वार्षिक रीपोर्ट
Annual Report

2014-2015

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

Annual Report

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सचिव, भारत सरकार
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
एवं
महानिदेशक, आई सी एम आर

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MESSAGE

It gives me immense pleasure to congratulate the Team-NICED consisting of scientists, administrative and other support staff for their overall achievements that have been reflected in this Annual Report of the National Institute of Cholera & Enteric Diseases (NICED) published for the period 2014-2015.

The report highlights an array of research activities covered by the Institute under basic and applied sciences, which were conducted on the urban and rural communities in wide geographical regions of West Bengal including those residing within the natural disaster prone terrain of Sundarbans. In addition to its traditional research on various aspects of enteric diseases including enteric pathogens, antimicrobial resistance, bacteriophages, environmental health; NICED, as part of institutional mandate, has conducted investigations on maternal and child health, nutrition, HIV-disease as well. The scientists of NICED have put their efforts both on innovation as well as facilitation of evidence based public health programming and policy making.

This premier institute of Indian Council of Medical Research (ICMR), as in other years, has served the nation through its unwavering support during 2014-2015 through various activities like training, outbreak investigations, providing rapid diagnostic services and assisting State Governments in situations of emerging infectious diseases like H1N1 influenza in the recent past. I hope, the Institute continues to strive for excellence in public health and moves ahead to carry out research in important areas to achieve its goals for a healthy and prosperous nation.

(Soumya Swaminathan)

From the Director's Desk

I have been associated with the steady growth and development of National Institute of Cholera and Enteric Diseases (NICED) for the last twenty one years. It is my proud privilege to be sharing my views for this annual report 2014-15. In recent years the research facilities of NICED has expanded tremendously with available modern amenities and excellent infrastructure for basic and applied research. This Institute is currently recognised as one of premier institutes under control of the Indian Council of Medical Research which is governed by Department of Health Research (DHR), Government of India. This is also a recognised WHO collaborating centre for training and research on diarrhoeal diseases.

The collaborative research projects brought in the extramural grants fromboth national and international agencies. Japanese International Collaboration Agency, Indo UK Technical Training Program, Japanese Counterpart exchange program with Sapporo Medical University and National Institute of Infectious Diseases, Japan, International Vaccine Institute, Korea, PATH Vaccine Solutions, USA and Okayama University, Japan spearheaded the improvement in varied areas of research and founded the diarrhoea diseases surveillance program at the institute with special emphasis on enteric pathogens and emerging infections. This has prepared the strong foot-hold to undertake the vaccine trials as well as to conduct clinical trials. The expansion of research was also achieved by receiving extramural grants from various national funding agencies such as CSIR, DAE, DBT, UGC, DST and ICMR.

The frontier areas of pioneering basic and applied research on enteric diseases were documented through commendable publications in peer reviewed national and international journals, national honours and awards, oration etc. to the scientists and scholars during the current period of report. The research work conducted at the institute is directly translated to improve the local public health activities through regular feed-back and direct communication with State Government authorities. The National Institute of Cholera and Enteric Diseases extends unanimous support at the time of national emergencies/outbreaks by expanding the services of timely diagnosis for containing the alarming situations affecting the environs during outbreaks caused by Avian Influenza, Swine Flu, Diarrhoeal diseases etc.

The training and extension component has richly served the nation by developing human resources through regular courses conducted at the institute for medical technologists, medical doctors, WHO fellows, research scientists and so on. Besides this the postgraduate and PhD program of the institute is the stepping stone to nurture future scientists.

I deeply acknowledge the immense support from all the staff and scientists of the institute and the overall cooperation and guidance from the headquarters of ICMR in collaboration with DHR. We shall thrive further to tread the path to attain better growth and scientific contribution in the days to come.

Dr.Shanta Dutta *MD, Ph.D., MAMS*
Scientist G and Director-in-Charge

CONTENTS

RESEARCH ACTIVITIES

Bacteriology

Division of Bacteriology has efficiently established its capacity in identifying more than 20 enteric pathogens with conventional as well as molecular methods from hospitalized acute diarrhoeal cases in the Infectious Diseases and Beliaghata General Hospital and outpatients treated in the Dr. B. C. Roy Memorial Hospital for Children, Kolkata. The Division provides laboratory support for diarrhoeal outbreaks/epidemic investigations carried out in West Bengal through IDSP and in other parts of the country. *Vibrio cholerae* O1, *Shigella spp.*, *Campylobacter jejuni*, and enteroaggregative *Escherichia coli* continued to be the most commonly isolated pathogens. Although *V. cholerae* were susceptible to the fluoroquinolones, *Shigella* strains were highly resistant to fluoroquinolones but susceptible to ceftriaxone. Most of the enteric pathogens remain susceptible to azithromycin. The weekly generated reports on the prevalence of diarrhoeal pathogens in these two hospitals are sent to State Govt. for better patient care and management, undertaking interventions for diarrhoea control. Antimicrobial resistance (AMR) mechanisms were extensively studied in understanding the role of mobile genetic elements in dissemination of AMR in other bacteria. Integron carriage was detected more in *Shigella* strains from diarrhoeal cases than from controls. A typical class 1 integron was detected exclusively in *S. flexneri*. In addition, the fluoroquinolone resistance encoding genes such as *aac(6')-Ib-cr* and *qnrS1* were also detected. EPEC/ETEC hybrid strain was detected for the first time, harbouring both *eae* and *elt* genes specifically present in the respective *E. coli*.

Studies have been initiated to characterize *Salmonella Typhi* clinical isolates from children attending Dr. B. C. Roy Memorial Hospital with provisional diagnosis of typhoid. A qPCR has been developed for rapid diagnosis of typhoid fever which was found to be very sensitive and specific. Currently, increase in fluoroquinolone resistance is a growing problem, which has restricted the use of this drug. Azithromycin may be used with caution for typhoid treatment. Molecular typing by PFGE of the isolates showed an association between the antimicrobial R profiles of the isolates with the pulsotypes which may be exploited to identify the source of the organism and eventually containment of the organism by appropriate intervention measures. Non typhoidal Salmonella (NTS) has been isolated both from clinical diarrhoea cases and environmental samples including raw poultry and dairy products. NTS was isolated from blood samples of fever patients, with blood dyscrasia and rarely with other systemic disorders. AMR profile and mechanism of resistance were studied. On the request of M/o Health and Family Welfare, as a member of Rapid Response Team (RRT) this division took part in visiting the SNTP Hospital, Gangtok, Sikkim to check the isolation facility for Ebola virus containment.

Seasonal dynamics of *Vibrios* and its phages in Gangetic riverine-estuarine ecosystem have been studied across districts of South Bengal. Virulence markers (*hlyA, tdh, vmh, toxR* etc.) specific for *Vibrio spp.* have been identified in the environmental *Vibrios*, indicating its possible role in the horizontal gene transfer. It was demonstrated that chitin in crabs of halophile condition assists *Vibrios* in concomitant colonization and toxin gene acquisition. Detection of antibiotic resistance genes among environmental *V. cholerae* suggests possibility of dissemination of AMR genes via mobile integrons. The drug resistance pattern of *V. cholerae* non-O1 isolates without harbouring any drug resistance gene indicated salinity induced antibiotic efflux system in *V. cholerae* of high saline zone. A salinity dependent zone demarcation has been established for eneteropathogenic *Vibrios* by evaluating the salinity gradients of the samples collected off the Gangetic system. While *V. cholerae* & *V. mimicus* showed higher abundance in low saline zone, *V. fluvialis, V. vulnificus, V. parahaemolyticus, V. alginolyticus* were mostly prevalent at high saline zone.

The request of the regional health authorities as well as was the community, routine microbial analysis of potable water samples was undertaken.

Divisional activities included identification and characterization of functional metabolic pathways in pathogenic *Vibrio*

cholerae and their eventual correlation to pathogenesis. Obligate involvement of the Entner Doudoroff (ED) pathway for gluconate (Gnt) utilization and functional ED pathway for pathogenesis of *V. cholerae* has been established. It was hypothesised that identification of enzyme(s) involved in different pathway(s) are good targets for developing newer drugs/ small molecule inhibitors for treatment of cholera.

The pathogenesis and drug resistance mechanisms of *Vibrio cholerae* and *Helicobacter pylori* including the development of simple PCR based assay for quick detection of hybrid variant virulent *V. cholerae* strains have been evaluated. This helps to track the dissemination of the new variant strains and to implement intervention strategies for preventing outbreaks in high-risk areas.

dupA gene of *H. pylori* was targeted to identify the probable biomarkers for duodenal ulcer in Indian population. Emergence of azithromycin resistance was observed in *Campylobacter jejuni* isolated from children in Kolkata. The A2075G mutation of 23s rRNA gene is responsible for the macrolide resistance in this region which is transferrable.

Carbapenem resistance has increasingly been identified in *Acinetobacter spp.* Both transmissible (carbapenemases) and nontransmissible (efflux pumps) resistance were studied. The emergence of NDM-1 along with an already existing repertoire of carbapenem-hydrolyzing-oxacillinases was noted. Compared with carbapenem-susceptible strains, the expression levels of efflux pumps were observed to be higher by real-time PCR. Sequence analysis revealed mutations in regulatory genes, which suggests the species as a threat in the hospital environment.

ESBLs and AmpCs may escape detection when they coexist with metallo-β-lactamases such as NDM-1. A phenotypic method was devised to detect other β-lactamases in NDM-positive isolates using a combination disk assay. The assay can be of use for routine diagnosis because of its reliability and specificity.

Protective immune response of different form of antigens, like outer membrane vesicles, heat-killed and live attenuated *Shigella* strains have been evaluated in different animal models. Use of different shigellae ΔtolA-OMVs will have valuable role in the development of next-generation non-living vaccines against shigellosis.

The heat killed multi serotype *Shigella* (HKMS) immunogen has been formulated 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). The immunogenicity and protective efficacy of HKMS immunogen was studied in Guineapig model. The short term and long term passive protection were confirmed in neonatal mice model.

During 2014-2015 identification and characterization of *V. cholerae* isolates along with phage typing has been done for 421 *V. cholera* strains, received at NICED phage laboratory from various national organizations of the country. A study on phage therapy was undertaken as a component of translational research which revealed prophylactic and protective efficacy of cocktail phage in different animal models.

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Swati Gupta

Dhrubajyoti Nag

Ritam Sinha

Priyadarshini Mukherjee

Debaki Ranjan Howlader

Nihar Ranjan Biswal

Sounak Sarkar

Awards

Ms. Anuradha Sinha received Ph.D. from Jadavpur University

Title of the Thesis: Development and application of species-specific PCR assay for identification of enteric pathogens from diarrheal stool specimens.

Soma Mitra received Ph. D. From Calcutta University

Title of the Thesis: Studies on Outer Membrane Vesicles of *Shigella sp.* as a Candidate Vaccine

Hospital based Surveillance on Diarrhoeal enteropathogens

Name of the Investigators: NICED Surveillance Group

During April 2014 to March 2015, a total of 6,948 children under five years of age received treatment in the B. C. Roy Memorial Hospital for children (BCRH), Kolkata and using sampling technique of every 5th patient of diarrhoea cases in OPD, 1,170 (16.8%) stool specimens/rectal swabs were obtained from the diarrheal children. During this period, in the Infectious Diseases Hospital (IDH), 21,344 diarrheal patients were admitted and out of them 1,164 (5.5%) were enrolled under surveillance. Among the total number of admitted cases, 101 (0.5%) were deceased in the IDH.

The number of hospitalized children under 5 years of age at the IDH was 5,665. About 6.4% (364 cases) of the stool specimens were collected before the initiation of antibiotic therapy. In BCRH, among OPD patients, about 61% diarrhoea cases presented with semisolid stool, 37 % cases with watery stools and only 2% cases with bloody stool whereas in IDH, among in-patients, most of the cases (90%) were observed with watery stool, 8.5 % cases with semisolid stool and 2% cases with blood only. In IDH, almost all the diarrhoea cases had dehydration at the time of admission (some dehydration: 99.7%, severe dehydration: 0.3%). The median duration of hospital stay among the IDH patients was 28 hours but about 37% of the cases had to stay more than 36 hours for treatment management. Vomiting was the main clinical symptom (88%) and also had abdominal pain in 61% cases, had fever in 33% cases among IDH patients but in BCRH, among OPD patients, it was 41%, 22% and 22% respectively.

In both the hospitals, rotavirus was identified as the major pathogen (~48%) followed by Adenovirus (7-17%), *Campylobacter* spp. (7-10%) and *Giardia lamblia* (~7%) among under 5 years age of children. Cholera and shigellosis were less in children (3 and 3-4%, respectively). Among children, polymicrobial etiology was detected in 17.7% of the diarrheal cases from the IDH, whereas in BCRH, 13.5% of the cases were infected with more than one pathogen. *Vibrio cholerae* O1 was identified among 117 cases (11.1%) in IDH.

Though the *V. cholerae* O1 remained susceptible for most of the fluoroquinolones but they were highly resistant to co-trimoxazole, nalidixic acid and streptomycin. *V. cholerae* O1 strains isolated from IDH were susceptible to ampicillin and similar picture was also observed to the strains isolated in the BCRH. Resistance of *V. cholerae* towards azithromycin was not seen in both the hospitals. Most of the *Shigella* strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone. This study helps in management of diarrhea at the hospital and also supports the basic research in our setting. This study helps in management of diarrhea at the hospital and also supports the basic research in our setting.

Studies on Fluoroquinolones and Azithromycin Breakpoints for *Salmonella enterica serovar* Typhi strains from Kolkata

Investigators: S. Dutta, S. Das

Due to emergence of resistance against commonly used antimicrobials (Chloramphenicol, ampicillin, cotrimoxazole) in *Salmonella* Typhi **(**S. Typhi) strains, ceftriaxone or a fluoroquinolone (FQ) have been recommended by the World Health Organization for the treatment of uncomplicated typhoid fever irrespective of the antimicrobial resistance (AMR) patterns of the isolates. Treatment failure cases with ciprofloxacin have been reported worldwide due to gradual decrease in ciprofloxacin susceptibility (DCS, MIC of ciprofloxacin 0.125-0.5 µg/ml) of *S.* Typhi over the years. In recent year emergence of ciprofloxacin resistant *S.* Typhi isolates is a growing concern in many countries including India. Azithromycin (Az) has been shown to be an effective alternative to FQs for treatment of uncomplicated typhoid in several clinical trials and *in-vitro* studies.

The Clinical and Laboratory Standard Institute (CLSI) has revised the minimum inhibitory concentration (MIC) breakpoints of Ci, Of and Le and the disk diffusion (DD) breakpoint of Ci for *Salmonella* spp, but DD breakpoints of ofloxacin or levofloxacin for *Salmonella* were not included in the document. In this study, the changing patterns of DD and MIC breakpoints of Ci, Of, Le and Az were determined for *S*. Typhi strains isolated from Kolkata, India.

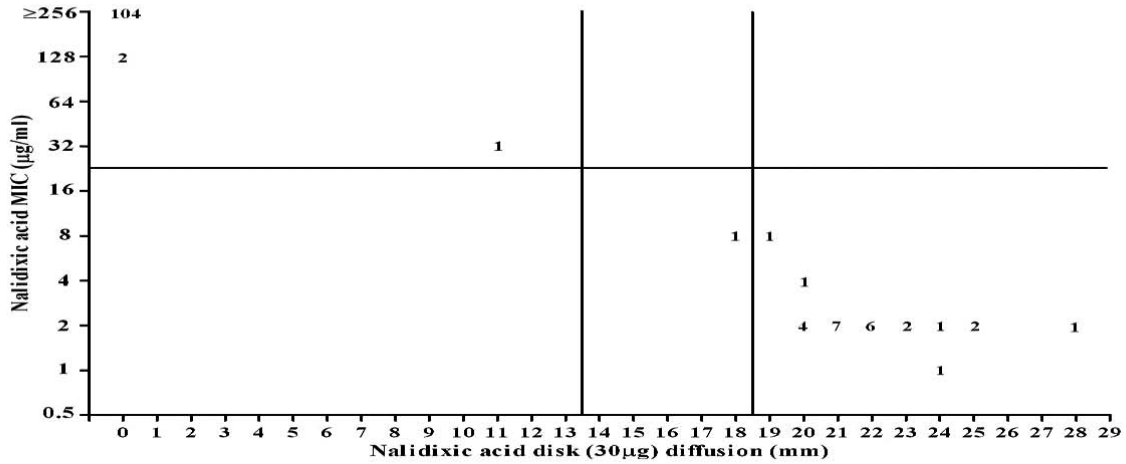
S. Typhi isolates used in this study were collected from the enteric bacterial repository of National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India. A total of representative 151 S. Typhi strains with various antimicrobial susceptibility patterns, isolated during 1998 to 2014, were selected for the study. The scatterplots of zone diameter and MIC of nalidixic acid (n=134), ciprofloxacin (n=146), ofloxacin (n=133) and levofloxacin (n=106) for S. Typhi isolates are shown in Fig. 1.

