. Multidrug-resistant, non-fermenting, gram-negative bacilli in neonatal sepsis in Kolkata, India: a 4-year study. Paediatr Int Child Health. 2014 Feb; 34(1):56-9.
67. Wajima T, Sabui S, Kano S, Ramamurthy T, Chatterjee NS, Hamabata T. Entire sequence of the colonization factor coli surface antigen 6-encoding plasmid pCss165 from an enterotoxigenic *Escherichia coli* clinical isolate. Plasmid. 2013 Nov; 70(3):343-52.
68. You YA, Ali M, Kanungo S, Sah B, Manna B, Puri M, Nair GB, Bhattacharya SK, Convertino M, Deen JL, Lopez AL, Wierzba TF, Clemens J, Sur D. Risk map of cholera infection for vaccine deployment: the eastern Kolkata case PLoS One. 2013 Aug; 8(8):e71173.

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 maintain ASSESSMENT of Scientists
- To promote under MACP scheme
- To maintain TA/LTC
- To make purchase of all consumable/non-consumable items
- To maintain Stores, Purchase & Maintenance work
- New Pension system in NICED
- To maintain Staff Canteen
- To control deployed Security and House Keeping Staff
- Online administration is in progress

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 63 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 63 and above Extramural Projects are going on. The total workload is carried out by the Administrative staff of the Institute.

(Subodh Karmakar)
Administrative Officer

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