Among the 71 *S*. Typhi isolates sequenced for detection of mutations in QRDRs of topoisomerase, 50 possessed a single mutation in *gyrA* at position 83 (S83F or S83Y) or at position 87 (D87N or D87Y) (Table 1). Four isolates had double mutations in *gyrA* at position 83 (S83F or S83Y) and *parC* at position 84 (E84G or E84K) or *parE* at position 502 (L502E). Triple mutations were detected among 15 S. Typhi isolates, two mutations in *gyrA* at position 83 (S83F) and at position 87 (D87G or D87N) and one mutation in *parC* at position 80 (S80I). PMQR genes were not detected among any of the 71 *S*. Typhi isolates.

The *S*. Typhi isolates (n=71) were accurately classified as susceptible, intermediate or resistant to FQs including nalidixic acid based on the topoisomerase mutations in the absence of PMQR (Table 1). This study demonstrated that the determination of MICs is a reliable option for susceptibility testing of FQs against *S*. Typhi isolates. *Salmonella* isolates with an MIC of ≥0.125µg/ml are likely to be resistant.

The correlation of QRDR mutations in S. Typhi with MICs of Na, Ci, Of and Levo is shown in Table 1. Among 71 isolates, two nalidixic acid susceptible (MIC 2 µg/ml) isolates had no QRDR mutations, but 69 nalidixic acid resistant (MICs =32 µg/ml) isolates possessed one or more QRDR mutations. Although mutation was not found in Ci susceptible isolates (MIC ≤0.06µg/ml), DCS isolates (MIC, 0.125-0.5µg/ml) possessed single or double mutations, and Ci-resistant isolates (MIC ≥1.0µg/ml) had double or triple mutations in QRDRs of *gyr* and/ or *par* genes. Similarly, single or double mutations were found in isolates with decreased susceptibility to ofloxacin and levofloxacin (MIC, 0.25-1.0µg/ml), whereas triple mutations were detected in resistant isolates (MIC≥2.0µg/ml).

In summary, this study provides insight about the correlation of disk diffusion and Etest results of FQs and Az for *S.* Typhi Kolkata isolates. Based on the result of this study ofloxacin and levofloxacin disk diffusion breakpoints may be recommended officially which is currently not included in the CLSI guidelines. Further research is necessary to evaluate the clinical relevance of the breakpoints of azithromycin with respect to the patients' outcomes like subsidence of fever, bacteria clearance etc.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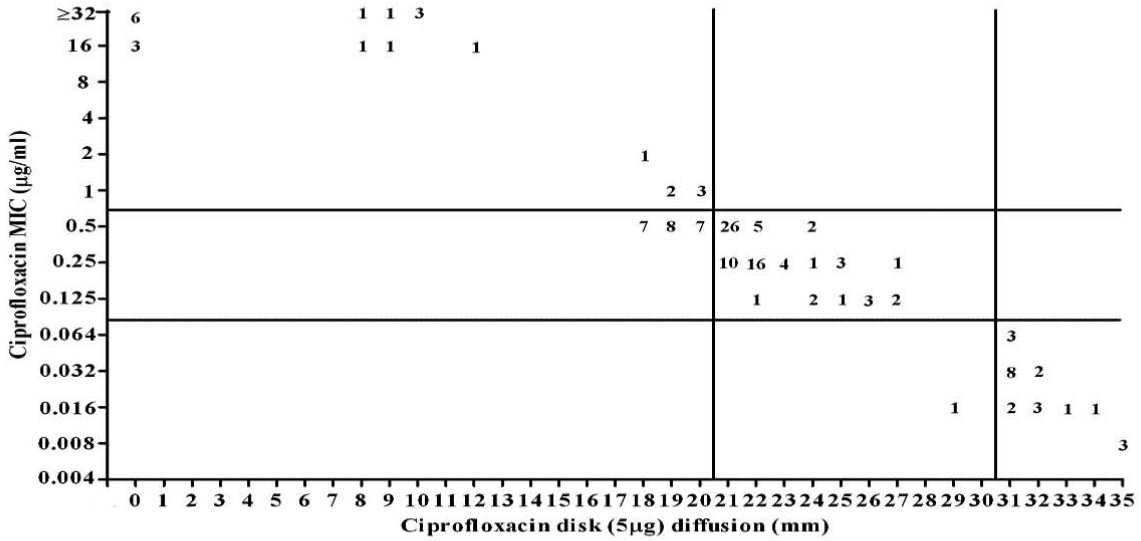
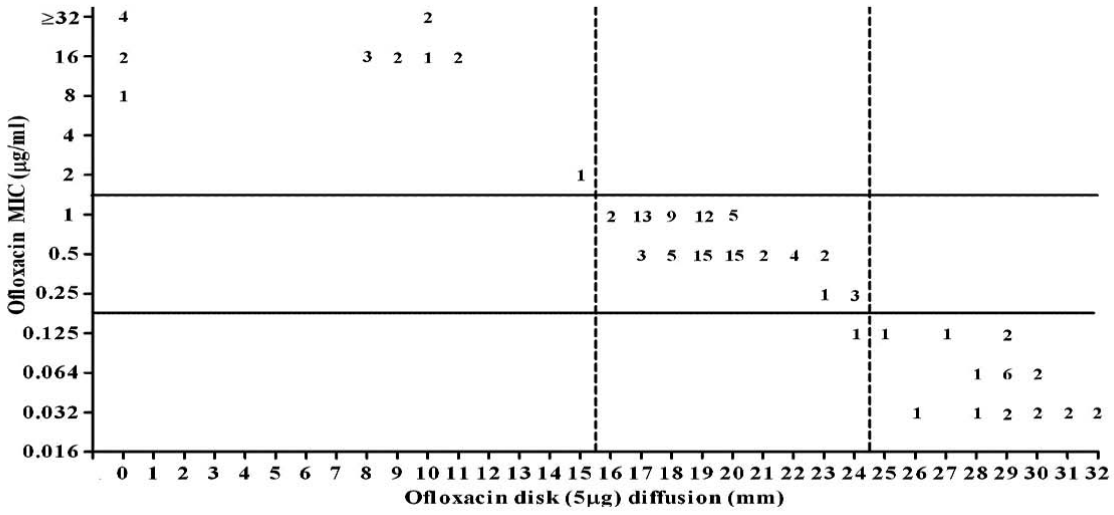
Nalidixic acid disk (30μg) diffusion (mm)


Ciprofloxacin MIC (μg/ml)
Ciprofloxacin disk (5μg) diffusion (mm)


Ofloxacin MIC (μg/ml)
Ofloxacin disk (5μg) diffusion (mm)

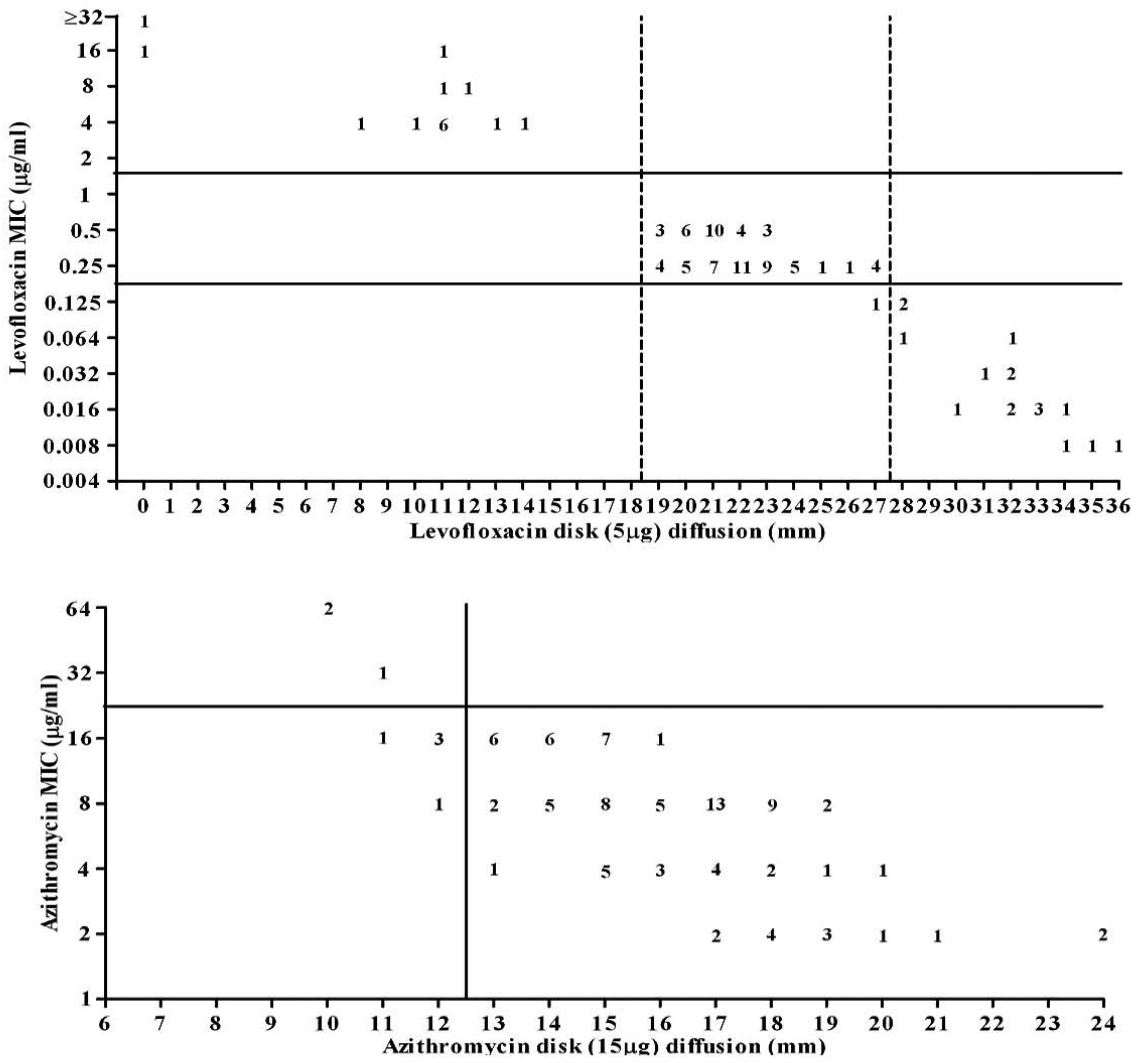


Fig 1. Scatterplots of zone diameter and MIC of nalidixic acid, ciprofloxacin, ofloxacin and levofloxacin for S. Typhi Kolkata isolates.

Table 1. Resistance mechanisms and fluoroquinolone MIC ranges for S. Typhi isolates (n=71) included in the study.[a]

QRDR mutation[b]	No. of isolates	MIC range (µg/ml)		
		Nalidixic acid	Ciprofloxacin	Ofloxacin
Absent	2	2.0	0.016-0.064	0.032-0.064
D87N (GyrA)	1	256	0.25	0.5
D87Y (GyrA)	2	128-256	0.25	0.5
S83Y (GyrA)	34	32->256	0.25-1.0	0.5-1.0
S83F (GyrA)	13	>256	0.125-0.5	0.5-1.0
S83Y (GyrA); L502E (ParE)	1	>256	0.5	1.0
S83F (GyrA); E84K (ParC)	1	>256	1.0	1.0
S83F (GyrA); E84G (ParC)	2	>256	1.0	1.0
S83F, D87G (GyrA); S80I (ParC)	1	>256	16	8.0
S83F, D87N (GyrA); S80I (ParC)	14	>256	16–=32	16–=32

[a] Plasmid-mediated quinolone resistance (PMQR) was absent.
[b] QRDR, quinolone resistance-determining region of GyrA, GyrB, ParC and ParE; D, aspartic acid; E, glumatic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; N, asparagine; S, serine.

Molecular characterization of *Salmonella enterica* serovar Typhimurium extra-intestinal isolates during 2010-2014.

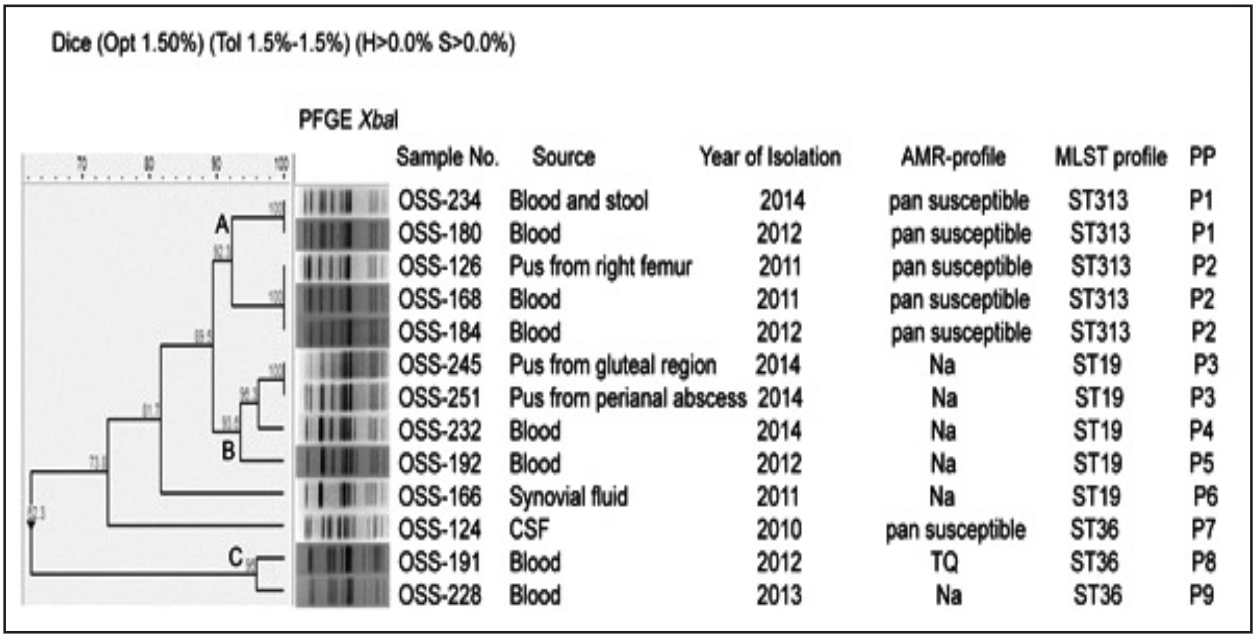
Investigators: S. Dutta, P. Jain

Non-typhoidal *Salmonella* (NTS) are important foodborne pathogens that primarily cause acute gastroenteritis. However extra-intestinal invasion occur in 5% of the patients usually with certain immunosuppressive conditions like malaria, malignancy, malnutrition, HIV-infection, etc., resulting in bacteraemia and focal infections. *Salmonella enterica* serovar Typhimurium (S. Typhimurium) is one of the serovars showing high predilection to cause invasive infections in humans and has been frequently reported from sub-Saharan African countries. Case fatality due to S. Typhimurium extra-intestinal infections has been reported to range from 38%-47%. The emergence of multidrug resistance in S. Typhimurium has further led to limited treatment options in humans. There are only few reports of S. Typhimurium, causing extra-intestinal infections, from India and those isolates were not well characterized. Therefore, the objective of this study was to characterize the S. Typhimurium extra-intestinal isolates with respect to their antimicrobial resistance, plasmids, virulence genes, PFGE and MLST profiles (Table 2).

Thirteen S. Typhimurium extra-intestinal isolates were collected from various laboratories and were included in this study. Two antimicrobial resistance profiles were seen among the isolates. Six isolates were pan susceptible. Among nalidixic acid resistant isolates, Asp87Tyr mutation in *gyr*A gene was most predominant. No mutations were seen in *gyr*B, *par*C or *par*E genes. *qnr*B1 gene was present in one isolate (OSS-191). This isolate showed reduced susceptibility

to ciprofloxacin (MIC: 0.38µg/ml) and ofloxacin (MIC: 0.75µg/ml). This isolate also showed presence of Class 1 integron with *dfr*A1+*orf*C gene as part of its gene cassette.

One or more plasmids were present in all the isolates (Table 2). spvB (virulence plasmid), spvC and pef (plasmid encoded fimbriae) were present in those isolates possessing the heavy plasmid (except OSS-124). The 13 isolates were assigned to three sequence types (ST313, ST19 and ST36) by MLST. PFGE of the isolates generated 9 pulsotypes (P1-P9) (Fig. 2) into 3 clusters (A, B, C) based on their MLST profile. Cluster A comprised of isolates belonging to ST313. It consisted of two pulsotypes P1 (n=2) and P2 (n=3). P1 and P2 showed 92.3% similarity. All ST19 isolates except one (P6) were clubbed under cluster B. This cluster consisted of 3 pulsotypes P3 (n=2), P4 (n=1) and P5 (n=1) which showed 93.6% similarity among themselves. Cluster A and B were 89.5% similar. Cluster C comprised of two ST36 isolates (P8 and P9) which were 96% similar.



Sample No.	Source	Year of Isolation	AMR-profile	MLST profile	PP
OSS-234	Blood and stool	2014	pan susceptible	ST313	P1
OSS-180	Blood	2012	pan susceptible	ST313	P1
OSS-126	Pus from right femur	2011	pan susceptible	ST313	P2
OSS-168	Blood	2011	pan susceptible	ST313	P2
OSS-184	Blood	2012	pan susceptible	ST313	P2
OSS-245	Pus from gluteal region	2014	Na	ST19	P3
OSS-251	Pus from perianal abscess	2014	Na	ST19	P3
OSS-232	Blood	2014	Na	ST19	P4
OSS-192	Blood	2012	Na	ST19	P5
OSS-166	Synovial fluid	2011	Na	ST19	P6
OSS-124	CSF	2010	pan susceptible	ST36	P7
OSS-191	Blood	2012	TQ	ST36	P8
OSS-228	Blood	2013	Na	ST36	P9

AMR, Antimicrobial resistance; Na, nalidixic acid; T, tetracycline; Q, co-trimoxazole; ST, Sequence Type: PP, PFGE pulsotypes

Fig 2. PFGE profiles of XbaI digested DNA of S. Typhimurium extra-intestinal isolates during 2010-2014, by cluster analysis.

Table 2. Molecular characterization of *Salmonella* Typhimurium isolates from extra-intestinal sites, India 2010-2014.

Sl. No.	Sample No.	Source	Place of Isolation	Year of Isolation	Antimicrobial resistance			Approximate size of Class 1 integron gene cassette (gene cassette array)	Plasmid		MLST profile	Virulence profile
					R-profile (MIC in µg/ml)[a]	Genes mediating antimicrobial resistance[b]	Mutation in the QRDR of *gyrA*[c]		Plasmid profile (in kb)	Plasmid typing by PCR		
1.	OSS-124	CSF	Bangalore	2010	Pan susceptible	-	-	-	215.4	untypable	ST36	*inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
2.	OSS-126	Pus from right femur	Bangalore	2011	Pan susceptible	-	-	-	157.1, 3.6	FIIS, FIB	ST313	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
3.	OSS-166*	Synovial fluid	Bangalore	2011	Na(>256)	-	D87Y	-	166.7, 7.0	FIIS, FIB	ST19	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
4.	OSS-168	Blood	Bangalore	2011	Pan susceptible	-	-	-	177.2	FIIS, FIB	ST313	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
5.	OSS-180	Blood	Bangalore	2012	Pan susceptible	-	-	-	159.8, 2.6	FIIS, FIB	ST313	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
6.	OSS-184	Blood	Bangalore	2012	Pan susceptible	-	-	-	159.4	FIIS, FIB	ST313	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
7.	OSS-191*	Blood	Bangalore	2012	T(>256), Q(>32)	*tet*B, *dfr*A1, *sul*1, *qac*EΔ1, *qnr*B1	-	1.2kb (*dfr*A1+*orf* C)	15.2, 8.9	untypable	ST36	*inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
8.	OSS-192*	Blood	Bangalore	2012	Na(>256)	-	D87Y	-	149.1	FIIS, FIB	ST19	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
9.	OSS-228*	Blood	Bangalore	2013	Na(>256)	-	S83F	-	28.9, 9.1, 3.4	untypable	ST36	*inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
10.	OSS-232*	Blood	Kolkata	2014	Na(>256)	-	D87Y	-	139.4	FIIS, FIB	ST19	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D
11.	OSS-234	Blood and stool	Bangalore	2014	Pan susceptible	-		-	139.1, 2.8	FIIS, FIB	ST313	*spv*B, *spv*C, *pef*, *inv*A, *stn*, *sop*B, *ssa*Q, *mgt*C, *hil*A, *spi*4D

[a]Na, nalidixic acid; Q, co-trimoxazole; T, tetracycline.

[b]*dfr*A1, co-trimoxazole resistance; *orf*C, open reading frame; *qac*EΔ1, quarternary ammonium compounds resistance; *qnr*B1, plasmid mediated fluoroquinolone resistance; *sul*, sulphonamide resistance; *tet*B, tetracycline resistance.

[c]D, aspartate; F, phenylalanine; S, serine; Y, tyrosine.

-, negative; *, these isolates showed reduced susceptibility to ciprofloxacin (MIC: 0.12-0.5µg/ml) and ofloxacin (MIC: 0.25-1.0µg/ml)

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

Investigator: A. Palit

Physico chemical Parameters:

Water temperature varied between 17.1^{O}C to 37.2^{O}C, with an elevated temperature at summer and comparatively lower at winter. pH level of the water samples at both study sites varied between 7.1 to 8.7 (alkaline in nature). Salinity varied greatly between the four (4) study sites. Highest salinity was recorded at Freserganj followed by the salinity at Kakdwip, the salinity at Diamond Harbour (0.0-2.4 PSU) was lower than the other two sampling stations. Salinity at Howrah site is too low to detect. **T**urbidity varied between 35 NTU to 550 NTU (at Howrah), 40 NTU to 900 NTU (at Diamond Harbour), 128 NTU -563 NTU at Kakdwip and 207 NTU – 484 NTU at Fresurganj.

Bacterial Preponderence:

Bacterial disposition pattern seems to be identical at all the study sites. TBC value varried mostly between 1 X 10^2 to 1 X 10^7 cfu/mL. While inland (HB & DH) bacterial load was upto 10^7 cfu/ml, estuarine environment restricted their bacterial preponderence upto 10^5cfu/ml. Cultivable vibrio count ranged between 1-1000cfu/mL at all the sampling sites, with a higher disposition at Howrah Bridge than that of other down stream sites. Dispositional variation of Vibrio organism at both sites indicate the role of different geographical settings as well as climatic and physico-chemical factors. The highest CVC disposition at Howrah indicate the factor of indiscremenate sewage disposal along with chunk of microbial pool. At Howrah Bridge we have observed a higher peak at rainy season (because of its inflow of higher volume of flood water along with fecal organic debris) in comparison to estuarine sites, where the peak has been observed at summer season (because of higher intrusion of marine saline water).

Diamond Harbour site was characterized with a higher tidal influence. Proximity to the sea mouth is the major contributing factor resulting in a clear visualization of tidal effect on the physico-chemical properties as well as on the predominance of different bacterial community (Fig 3). Thus a nice variation of bacterial prevalence can be noticed along with the tidal changes.

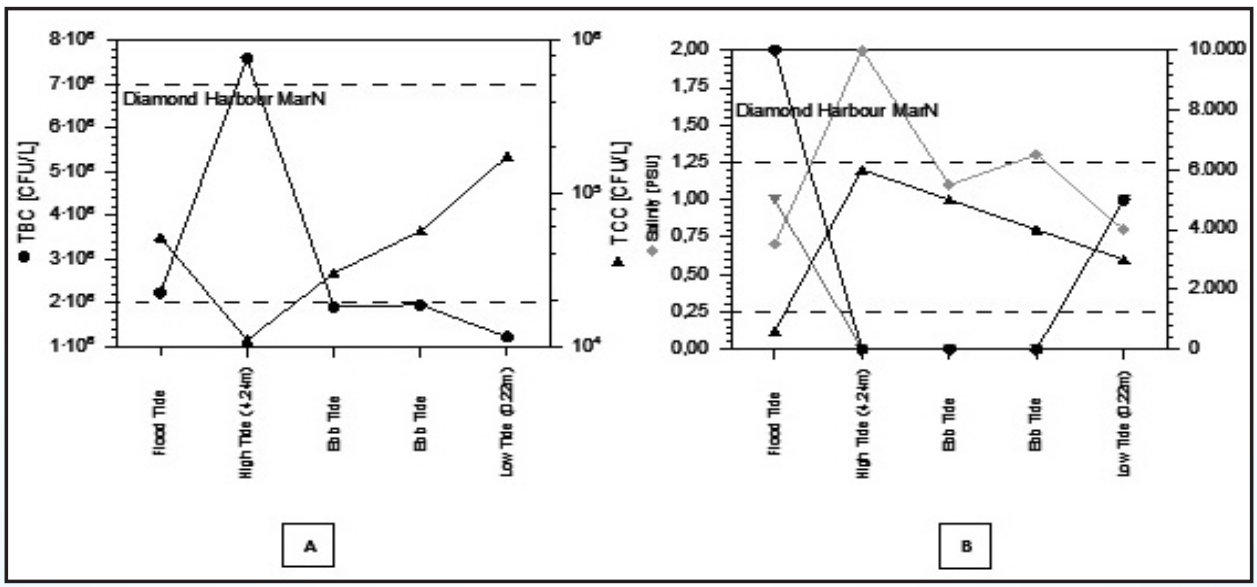


Fig. 3 : Tidal impact on bacterial as well as physico-chemical parameters.

100 odd suspected *Vibrio* samples have been isolated, irrespective of study focus, *V. cholerae, V. parahaemolyticus* seems to be the most prevalent species among all other *Vibrio* organisms. *V. mimicus, V. alginolyticus, V. vulnificus* (other than *V.cholerae* and *V.parahaemolyticus*) has also been identified from different zones. Altogether 40 samples

were positive for vibriophages. Vibriophage preponderance has been increased along with water temperature (during summer) and reached at its peak during monsoon.

The present study, in the dimensions of a longitudinal and systematic approach addresses the issues of correlation between seasonal, physico-chemical factors, tidal influences, lunar positions along with the preponderance of *Vibrio* and heterotrophic bacterial density in a flowing riverine-estuarine ecosystem in Indian scenario with persistent high diarrheal incidence reports. Therefore, the present findings will of immense importance to underline a hitherto unexplored and unreported fact that seasonal variations coupled with atmospheric oscillations, tidal amplitude (spring tide, neap tide, etc.), lunar cycle, associated physico-chemical changes are the convincing bioenvironmental determinants significantly related to the disposition of the Vibrio community in particular and the total bacterial preponderance as an entity. The abundance of *Vibrio* and TBC also showed a positive dependence on turbidity which in turn can be influenced by tidal regime, turbulence and runoff. Further characterization will help to conclude the role of *Vibrio* dynamics in riverine-estuarine ecosystem in south Bengal diarrheal incidence.

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

Investigator: A. Palit (PI)

Results:

Water samples collected from the riverine estuarine sources were analysed for different physico-chemical indices as well as for the entero-pathogenic Vibrios and for Vibriophages. Throughout the study period, 148 water samples from the two sites of the Hooghly River (Howrah and Diamond Harbour) were collected. Water temperature oscillated between 17.3 to 34.7ºC at Howrah, between 15.1 to 36.6 ºC at Diamond Harbour, without any significant variation amongst respective sampling sites. pH level varied between 7.10- 8.13 at Howrah site, 7.1-7.96 at Diamond Harbour respectively with a basic alkaline drift. Salinity level at Site I (Howrah) always remained <0.1ppt and yearlong salinity gradient varied between 0.2-4.7 ppt. at Site II (Diamond Harbour). Turbidity was slightly higher at Diamond Harbour varying between 86 to 723 NTU, compared to Howrah (32 to 589 NTU).

Enteric Vibrios

V. parahaemolyticus were mostly prevalent in the samples of high saline zone (Site III), to some lesser extent at Diamond Harbour and was completely absent at Howrah. Both *V. alginolyticus* and *V. vulnificus* could be detected from all the three sampling sites, with a slightly higher preponderance at Site III. *V. mimicus* was isolated with highest preponderance from low saline zone, followed by mid and saline zone respectively. *V. cholerae* non-O1/O139 was the most prevalent among all the five species of non-cholera *Vibrios* being present in the water samples. Highest abundance of *V. cholerae* non-O1/O139 was also observed at Site I at salinity <0.1ppt. Seasonal prevalence of *Vibrios,* gradually decreased from the monsoon months (20-34ºC), followed by summer (24-36ºC) and winter (13-18ºC) seasons respectively. Irrespective of any organism or any site, monsoon seems to be the most favourable condition with maximum isolation rate achieved during the period.

Thereby it is convincingly established that Gangetic riverine-estuarine aquatic ecosystem regulates the survival, distribution and transmission of diarrheogenic *Vibrios* from its saline habitat to inland fresh water riverine ecosystem, where it can adversely affect human health. Current observation of aquatic environmental circulation of enteropathogenic *Vibrios* along the riverine-estuarine gradient also necessitates further long term studies on assumed benthic *Vibrio* dynamics including the pathogenic ones for a comprehensive understanding of the seasonal occurrence of *Vibrio* induced diarrhoea in this endemic focus.

Entero-pathogenic *Vibrio* dynamics in relation to salinity gradient in south Bengal riverine and estuarine environment: impact on coastal health population

Investigators: A. Palit

Results Obtained so far:

Based the yearlong seasonal variation of physico-chemical properties at riverine-estuarine ecosystem, the study sites can be divided into three following catagories:

i) High Saline Zone- The zone has been demarcated with a salinity ranged between 10 to 30 ppt. In the present study, Gosaba is such a site which can be categorised as high saline area. Simultaneously, the turbidity level was also significantly low (25±10 NTU, except unique increase after catastrophic event) than that of other study sites. Seasonal impact either by means of heavy rain fall/down pour could be visualized during rainy period, when salinity get reduced (upto 12ppt) or due to storm during summer period (Fig-4, Table-3) resulting in high turbidity (up to 960 NTU).

ii) Mid Saline Zone- The zone has been demarcated with a salinity ranged between 1 to 10 ppt. In the present study, Kakdwip and Diamond Harbour is such a site which can be categorised as mid saline area. Although, a distinct fluctuation of salinity has been observed at both the stations (10±5 ppt at Kakdwip and 4±3 ppt at Diamond Harbour) (Fig-4, Table-3).

iii) Low Saline Zone- The zone has been demarcated with a salinity ranged up to 1 ppt. In the present study, Howrah is such a site which can be categorised as low saline inland area. No distinguishable fluctuation of salinity has been observed at this station and salinity has remained <0.1 ppt throughout the year (Fig-4, Table-3).

The turbidity level was also very high (50 to 960 NTU) at the mid saline zone, greater than that of other study sites. Seasonal impact either by means of heavy rain fall/down pour could be visualized during rainy period, when salinity as well as turbidity get reduced or during summer period, when higher marine water intrusion causing greater turbulence resulting in higher salinity and turbidity. The concentrations of suspended matter (denoting turbidity) are usually high at the river-end of the estuary and decrease seaward with increase in salinity. Contrastingly, at two di?erent periods, viz., June–September and February–April, the concentrations of SPM at sea-end stations are orders of magnitude greater suggesting that this part of the channel represent zone of estuarine turbidity maximum (ETM).

Conductivity is the proper alternative varied between 160 to 600 µs/cm with a peak during winter and early summer. The winter/ early summer peak indicate the minimum or no effect of saline water intrusion. Rather, seasonal impact could be measured during rainy period, when conductivity get reduced drastically. However, turbidity level varied between 50 to 550 NTU, showed its peak during rainy season. Heavy rain fall followed by flood water intrusion along with huge organic debris facilitate the re suspension of the sediments resulting in higher turbidity during monsoon.

Since flood water discharge (within river track) is greatest during the South West monsoon there may be an increase in both stratification and strength of the inland riverine circulation. The seaward moving dominant surface water river flow counteracts the landward moving bottom flow, intensified winds and wind-induced waves and tidal current activity. The opposing marine and fluviatile processes are sufficiently intense to move sediment up the inland often causing for the development of turbidity maximum.

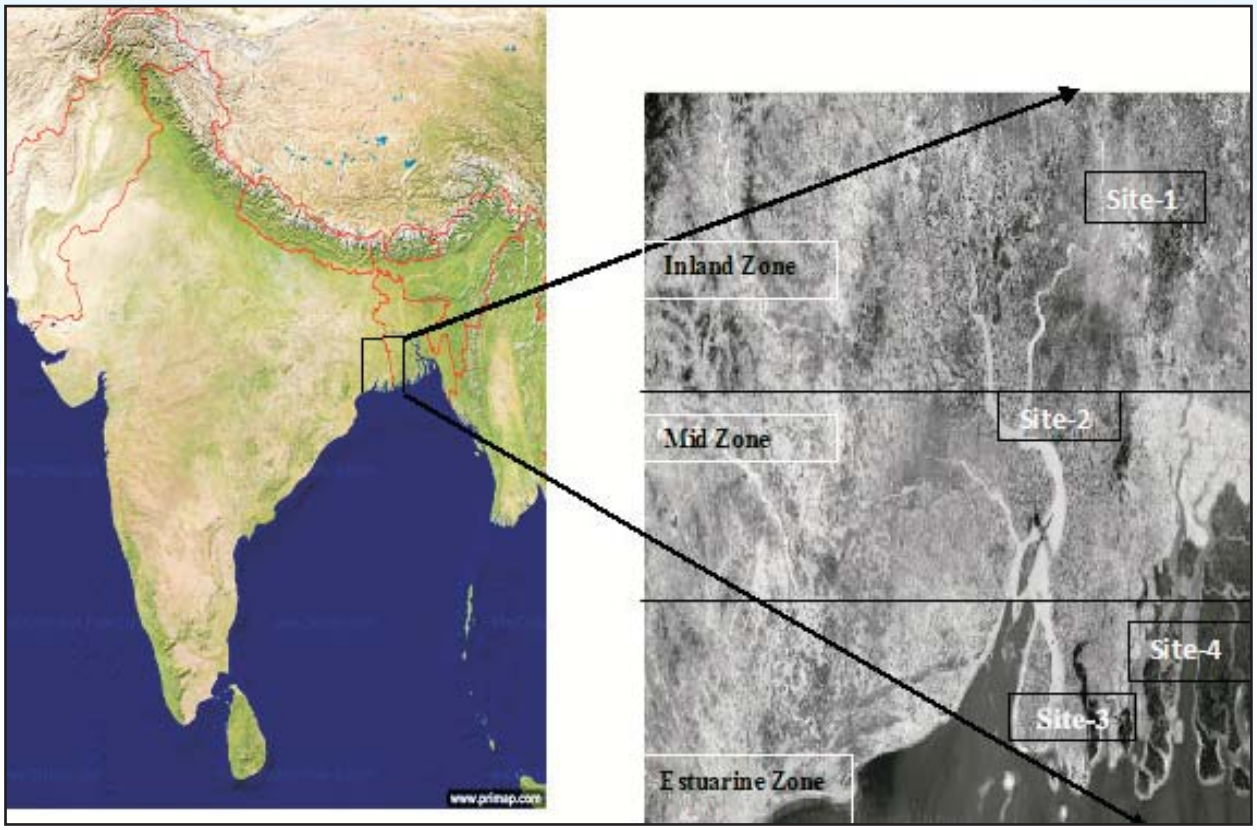


Fig. 4 : Zonation of South Bengal river in e-estuar in e-ecosystem.

Table-3 : Physico-chemical properties of river in e-estuarine water samples.

Season	Estuarine zone	Mid zone	Inland zone
Summer	pH:7.7-8.4 Salinity:25-30ppt Turbidity:10-150 NTU Temperature:26-34°C	pH:7.6-81 Salinity:1.5-8ppt Turbidity:20-920 NTU Temperature:26-37°C	pH:7.6-8.6 Salinity:<0.1ppt Turbidity:35-275 NTU Temperature:23-37°C
Monsoon	pH:7.2-8.6 Salinity:7-26ppt Turbidity:5-100 NTU Temperature:29-31°C	pH:7.1-8.0 Salinity:0.1-0.7ppt Turbidity:100-650 NTU Temperature:30-35°C	pH:7.0-8.0 Salinity: <0.1ppt Turbidity:75-550 NTU Temperature:29-31°C
Winter	pH:7.6-8.4 Salinity:16-25ppt Turbidity:10-100 NTU Temperature:19-29°C	pH:7.2-8.2 Salinity:0.1-1.7ppt Turbidity:150-420 NTU Temperature:21-28°C	pH:7.7-8.7 Salinity: <0.1ppt Turbidity:40-200NTU Temperature:16-26°C

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

Investigator: B. L. Sarkar

The strains of *V. cholerae* isolated from patients, environment and outbreak sent to us from different institutes across the country. A total of 421 strains of *V. cholerae* were received from different parts of the country during the current year

for serotyping, biotyping and phage typing at Phage Laboratory (Table 4). All the 421(100 %) strains were confirmed as *V. cholerae* O1 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 345 (81.9 %) followed by Inaba 42 (9.97%). A total of 16 (3.8%) strains were found to be untypeable with the conventional scheme of 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 (68.03%) followed by type 26 (6.51 %), type 23 (5.65 %), type 13 (3.94 %) and type 24 (2.85 %) respectively. It has been shown that type 27 was the predominant phage type circulating in this country. For the last couple of years, *V. cholerae* O139 is absent as we did not receive any strains from any parts of the country.

Table-4 : Biotype, Serotype and Phage Type of *V.Cholerae* Strains received during 2014-15.

State	No of Strain	Biotype		Serotype		Basu & Mukherjee			New Phage													
		El Tor	Classical	Ogawa	Inaba	T-2	T-4	UT	3	7	10	13	19	20	21	22	23	24	25	26	27
Assam	24	24		24		-	22	2				1					-	1		3	19
Andhra Pradesh	33	33	-	29	4	2	31	-	-	-	2	2	-	1	-	-	2	2	-	3	21
Gujarat	79	79	-	45	34	32	45	2	3	3	2	3	5	-	-	3	7	3	1	7	42
Madhya Pradesh	28	28	-	21	7	-	27	1	-	-	-	2	-	1	-	-	2	2	1	3	17
Maharashtra	84	84	-	67	17	13	64	7	2	1	-	2	-	-	1	1	4	1	1	4	67
Punjab	56	56	-	56	-	18	38	-	2	-	2	1	2	1	2	3	4	-	3	3	33
Rajasthan	77	77	-	68	9	-	73	4	2	2	-	3	1	-	2	2	3	2	3	3	54
West Bengal	40	40	-	32	8	6	34	-	-	-	-	1	-	1	-	-	1	1	-	2	34
Grand Total	421	421	-	342	79	71	334	16	9	6	6	15	8	4	5	9	23	12	9	28	287
Total %	100	100	-	81.2	18.7	16.8	79.3	3.8	2.1	1.4	1.4	3.5	1.9	0.9	1.1	2.1	5.4	2.8	2.1	6.6	68.1

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

Investigator: B. L. Sarkar

A total of 300 *V. cholerae*, biotype El Tor strains were taken for this study from the year 1990 – 2014. Most of the strains were found to be Ogawa (91 %) and the Inaba (9%) serotype was dispersed among very few isolates. According to new phage typing scheme, type 27 was widely distributed throughout the study, followed by type 26, type 14 and type 23. Only 4.23 % strains were found to be sensitive to all of the antibiotics but 91.25 % strains were resistant to streptomycin and sulfamethoxazole/trimethoprim. Among the 300 strains almost 252 strains was found to contain toxigenic traits like ctxA, sxt, zot positive. MAMA-PCR result showed the presence of toxin gene, Classical *ctx*B in 98.3 % of the strains of *V. cholerae* where as 0.6 % strains were found to be positive for El Tor *ctxB*. From the year 2006, haitian ctxB positive strains (1.1%) were found among the clinical isolates. The experiment for analysis of fluid accumulation for different *V. cholerae* strains in the rabbit ileal loop model was performed to determine the toxicity of the strains. A total of 10 *V. cholerae* strains of 10^8 CFU were inoculated in RIL model. All except one strain were found to be toxigenic in RIL. Results were expressed as fluid accumulation per loop length (FA). The clinical isolates from the recent decade (2003-2014) showed increased levels of fluid accumulation in rabbit ileal loop assay compare to the previous decades. These observations revealed that current strains of *V. cholerae* were more toxigenic compare to previous years. This study found that genotypic changes can influence the phenotypic behaviour as it is visibly seen in case of phage typing and rabbit ileal loop assay. The evolutionary scenario depicts that the *V. cholerae* strains are rapidly evolving with genetic aspects. These epidemiological findings would fill the gaps in future research arena of *Vibrio cholerae.*

Development of a bacteriophage-based biocontrol technology for the treatment of cholera

Investigator: B. L. Sarkar

The Indo-UK collaborative project between NICED-UKERI, UK is ongoing. In an around Kolkata, a total of 55, O1 specific vibriophages were isolated. Later on, the restriction digestion (EcoRV) pattern of the phage DNA has indicated only five dissimilar phages. These five phages also showed distinct plaque morphology compared to each other on nutrient agar plates. The *in vitro* lytic activity of these five vibriophages showed a maximum lytic activity for A12, A13 for two hours after infection whereas, other three phages (A2, A4, A10) represented the maximum inhibition up to three hours of infection. All the five vibriophages isolated were totally new and different from the previous *V. cholerae* phages routinely in use in our laboratory. These environmentally isolated phages are highly lytic and having broad host range.

Development of a high throughput screening (HTS) and identification of small molecule inhibitors of *Vibrio cholerae* pathogenesis.

Investigators: R.K Nandy (NICED), Dr. W. Tegge, (HZI, Germany)

A high throughput screening (HTS) assay was developed for identifying compounds that can reduce the secretion of the major pathogenicity factors of *Vibrio cholerae*, cholera toxin (CT) and toxin co-regulated pilus (TCP). *V. cholerae* O139 strain MO10 carrying the construct (pAKSB) capable to exhibit conditional kanamycin resistance (Km^r) under the control of *aphA* like gene promoter was utilized for this assay. Approximately 20300 compounds were screened by newly developed HTS assay to identify compounds that caused growth reduction in the presence of Km but not in its absence and were further investigated for their effect on CT expression. Six compounds that exhibited growth inhibitory half maximal concentration (g-IC_{50} value in low mM range) in the presence of Km but not without Km containing media, were considered as active compounds. These most active compounds caused reduction in CT secretion and had low μM CT-IC_{50} values. The transcriptional inhibition was evident by qRT-PCR for *aphA*, *ctxA* and *tcpA* in cells grown in the presence these compounds. Identification/ characterization of compounds with potential to attenuate pathogenesis is an emerging area for interest towards development/ designing of new drug(s) to overcome concerns on emerging multidrug resistance pathogens.

Haitian Variant *tcpA*: Evidence for the sequential event in the evolution of *Vibrio cholerae* in India

Investigator: A. K. Mukhopadhyay

Cholera still continues a substantial health problem due to inadequate hygiene and sanitation, especially in Africa and Asia. This severe, dehydrating diarrheal disease is caused by the Gram negative bacterium *Vibrio cholerae*. In recent years, novel pathogenic variants of *V. cholerae* O1 have been emerged and disseminated throughout the world. This indicates a cryptic change in the genome of *V. cholerae* subsequently modified the epidemiology of cholera. The devastating cholera outbreak during 2010 in Haiti, for the first time in almost a century, placed this ancient scourge at the forefront of the global public health agenda. More than 5 lakh people got sick with this outbreak and around 8000 people were died. Many published reports suggest that the origin of cholera in this region may be from Asian countries and/or due to indigenous strains. The World Health Organization recognized the re-emergence of cholera as a significant global public health problem and called for the implementation of an integrated and comprehensive global approach to cholera control.

Whole genome sequencing analysis of *V. cholerae* strain in Haiti revealed some unique mutations in different segments of their chromosomes. These include structural variation in superintegron, VSP-2, and SXT as well as SNPs in the *ctxB* allele which codes for the B subunit of the cholera enterotoxin (CT).Further analysis of the sequencing data and BLAST results showed presence of another mutation at the 266th nucleotide position of the *tcpA* allele, which codes for the major structural protein of the toxin-co regulated pilus, the second major virulence factor of *V. cholerae*. Our previous studies showed that the El Tor variant strains of *V. cholerae* O1 producing classical CT have completely replaced the prototype El tor biotype strains in Kolkata, India since 1995 and followed by the appearance of Haitian type *ctxB* in 2006.

These results together with novel genetic variations in the Haitian isolates motivated us to further investigate the emergence and dissemination of variants carrying this novel mutant of *tcpA* allele if any, in Kolkata. We have developed a simple PCR-based assay by exploiting the sequence variation to accurately discriminate the Haitian, El Tor, and classical type *tcpA* alleles(Fig 5).

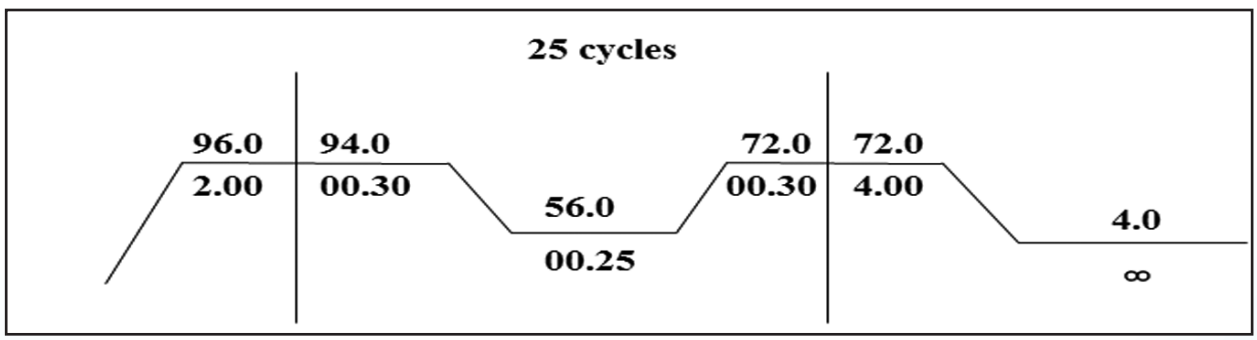


Fig. 5 : Different sets of PCR were done using variation in the temperature and timing with the control strains. Finally, this standardized PCR condition provided the best result.

Further, this newly developed method was utilized in the retrospective analysis of *V. cholerae* O1 strains isolated from cholera patients over the 12 years (2001-2012) in Kolkata, India to understand the advent of new allele of *tcpA* along with the bioinformatics based analysis to elucidate the influence of this new alleleon the pathogenesis(Fig 6 and Fig7).

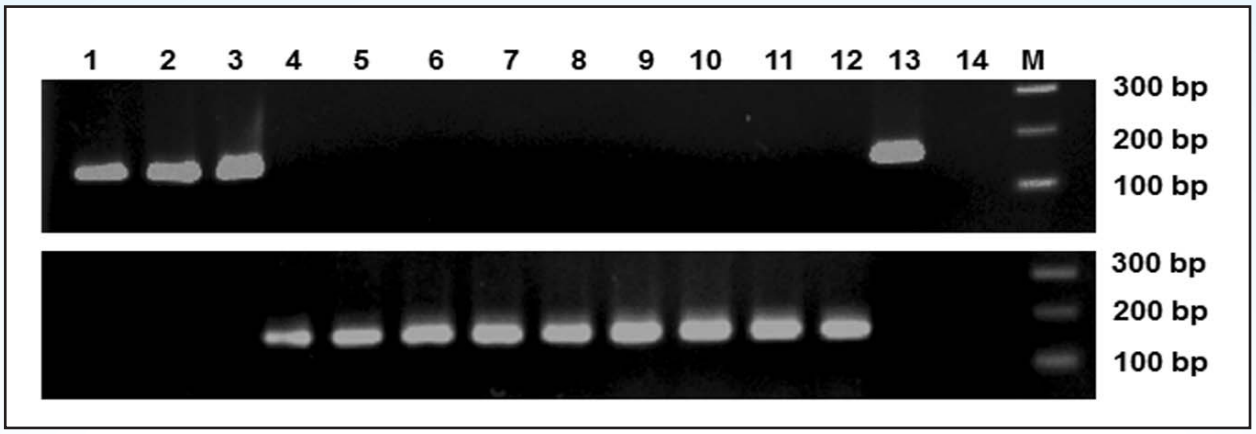


Fig. 6 : Standardized PCR assay to detect the type of *tcpA* allele in representative *V. Cholerae* O1 strains of Kolkata using primers (*tcpA*F1/*tcpA* EL-Rev) for El Tor *tcpA* allele (Upper panel) and (*tcpA*F2/*tcpA* EL-Rev) for Haitian *tcpA* allele (Lower panel).

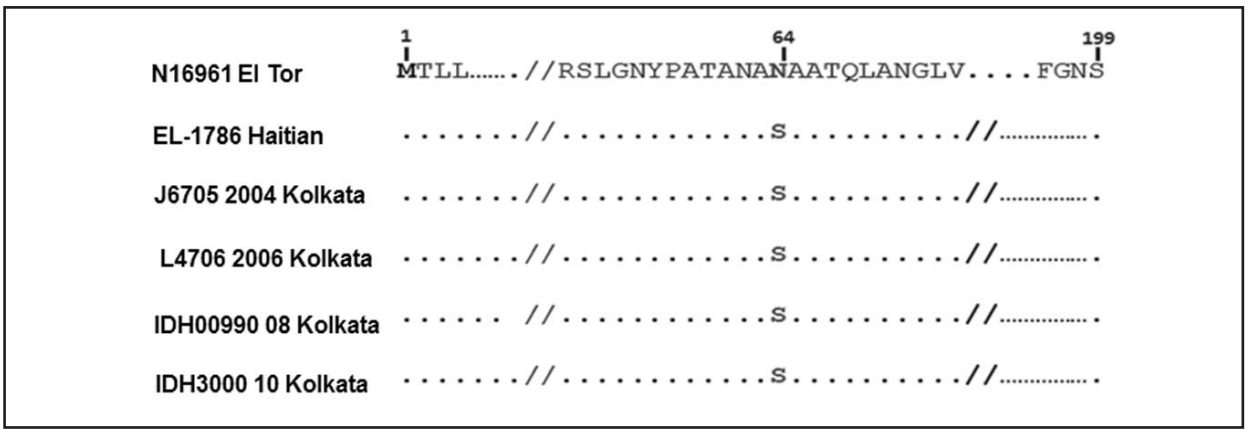


Fig 7. The deduced amino acid sequence of *TcpA* of representative Kolkata isolates were found to be identical to the amino acid sequence of the matured *TcpA* of the El Tor reference strain N16961 except for an asparagine to serine substitution at the 64th position of the sequence confirming its identity with the Haitian type *TcpA*.

Our results showed that Haitian *tcpA* first appeared in Kolkata during October, 2003, and interestingly soon after its appearance; this new variant *tcpA* displaced the canonical El Tor *tcpA* completely in the following years(Fig 8).

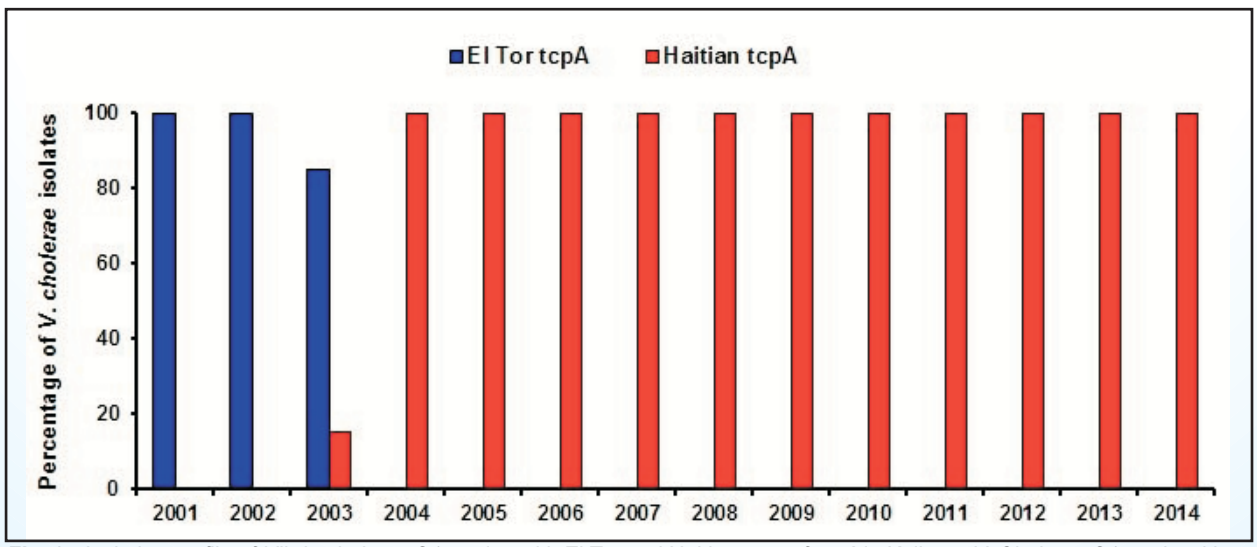


Fig. 8 : Isolation profile of *Vibrio cholerae* O1 strains with El Tor and Haitian type of *tcpA* in Kolkata. *V. Cholerae* O1 strain with Haitian type *tcpA* was first time isolated in Kolkata during October 2003.

Our bioinformatics based analysis depicted that among the three different mutations present in 89thposition, only Asparagine to Serine is positively selected.The particular mutation (Asn->Ser) at the 89th amino acid of whole TcpA (or 64th amino acid of mature TcpA) is the result of transition, i.e., purine-purine conversion. This pattern is conserved natural selection, since a transition bias (i.e., purine-purine conversion) is expected to reduce the incidence of potentially harmful mutations and thus evolutionarily preferred.Our previous study indicated that the Haitian *ctxB* first appeared in Kolkata during April, 2006. Therefore, a certain proportion of *V. cholerae* strains in Kolkata acquired the combination of Haitian *ctxB* along with Haitian *tcpA* from April 2006 onwards. It should be noted however that this occurrence (acquisition of Haitian *ctxB* and *tcpA*) does not always occur in tandem.This Haitian variant strain may be theresult of the sequential genetic events in the evolution of *V. cholerae* strain in the Indian subcontinent. Our results highlight a significant event in the evolution of recent variants of *V. cholerae*. Finally,this finding not only shows a cryptic change in the epidemiology of cholera but also raises questions about the origin of this variant of *V. cholerae* O1 El Tor.

Studies on carbapenem resistance in Acinetobacter from cases of neonatal sepsis and establishment of a reliable phenotypic test

Investigator: S. Basu

Carbapenem resistance has increasingly been reported in *Acinetobacter spp*. In addition to the studies on carbapenem resistance in Enterobactericae we also evaluated the mechanism of carbapenem resistance in *Acinetobacter spp* causing neonatal sepsis. Both the transmissible (carbapenemases) and nontransmissible (efflux pumps) modes of resistance were studied. We have been able to gain a unique assessment of the diverse genetic determinants responsible for carbapenem resistance in these isolates. The emergence of NDM-1 along with an already existing repertoire of carbapenem-hydrolyzing-oxacillinases was noted. Analysis of the efflux pump genes such as AdeABC, AdeIJK, AbeM and AbeS in the *Acinetobacter* were carried out and proved to be positive in these isolates. Compared with carbapenem-susceptible strains, the expression levels of AdeB and AbeS were observed to be higher by real-time RT PCR. Sequence analysis revealed mutations in regulatory systems of these pumps which might be responsible for their over expression. The diversity of the resistance mechanisms makes this species a threat in the hospital environment. Further, a phenotypic test was devised to detect other β-lactamases in NDM-harbouring isolates. ESBLs and AmpCs may escape detection when they coexist with metallo-β-lactamases such as New Delhi Metallo-β-lactamases-1. A combination disk assay was established using cefotaxime, cefotaxime/clavulanic acid, cefotaxime/clavulanic acid/cloxacillin, cefoxitin and cefoxitin/phenylboronic acid/cloxacillin on Mueller Hinton agar supplemented with dipicolinic acid for determination of β-lactamases in the presence of NDM-1. The assay, therefore, can be suggested for routine diagnostic application because of its reliability, specificity and very good discriminatory potential for different β-lactamases (Fig.9).

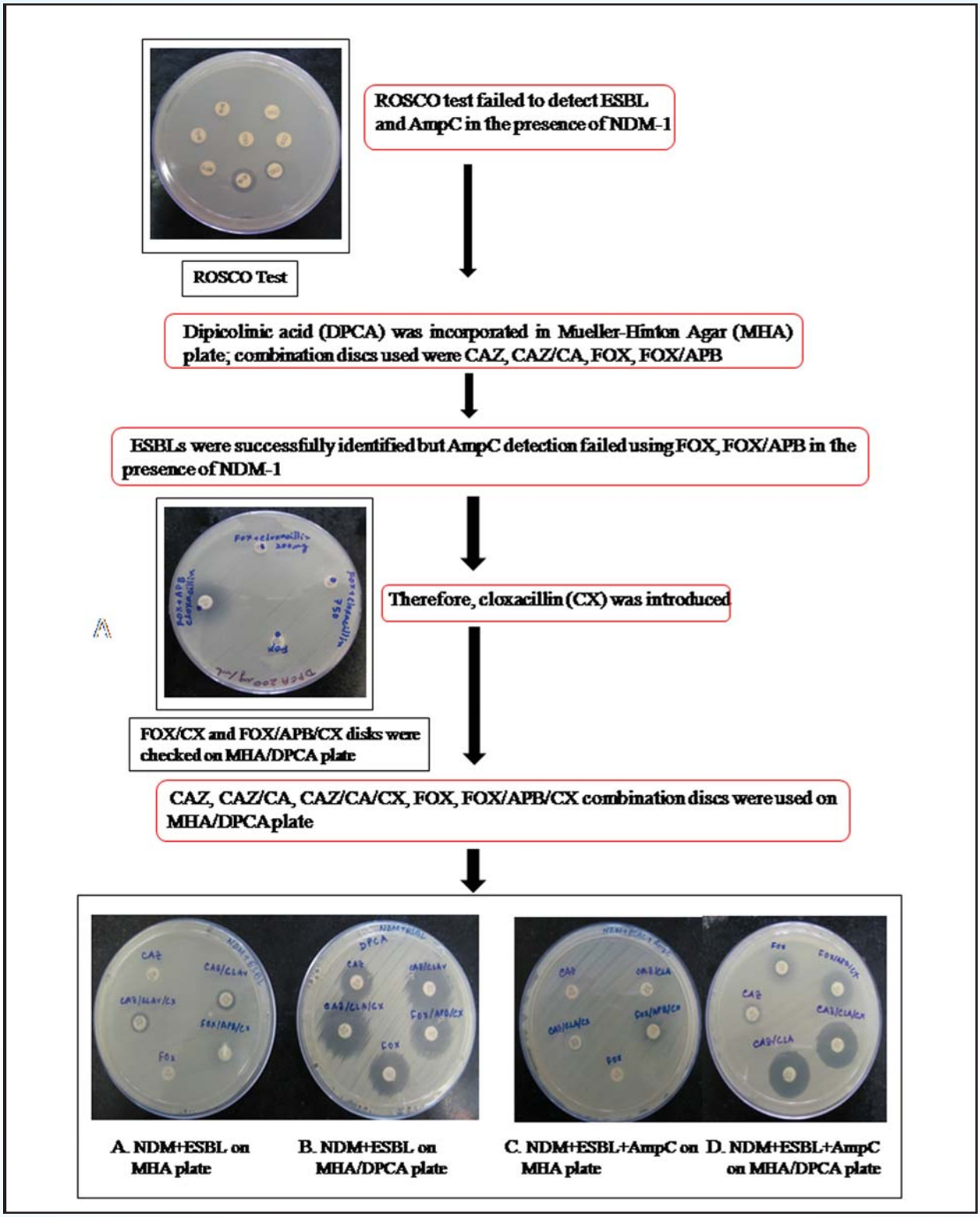


Fig 9. Diagrammatic representation of the phenotypic assay for detection of ESBL and AmpC in MBL-producing gram-negative bacteria with the use of aminophenylboronic acid, dipicolinic acid and cloxacillin

tolA gene deleted *Shigella boydii* type 4 mutant releases more outer membrane vesicles than wild type

Investigators: H. Koley, S. Mitra, D. Nag, R. Sinha, P. Mukherjee, D. R. Hawlader, D. R. Saha.

Vaccines in use today were developed using techniques pioneered more than 100 years ago and do not reflect the full potential of the field. With the introduction of developing biotechnologies such as genetic engineering, rapid advances have been made in the design, synthesis, and application of modern vaccines against a myriad of infectious diseases. Scientist, Academician and thinking of a modern vaccine has two simple objectives: replace the vaccine target with a simplified molecular identity (antigen) that the immune system can use to recognize the target in the future and replace the inherent immunostimulation of a pathogen's infection with a less dangerous stimulus (adjuvant). Among them, we have observed OMVs contain a variety of immunoactive virulence factors, became interested in their potential to be used as modern vaccines.

Our previous studies on outer membrane vesicles based vaccine development against shigellosis, we observed the inefficient of *Shigella* to secrete significant amount of outer membrane vesicles naturally, during growth, making the study very time consuming and costly. To overcome this trouble, we disrupted *tolA* gene, necessary to maintain outer membrane integrity from four *Shigella sp* (Table 10). It was observed more than 60% increase of OMVs secretion was noticed in the *tolA* mutants than the wild type. Moreover, Δ*tolA*-OMVs played better stimulatory role to macrophage and epithelial cells inducing more different cytokines secretion than the wild type OMVs. Over all cytokine profile has made clear the Th1 biased immune response by OMVs of *Shigella*. This study has efficiently established a new technique for better production of OMVs in shigellae. In-combination use of different shigellae ΔtolA-OMVs will be valuable role in the field of next-generation nonliving vaccines against shigellosis.

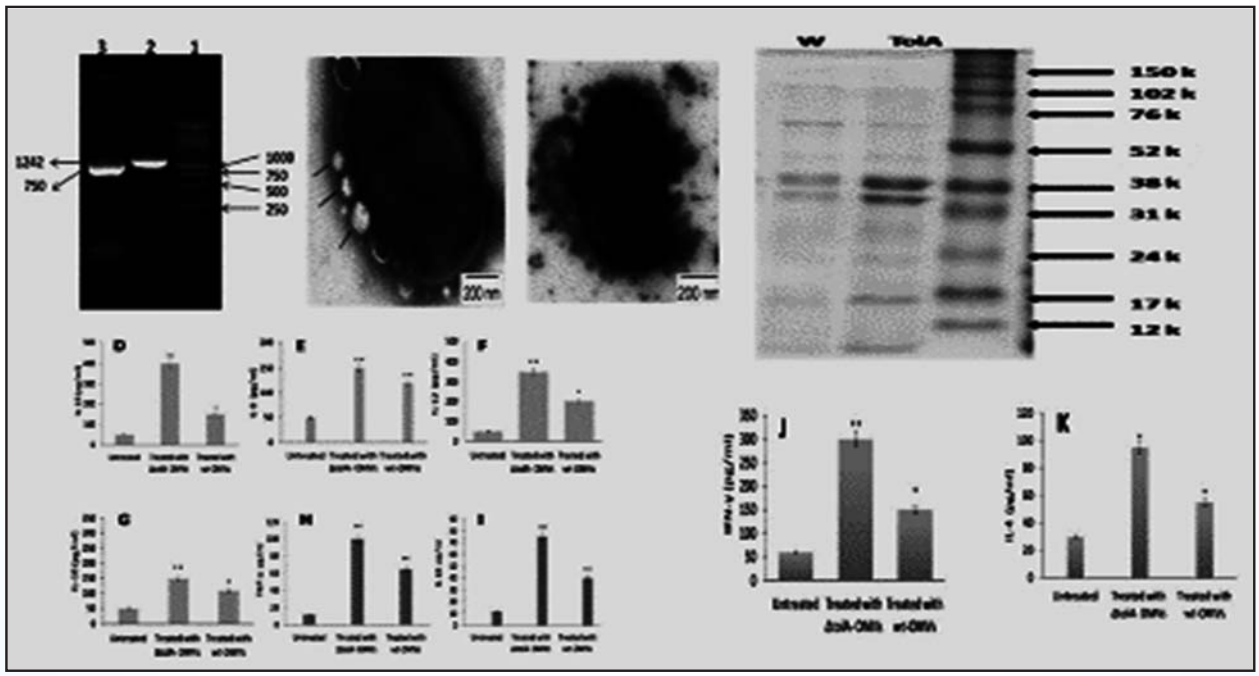


Fig. 10 : A) PCR verification of the tolA mutant of representative shigella strain. Electron micrograph of outer membrane vesicles attached to the bacteria *Shigella boydii* (B) and tolA mutant (C) and SDS Page profile. Supernatant from overnight grown culture was negatively stained and observed under transmission electron microscope (Bio Twin Transmission Electron Microscope, FEI, Netherlands) operating at 80 KV (×20 magnification). *In-vitro* assay of various cytokine. Mouse peritoneal macrophages were treated with wt-OMVs or ΔtolA OMVs and IL-18 (A), IL-6 (B), IL-12 (C), IL-10 (D), TNF-α (E), IL-1β (F) were measured in the supernatant. In another experiment co-culture of peritoneal macrophages and CD4+ T cells were treated with wt-OMVs or ΔtolA OMVs; IFN-γ (G) and IL-4 (H) were measured in the supernatant after four days. In both the cases ΔtolA OMVs were found to be more immunogenic.

Heat killed multi serotype *Shigella* immunogen induces humoral and adaptive immunity and offers broad spectrum and long term protection in animal models.

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

The recent vaccine developed with single serotype of *Shigella* does not give total protection against all serotypes. Keeping such idea, 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). The immunogenicity and protective efficacy of HKMS immunogen was studied in guinea pig rectal challenge model. The short term and long term passive protection offered by the HKMS immunogen was confirmed in neonatal mice model. After getting successive homologous protective efficacy of HKMS immunogen, we have studied the heterologous protection in rabbit model. The humoral immune response and long term plasma cell responses were confirmed by antibody in lymphocyte supernatant (ALS) assay.

In our recent work we have immunized mice with two doses of HKMS immunogens (Fig 11). Peritoneal macrophages, bone marrow derived dendritic cell (BMDC) and CD4+ T-cells were isolated from immunized mice on different time interval after immunization. Production of NO from peritoneal macrophages and different cytokines such as IL-12p70, IL-1β, IL-6 and IL-23 from peritoneal macrophages and BMDC of immunized mice upon stimulation with HKMS immunogens which conferred that the immunogens induced innate and adaptive immune system in mice model. Furthermore, incubation with HKMS immunogens with HKMS-primed splenic $CD4^+$ T cells enhances the production of IFN-γ, IL-10 and IL-17 represented that HKMS immunogens may induce Th1 and Th17 cell mediated immune responses.

In summary we can conclude that the HKMS immunoegen could be a promising broad spectrum vaccine candidate that confers a long term protection against all *Shigella* serogroups and serotypes 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.

Protective efficacy of live Δhfq *Shigella flexenari* 2a vaccine strain in passive protection model

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

Current vaccines for bacterial diseases have a sero-specific direction that limits the effect of vaccination to a narrow range of bacteria within the same species. An attempt to develop vaccine against broad serogroups and serotype is worthwhile but difficult. Pathogenicity of Shigella depended on Type III secretion system which is tightly regulated by RNA binding protein Hfq. Our previous study showed that the *hfq* mutant of *Shigella flexenari 2a,* 2457T (MF4853) less virulence than wild type strain. In this study, using guinea pig cecal loop model to determine nature invasion ability of vaccine strain, MF 4853. Wild type *S. flexenari* 2a 2457T strain showed hemorrhagic thick fluid whereas loop of vaccine strain showed minimum fluid accumulation like the control loops treated with phosphate-buffered saline (Fig.12 A). Massive hemorrhage with neutrophilic infiltration in the lamina propria extending up to the submucosa with congested and dilated crypts was observed (Fig.12 B). Sections from tissues treated with the vaccine strain did not reveal such changes and maintained normal villous contour. We also observed, vaccine strain more invasive potential than wild type strain at initial infection stage (6 hrs). But invasion ability of vaccine strain were decreased after 24 hrs than wild type strain (Fig 12 C). To confirmation of our data, we also studied passive protective efficacy of vaccine strain MF4853 in suckling mice model after four doses of oral immunization (Fig.12 D). Moreover, our results showed a satisfactory 80-75 % protective efficacy induced by vaccine strain against different recent circulating Shigella strains and also *Enteroinvasive E.coli* (EIEC) strain (Fig. 12 E) . Live Δhfq *Shigella flexenari* 2a (MF4853) vaccine strain could be a useful live vaccine candidate against shigellosis in our future.

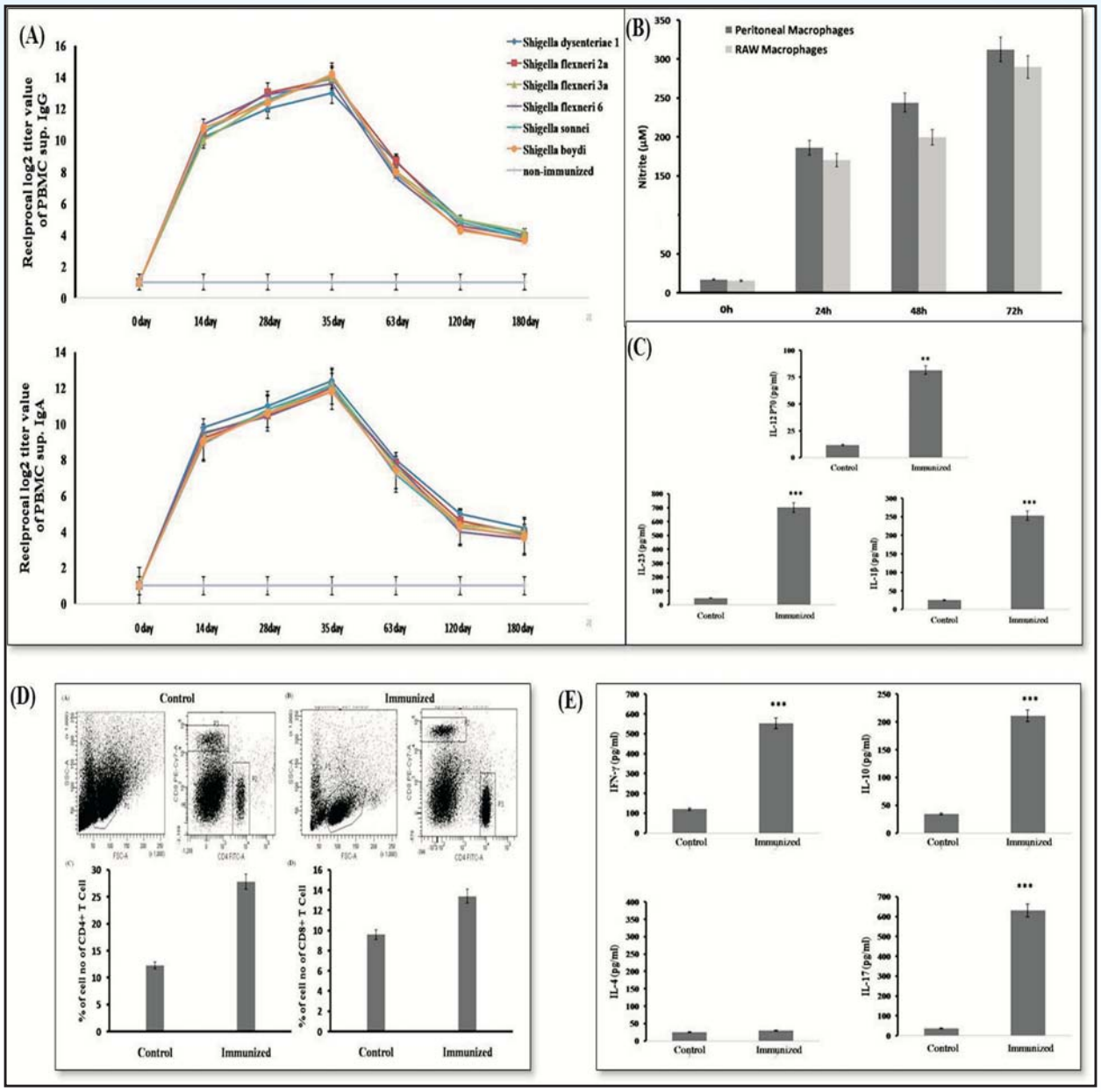


Fig. 11 : Immunological responses of HKMS immunogen. (A) IgG and IgA titer of PBMC supernatant (which were isolated from immunized and non-immunized rabbit and cultured at 37^0C with 5% CO_2 for 72 hours) against six Shigella challenge strains. (B) Cell-free supernatants were collected at 24, 48, and 72 h after incubation of peritoneal macrophages and RAW264.7 macrophages with HKMS and NO production in the supernatant was determined using a Griess assay. HKMS induced NO secretion was increased over time. (C) BMDC of immunized mice was treated with antigen for 24 h *in-vitro*. The production of Th1 (IL-12p70 and IL-1β) and Th17 (IL-6 and IL-23) cytokines from culture supernatant was measured by ELISA (n = 3, each group). (D) Graphical comparison of percentage of CD4+ and CD8a+ T cells counted in FACS Calibur flow cytometer (BD Biosciences, USA) from splenocyte of control and HKMS-immunized mice stained with CD4-FITC and CD8a–PE-Cy and generated using Cell Quest software. The same result was shown in a bar diagram (n=5, each group). (E) CD4+ T cells were isolated from the spleen of immunized mice 1 week after the final immunization and then stimulated with HKMS for 72 h. The levels of IFN-γ, IL-17, IL-4, and IL-10 in the cell supernatants were measured by ELISA assay (n = 3, each group). **p<0.005, ***p<0.0005.

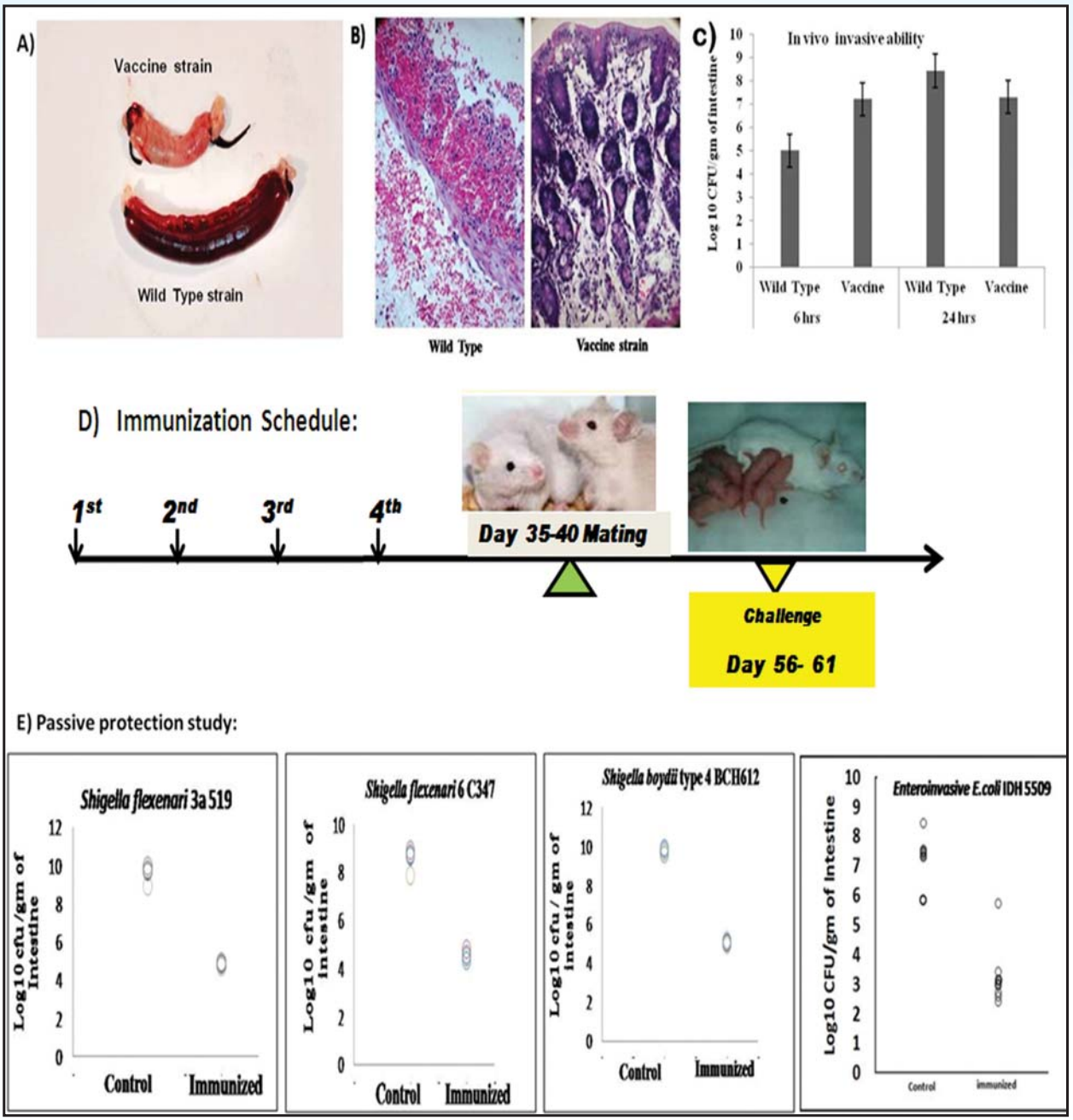


Fig-12: Guinea pig cecal loop model: Wild type *Shigella flexenari* 2a, 2467T and vaccine strain, MF4853 (1X10^9 cells/ml) were injected into cecal loop, after 6 hrs of inoculation, we measured number of invasive bacteria in cecal portion by gentamicin treatment expressed in log of CFU/gm of intestine. A) Pictorial picture showed nature of fluid accumulation with haemorrhage among two strains. B) Nature of histopathology of infected cecal portion. C) Invasive potential among wild and vaccine strains. D) Immunization schedule and E) Comparative data of protective efficacies between control and immunized neonates, after challenging with wild type Shigella strains: Each circle represents the colonization data obtained from a single suckling mouse. Data were expressed as Log10 of recovered colony forming unit (CFU)/gm of intestine of each mouse. Pups were challenged according to the challenge dose ~10^9.

Awards/ Honours Received

S. Dutta

Acted as an invited reviewer for following international/National journals/ Internatinal projects :

- Invited reviewer of project submitted to the US-Israel Bilateral Agricultural Research & Development (BARD) for funding
- Diagnostic Microbiology and Infectious Diseases (DMID)
- J of Antimicrobial Chemother (JAC)
- J of Applied Microbiology (JAM)
- J of Medical Microbiol
- BMC infectious diseases-a BMC series journal,
- PLoS One, PLoS Neg Trop Dis
- International J of infectious Diseases (IJID)
- Indian Journal of Medical Research (IJMR)
- Reviewer of STS, ICMR proposals

Invited as Editorial Board member of following International Journals :

- IBIMA Publishing, USA
- Austin J of Infectious Diseases, USA
- J of Bacteriology and Mycology
- International Archives of Microbiology and Immunology

A. Palit

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

S. Basu

- Served as invited reviewer of Pediatrics, New Infection and Microbes, BMC Microbiology, Microbial Drug Resistance and Journal of Infections in Developing Countries.

H. Koley

- Invited Lecture for Integrative Life Sciences: Destiny in UGC Academic Staff College, University of Calcutta, University College of Science and Technology, Kolkata - 700009 on 28 March, 2015.

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

S. Dutta

- Attended 38th National Conference of Indian Association of Medical Microbiologists (Microcon) held at Jaipur from 15-19 October 2014 and delivered a talk titled “Evaluation and comparison of performances of diagnostic PCRs for typhoid fever.”
- Attended 9th Annual Conference of Indian Association of Medical Microbiologists (West Bengal Chapter) held at NRS Medical College and Hospital, Kolkata on 7 Dec 2014 and participated as judge for reviewing the posters presented in the award category session.

- Invited to attend one symposium on "Impact of environment on health" held on the occasion of World Environment Day on 5 June 2014 organized by M/O Health and Family Welfare, Govt. of India and delivered a talk on "Drinking water quality and Diarrhoeal Diseases".
- Invited as a member to attend the twenty first meeting of Drinks and Drinking water Sectional Committee, FAD 14 of BIS, on 2 July, 2014 at Bureau of Indian Standards, New Delhi.
- Invited as an Expert by National Innovation Foundation_DST organization - India in the Research Advisory Committee (RAC) meeting held at CSIR Science Centre, New Delhi on 3 Dec 2014.
- Attended a joint meeting of the steering group and the technical advisory group (TAG) for review and final approval of the draft protocol "Multicentric Pneumonia Aetiology Study" on 15-16 January 2015 at ICMR HQ, New Delhi.
- Attended DST approved General Management Program for Women Scientist organized by Administrative Staff College of India (ASCI), Hyderabad from 26 Jan 2015 to 6 Feb 2015.
- Invited to participate in the workshop on "Systematic review of evidence on burden and strategies for control of typhoid fever in India" held on 10-12 March, 2015 at Christian Medical College, Vellore supported by the Indian Council of Medical Research.
- Invited to participate in the discussion on "All funders meet- Antimicrobial Resistance-A PPP Consortia for Innovation Research" on 19 March, 2015 at IHC, Lodhi Road, New Delhi.

A. Palit

- Invited to participate in a panel discussion organized by Asiatic Society, Kolkata in association with National Academy of Vector Borne Diseases and Post graduate department of Zoology, Ashutosh College, Kolkata at Vidyasagar Hall, Asiatic Society, 9 Decemeber, 2014.
- Invited & participated in a PANEL DISCUSSION on "Arboviral diseases in the changing Environment" organized by Asiatic Society, National Academy of Vector Borne Diseases, Eastern India chapter and Asutosh College, at Vidyasagar Hall, Asiatic Society, Kolkata on 9 December,2014

B. L. Sarkar

- Delivered lecture entitled "Bacteriophages: a weapon in the treatment of *Vibrio cholerae* infection" at 12th National conference of IAAM held in Chennai on 5- 6 December, 2014.
- Attended at 9th IAMM conference held in NRS Medical College, Kolkata on 7 December, 2014.
- Delivered lecture on 'Cholera and Diarrhoea" at Periyar University, Salem on 29 Jan, 2015.
- Attended 4th Annual Conference on Molecular Pathology Association of India (MPAI) at Tata Medical Centre, Kolkata on 14-15 Feb, 2015.

R. K. Nandy

- Oral presentation "Rotavirus vaccination: systemic and mucosal responses among infants in Kolkata, India" at 11th Rotavirus symposium 2014 at New Delhi during 3-5 September, 2014.
- Attended 14th World Congress on Public Health at Kolkata during 11-15 February, 2015.
- Attended 3rd Annual NKN Workshop 2014 at Guwahati, during 15-17 December, 2014 to represent NICED as a part of NKN connected Institute.

A. K. Mukhopadhyay

- Oral Presentation on "Major Genetic events in the evolution of Vibrio cholerae O1 for the last two decades at Kolkata, India" in the "The International Union of Microbiological Societies (IUMS 2014) – XIVth International

Congress of Bacteriology and Applied Microbiology, XIVth International Congress of Mycology and Eukaryotic Microbiology, XVIth International Congress of Virology held in Montreal, Canada during 27 July –1 August, 2014.

H. Koley

- H. Koley, D. Nag, R. Sinha, P. Mukherjee, S.Mitra. Studies on immunogenecity and passive protective efficacy of oral live transconjugantShigella strain in mice modelAnnual Conference of The Physiological Society of India (PSI) Babarampur, December 2014.
- Nag, R. Sinha,S.Mitra, S.Shinoda, H. KoleyTh1 cell mediated adaptive immunity and heterologous protection offered by heat killed multi-serotype Shigellaimmunogens in rabbit model. 83 Annual Meeting of Society of Biological Chemists (INDIA), Bhubaneswar Orissa, 18-21 December, 2014
- Organized a campaign for promoting awareness related to Health and Hyegine, an initiative by National Institute of Cholera and Enteric Diseases, Kolkata on 20 January 2015 at Kolkata, Gobra Kaji Nazrul Satabarshi Sikhayatan, 12/13/14 Mahendra Roy Lane, Kolkata -700046

Biochemistry

The Division of Biochemistry primarily focuses on in-depth understanding of themolecular mechanisms of host pathogen interaction using biochemical andbiophysical approaches. The molecules of interest are *Vibriocholerae* cytolysin / hemolysin(VCC), chitinases, chitin-binding proteins and colonization factors of enterotoxigenic *Escherichia coli*. Scientists of this division address characterization of these microbial proteins in relation to structure and pathogenesis of enteric diseases and host response. Knowledge generated has already begunto provide a greater understanding of the complexity of bacterial pathogenesis and aim to translate the knowledge in developing novel therapeutic intervention strategiesagainst enteric infections in near future. Further, know-how gathered is being applied to establish molecular tools for detection of virulence markers in pathogenic strains.

Scientist:

Dr. N. S. Chatterjee, Scientist E

Staff:

R. Naik, Technical Assistant

Post-Doctoral Fellow:

Epshita Chatterjee

Pre-Doctoral and Fellow:

Moumita Mondal

Anusuya Debnath

Sudipta Mondal

Rhishita Chourashi

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

Investigator: N. S. Chatterjee

Vibrio cholerae O1, a cause of epidemic diarrheal diseases, normally resides in aquatic environment utilizes chitin as the sole carbon and nitrogen source and remains associated with the chitinous exoskeletons of zooplankton. The principal objective of our study is to understand the mechanism how these bacteria adhere to the gut and survive in the environment using some common factors. Presently, we focused our study on a sensor histidine kinase ChiS to understand its role in *V. cholerae* survival in natural and host environment. ChiS is a 133 kDa protein located in the inner membrane of *V. cholerae* and is produced from locus VC0622. It is the master regulator of the chitin utilization proteins.

During *chiS* RNA expression analysis at different temperature, pH and salinity, we found *chiS* expression was lowest at 20C. RNA expression gradually increased with the temperature shift. It was the highest at 30°C and then decreased with further temperature shift. The *chiA2* expression at pH 5.5 was the lowest. It increased gradually with increasing pH, being optimum at pH 7.5 and then the increase in pH caused decrease in *chiA2* expression. Similarly, the *chiS* RNA expression increased with gradual increase in salinity from 100 mM to 400mM and then decreased with increasing salinity till 600 mM.

Molecular characterization of Enterotoxigenic *Escherichia coli* colonization factors

Investigator: N. S. Chatterjee

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. Our laboratory has been studying different aspects of this colonization factor and aims in developing simple methodologies for detection of CS6 and intervention against ETEC pathogenesis. Previously we demonstrated ETEC could induce expression of IL-8 and TNF-α in HT-29 and Caco-2 intestinal cell lines following infection. We also showed ETEC adherence is important to mediate cytokine response during pathogenesis. We further demonstrated that the increased pathogenicity of ETEC expressing CS6 subtype AIBI was correlated with cytokine secretion in the intestinal cells in comparison to that of AIIBII. We found that cytokine secretion profiles for AIBI and AIIBII-expressing ETEC varied significantly. HT-29 or Caco-2 cells when incubated with 1×10^7 CFU/ml of ETEC variants for 0 to 20 h showed increased secretion of IL-8 and TNF-α in a time dependent manner. In case of IL-8 and TNF-α 4-fold of difference in cytokine production was found between these two strains of ETEC. This result in collaboration with the binding data suggests that AIBI subtype of CS6 helps in stronger binding of the bacteria with epithelial cells compared to AIIBII, which indirectly affect the consequent difference in cellular response by these two strains of CS6. Reduced IL-8 and TNF-α secretion occurred during infection with ETEC expressing AIIBII in animal models. NF-κB was found to be the main downstream effector in inducing cytokine secretion during bacterial infection.

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

N. S. Chatterjee

- Presented a talk on "Assembly of colonization factor CS6 of enterotoxigenic *Escherichia coli*: Role of specific residues" at the 14th Congress of the International Union of Microbiologists (IUMS 2014) held at Montreal, Canada during July 27-August 1, 2014.

Clinical Medicine

The Clinical Medicine division is conducting the two major institutional studies on hospital based surveillance of diarrhoeal disease. One surveillance project is conducted at Infectious Diseases Hospital where every 5th hospitalized patient of all age groups is surveyed on randomlyselected 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 and working on on host-pathogen interactions during human Salmonellosis, especially *Salmonella*Typhi infection. The major emphasis is on the identification of novel microbial virulence factors and the host immune responses. Another focus area is studies on the regulation of cationic antimicrobial peptide expression at the mucosal surfaces and their role in protection against inflammatory disorders. In addition, synthetic peptides are being designed and tested as novel antimicrobial therapies. A third focus area of research involves screening of indigenous probiotic strains for their role in mucosal inflammatory diseases of the intestine and studies on the underlying mechanism of their beneficial functions.

Scientists have also conducted different facet of clinical research projects funded by external funding agencies. The clinical medicine division is closely associated with the scientist of different division in the research project. Scientists are involved in investigation of epidemics of diarrhoeal diseases and unknown fever. They are also involved in human resource development by providing training to the service providers like doctors and para-medical staff.

Scientist

1. M. K. Bhattacharya, Scientist 'F'
2. S. S. Das, Scientist 'E'
3. P. Indwar, Scientist 'B'

Staff

1. A. Pal, Technical Officer
2. P. Bhowmick, Technician 'A'
3. K. G. Saha,Technician 'B'
4. S. Turi, MTS
5. S. Routh, MTS
6. S. Dey, MTS

Project Scientists/ Fellows

Dr. Soumendra Moitra (SRF, Medical)

Dr. Diptoman Nandy (SRF, Medical)

Dr. Himanshu Sekhar Das, SRF

Dr. Ankita Bhattacharya (SRF, Medical)

Dr. Ayan Lahiri (Research Associate: CSIR Project)

Dr. Amita Barik (Scientist II: ICMR project)

Rahul Shubhra Mondal (Scientist I: ICMR project)

Ranjan Kumar Barman (Research Assistant: ICMR project)

Pre-doctoral Fellows:

Theya Nagaraja

Pujarini Dutta

Bhupesh Kumar Thakur

Atri Ta

Nirmalya Dasgupta

Asim Biswas

Sayan Das

Rimi Chowdhury (Research Assistant)

Hospital based surveillance system for diarrhoeal diseases

Investigator: M. K. Bhattacharya (PI)

Objectives of the study are

1. To monitor changes in disease pattern,
2. To create a database on diarrhoeal diseases,
3. To provide regular reports to the Govt. and other agencies and to improvement in better patients care and preventive measures.
4. As an Principal investigator my role is to conduct the study, check the data and prepare reports for sending to appropriate authorities.

Result obtained so far:

From April 2014 to March 2015, a total of 1164 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), 1170 specimens were 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 (85.6% vs. 37.3%), bloody (5.4% vs. 1.9%) and semi-solid (8.7% vs. 60.8%). 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, isolation of rotavirus was found 45.2% in ID Hospital where as 19.0% in B. C. Roy Post Graduate Institute of Paediatric Sciences. *Vibrio cholerae* O1 (5.2%) 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 and partially azithromycin. NDM-type carbapenemase were detected in 27 strains of *V. fluvialis* strains isolated from 2014-2015. 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.

Bacterial CpG unmethylated DNA protects from S. Typhimurium colitis through the induction of cathelicidin antimicrobial peptide

Investigator: S. S. Das

Bacterial DNA up regulates cathelicidin expression via TLR9-mediated activation of ERK-MAP kinases in the intestinal epithelial cells. Enhanced expression of endogenous cathelicidin by bacterial DNA was suggested to modulate intestinal inflammatory responses. We observed that intracolonic administration of bacterial DNA in BalB/c mice ameliorates *S.* Typhimurium-induced colitis as evident from the macroscopic and histopathological examination of the caecum. This is accompanied by significantly reduced colonization of the intestine by the bacteria and increased number of T-regulatory cells ($CD4^+CD25^+FoxP3^+$) in the lamina propria. All the above features were largely reversed by the concomitant use of CRAMP antibody. This data confirms the role of murine cathelicidin (CRAMP) in the protection against colitis caused by *S.* Typhimurium (Fig 13).

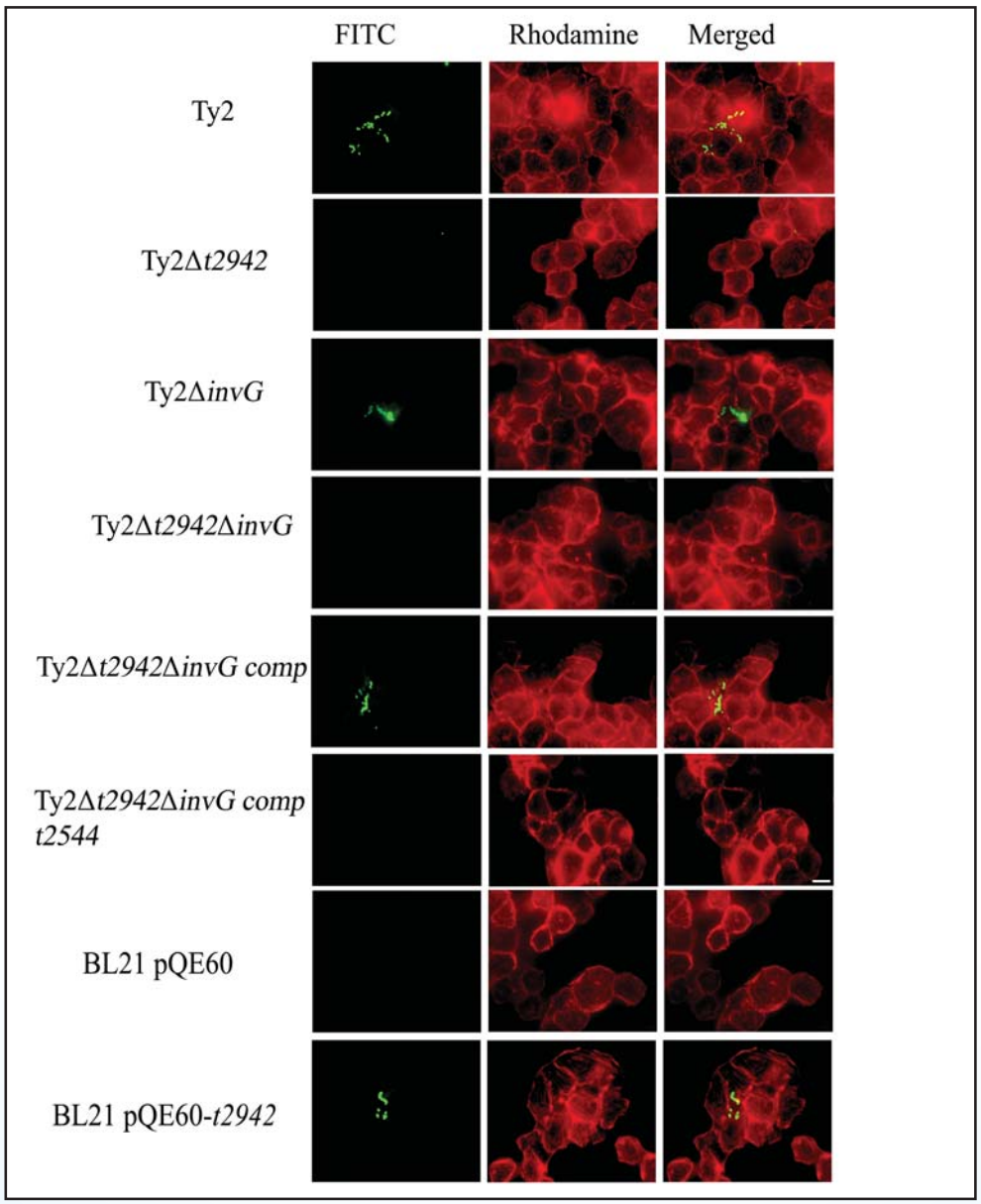


Fig 13. Epifluorescence microscopic images of HT-29 cells infected with the wild type or genetically engineered strains of *S.* Typhi and *E. coli* to visualize internalization of the bacteria. Cells were infected for 30 min, washed and cultured in the presence of gentamicin (200µg/ml) for 1 hr to kill the extracellularly adhered bacteria. F-Actin filaments and bacteria were stained with Phallodin-Rhodamine (red) and polyclonal Salmonella antisera followed by FITC-conjugated secondary antibody (Green), respectively.

Role of Type Three Secretion System-1 (T3SS-1)-independent mechanisms in the pathogenesis of *Salmonella enterica* serovar Typhi (S. Typhi)

Investigator: S. S. Das

Intracellular pathogens like *Salmonella* employ multiple mechanisms to invade the intestinal mucosa. T3SS-1 is thought to be the prototype invasion apparatus for epithelial invasion by *Salmonella*. However, recent evidences suggest critical and T3SS-1-independent role is played by other epithelial entry mechanisms in various *Salmonella* serovars including the clinical strains. We found that T2942, an AIL-family protein of *Salmonella* Typhi is essential for invasion of intestinal epithelium. It functions autonomously of T3SS-1, but cooperates with it for invasion of epithelium (Fig 14). T2942, when expressed in non-invasive *E. coli* BL21, mediates invasion of the epithelium. It requires a 20-amino acid long outer membrane loop. The mutant strain *S.* Typhi Ty2Δt2942 displays significantly reduced pathogenicity in mice that is reversed by T2942 complementation of the strain. Further studies revealed that T2942 functions through binding to a cell surface receptor, leading to localized actin polymerization.

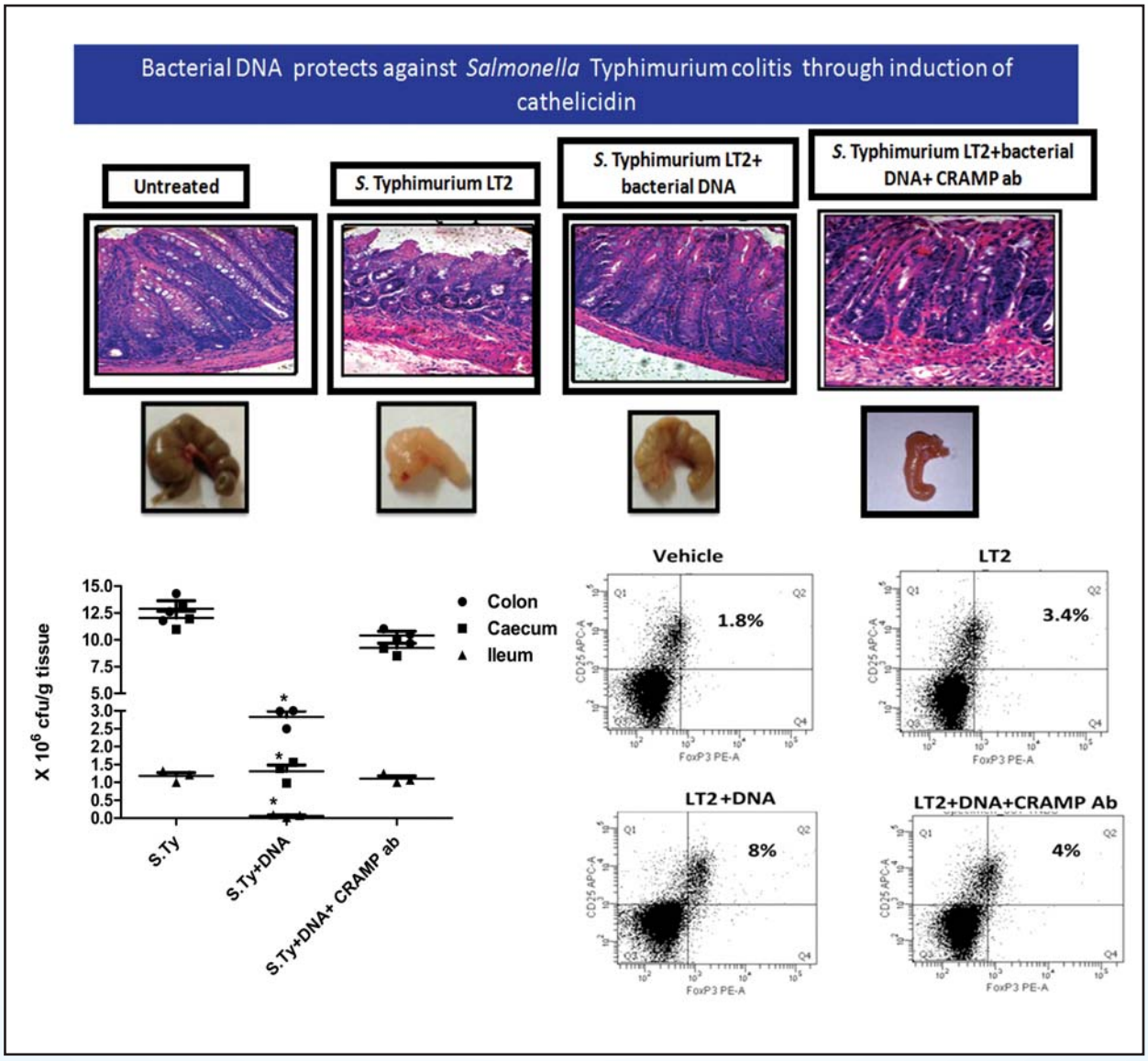


Fig 14. (Upper panel) Histopathology of mouse caecum isolated 72 hrs after oral infection with *S.* Typhimurium and stained with hematoxylin and eosin. Bacterial DNA was administered intra-rectally 6hrs post-infection. CRAMP antibody, whenever used was administered 2 hrs before bacterial DNA injection. (Middle panel) Caecum of mice isolated as above. (Lower panel) Bacterial colonization of mouse intestinal tissue isolated as above (left). Flow cytometry to analyze T-regulatory cells isolated from the mesenteric lymph nodes of mice treated as above (right).

Detection of Rotavirus RNA in CSF in children aged < 5years hospitalized with acute gastroenteritis with neurological manifestations in Kolkata.

Investigators: P. Indwar (PI),

Co-PI: M. K. Bhattacharya, M. C. Sarkar, B. Ganesh, T. Ramamurthy, S. Ganguly, S. Banerjee

This study was initiated as Pilot study to detect rotavirus RNA in CSF of children admitted with acute watery diarrhea with neurological manifestations. We identified patients presenting with acute watery diarrhea and with neurological symptoms and were able to detect rotavirus in CSF as well as in stool of some of these cases. This study was concluded last year.

With the findings of above mentioned study we started a new Project Titled "Clinical Study to evaluate the association of neurological manifestations in rotavirus diarrhea among hospitalized children under three years of age". Preliminary work of this study has been initiated. Awaiting Institutional ethics committee approval.

Therapeautic effects of Dioctahedral Smectite on acute watery diarrhea in children under five year.

Investigators: P. Indwar, M. K. Bhattacharya, M. C. Sarkar, A. K. Mukhopadhyay, S. Ganguly

This study is in progress and will be concluded this year.In this study we are evaluating the efficacy of an adsorbent Dioctahedral Smectite on duration of diarrhea, specially its efficacy in cases of Rotavirus diarrhoea.

Awards/ Honours Received

M. K. Bhattacharya

Awarded as Honorary Fellow of Indian Public Health Association in 12th World Congress on Public during 09-15 February, 2015 at Science City, Kolkata, India.

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

S. S. Das

- 2nd International Meet on Advanced Studies on Cell Signaling Network (*CeSiN*) 2014 held at CSIR-Indian Institute of Chemical biology, Kolkata on December 13-15, 2014 (Organizing Committee Member and Invited Speaker).
- Symposium on Probiotics and Multiscale Bioscience and Bioengineering to be held at Indian Institute of Technology, Bhubaneswar on December 12, 2014 (Invited Speaker).
- National Level Seminar on 'Frontiers of Microbiology: Prospects and Challenges' at Ramakrishna Mission Vidyamandira, Belur Math, Howrah, West Bengal on 20-21 November 2014 (Invited Speaker).
- 2nd conference of the Probiotic Association of India and International Symposium held in New Delhi on November 3rd to 4th, 2014 (Invited Speaker).
- Research Workshop on Diagnostic and Therapeutic Immunology, College of Medicine & JNM Hospital, Kalyani on September 19-20, 2014 (Invited Speaker).
- Organized workshop entitled "Design and Statistical Analysis for Biomedical Research" at NICED on September 16-18, 2014 (attended by 20 participants).
- Rahul Shubhra Mondal, PhD Student presented a poster at SYSCON-2014 held at JLN Auditorium, AIIMS, New Delhi, India on 10 December, 2014.

- Rahul Shubhra Mondal, PhD Student presented a poster at the 12th BioAsia Innovation Award, 2015 held in Hyderabad on 2-4 February, 2015 (received the 2nd Prize).
- Bhupesh Kumar Thakur, Nirmalya Dasgupta and Pujarini Dutta, PhD Students presented posters at the 2nd International Meet on Advanced Studies on Cell Signaling Network (*CeSiN*) 2014 held at CSIR-Indian Institute of Chemical biology, Kolkata on 13-15 December, 2014.
- Piu Saha, Postdoctoral Fellow attended the 2nd Annual conference of the Probiotic Association held in New Delhi on November 3rd to 4th, 2014 (Received the Best Poster Award).
- Rahul Shubhra Mondal, PhD Student presented a poster the 5th ACM Conference on Bioinformatics, Computational Biology and Health Informatics held at Newport Beach, California, USA, during 20-23 September, 2014 (Received International Travel Support from the Department of Science and Technology, India).
- Theeya Nagaraja, PhD Student attended International Conference on Host-pathogen Interaction held at National Institute of Animal Biotechnology, Hyderabad on 2-4 July, 2014 (Received the Best Poster Award).

Biostatistics and 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 PATH vaccine solutions.

This division always rendered statistical help for epidemiological, clinical and microbiological research as well as to Ph.D. students for their thesis.

There are also future plans to conduct local and country level courses on research methodology, biostatistics use in laboratory science, sample size determination for randomized Clinical trial for health researchers.

Final goal is to publish the research findings using modern and appropriate statistical techniques in peer reviewed journals.

Scientists:

Dr. B. Manna, Scientist 'F'

Dr. K. Rajendran, scientist 'C'

Generation of a database on cholera outbreaks in India

Investigator: B. Manna

A huge number of diarrhoeal outbreaks have been reported and investigated in different parts of India during last 30 years. All the investigation reports are usually submitted to the respective State Government as well as Ministry of Health, Govt. of India. But unfortunately, some of the outbreak reports are published in the indexed journal depending

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

The published articles on diarrhoea outbreak /epidemic were collected through Freemedicaljournals, Medexplorer, Medscape, Medhunt and PubMed. Attempts were also 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 were started from different published articles and unpublished documents. During the outbreak period from 1980 to 2014, a total of 154 articles have been published so far and out of 154, 96 full text articles were collected and data were entered into the database and analyzed. From Fig 15, it was observed that *Vibrio Chloera* O1 is the most causative organism (60.4%) for diarrhoeal outbreaks. We also looked at the Integrated Disease Surveillance Project (IDSP) data from IDSP –web site. Since July 2009 to December 2014, a total of 2,624 outbreaks have been reported in the IDSP web-site. The maximum number of outbreaks was reported during June – August (monsoon season) in every year. From the fig. 16, it was noted that in 2013, maximum number of outbreaks occurred in West Bengal followed by Karnataka, Orissa and Maharashtra.

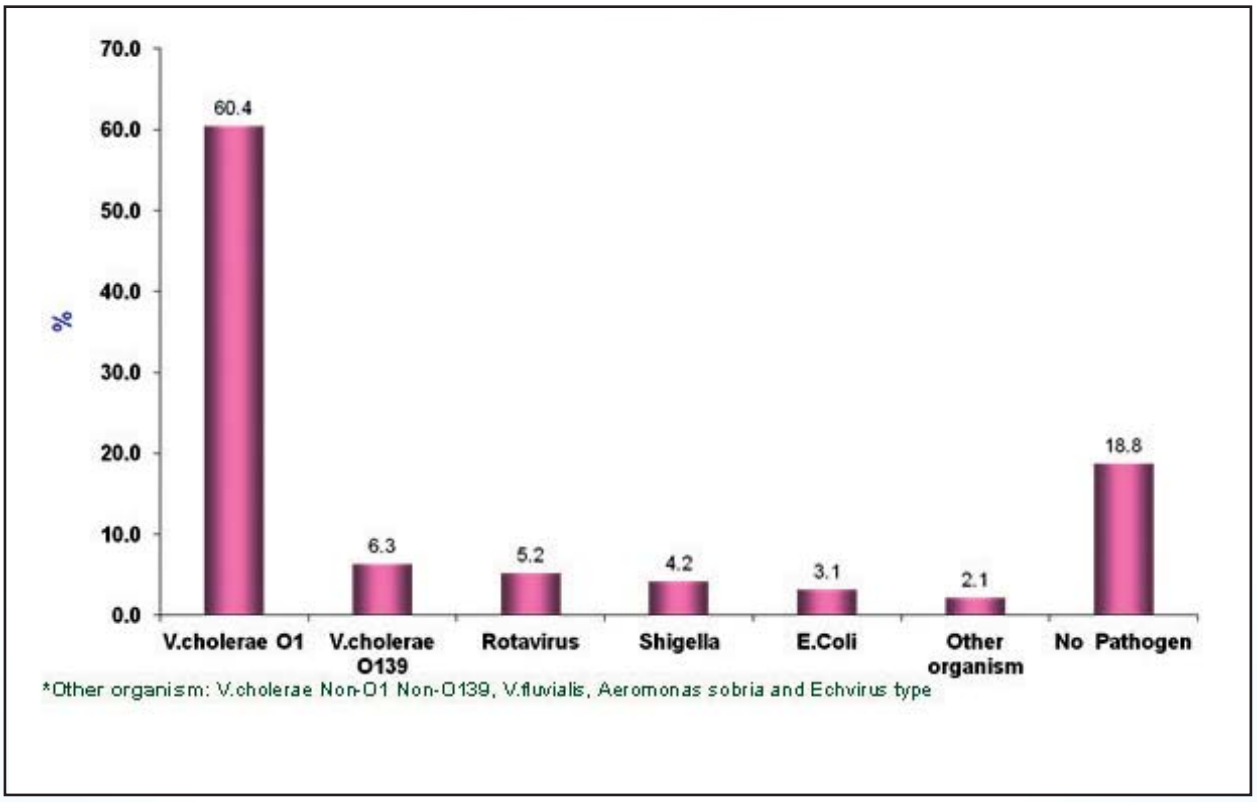


Fig 15. Reported causative organism (%) for diarrhoea outbreaks (n=96) during 1980-2014

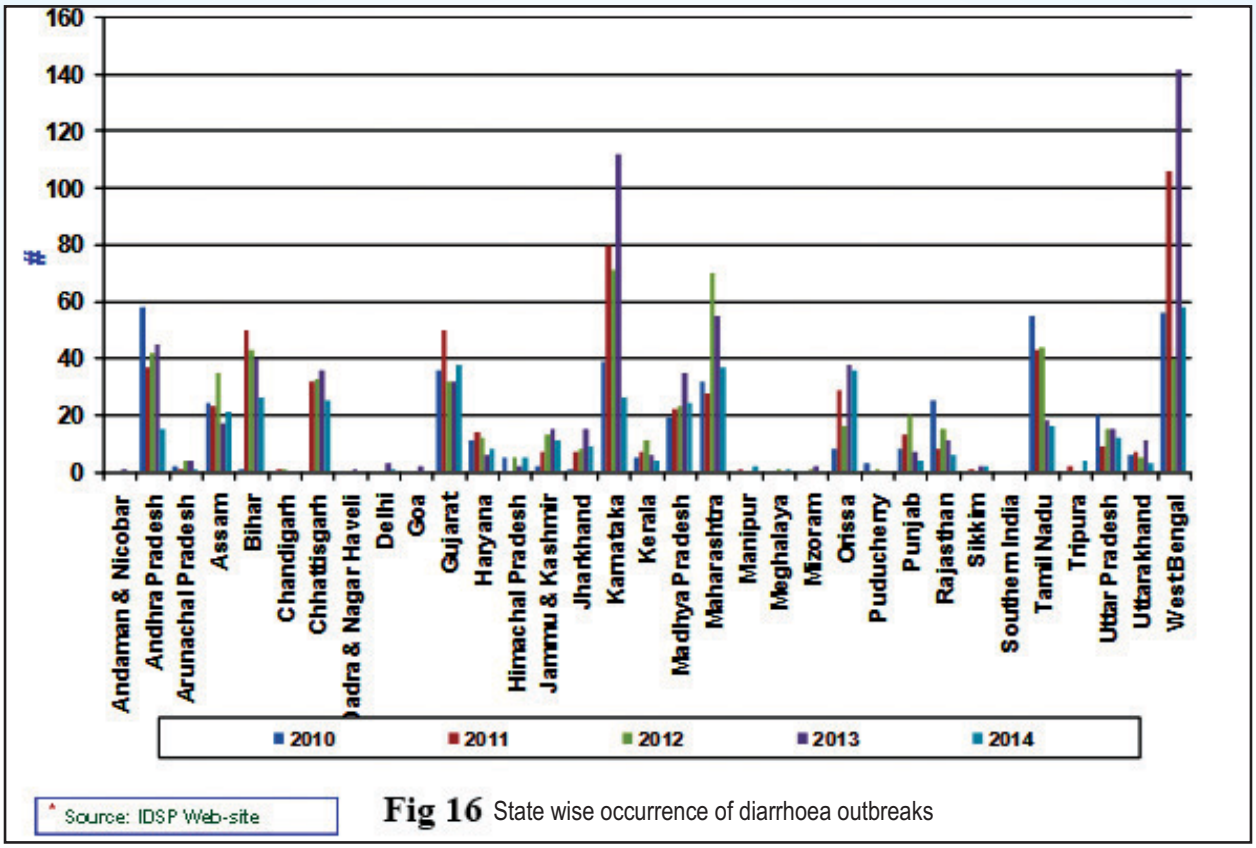


Fig 16 State wise occurrence of diarrhoea outbreaks

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

B. Manna

- Attended the 14th World Congress on Public Health at Science City in 11-15 Feb, 2015 in Kolkata, India and delivered lecture on "Inferential Statistics" in the preconference on Research Methodology Workshop at International Student Meet on Public Health in the 14th World Congress on Public Health at Science City on 11 February, 2015 in Kolkata, India.
- Attended Eleventh International Rotavirus Symposium 2014 during 3 - 5 Sep, 2014 at New Delhi, India.
- Attended and presented data management update in the Annual Meeting for the NICED-IVI collaborative project on 'Exploration of the Biologic Basis for underperformance of Oral Polio and Rotavirus Vaccine in India' during 5-7 July, 2014 at Seattle, USA.
- Delivered lectures on "Application of Statistics in Medical Research" for post graduate students under DM, MD programme at Dr. B C Roy Post Graduate Inst. Of Pediatric Sciences , Kolkata on 28 September 2014.
- Delivered lecture on "Introduction to Biostatistics" for research fellows under Ph.D programme of this institute on 16th December 2014.
- Acted as a resource person in the Workshop on Essentials of Epidemiology and Research Methodology, organized by the INCLEN Trust International & IAP Research in Child Health Subspeciality Group at IMA House, Bhubaneswar, Odisha during March 14-15, 2015 and delivered a series of lectures in biostatistics.
- Worked as a team member from NICED with ICMR group to organize Scientists-Group B examination, held on 8th February, 2015 at Santragachi Kendriya Vidyalay, one Kolkata Centre.

Epidemiology

Division of Epidemiology is associated with working on all sorts of nationally as well as locally prevalent public health problem(both communicable as well as non-communicable health disorders) that threatens health of our country people apart from specific institutional mandate of research on enteric infections and HIV/AIDS. This division is entrusted with combating all major public health emergencies taking place in eastern & north-eastern India from time to time such as epidemics caused by Dengue, Chicunguniya, Japanese Encephalitis, swine flu, bird flu, cholera, HIV, STIs etc. Epidemic investigations through institutional epidemic investigation team members are routinely carried out on request that usually comes from local as well as national health authorities. This division is also actively involved in providing public health training to various categories medical & para-medical health professionals to improve their capacity and thereby helps in strengthening public health services. This division also takes part in all major nation-wide surveys of eastern-India such National Family Health Surveys, District Level Household Surveys etc. Some of the recent major contributions of this division are working on assessment of perceived illness & health care seeking behavior of a backward community populationof West Bengal, studying problem of diabetes, hypertension & arsenicosis in an under-privileged community and investigating problem of malnutrition among school children of all districts of West Bengal. The later was done at the request of Govt. of West Bengal to improve the quality of school children's nutritional status by strengthening Cooked Mid-day Meal Programme activities. Following completion of this study, aseries of dissemination workshopsare being planned to be conducted in all districts of West Bengal to sensitize the district school teachers, mid-day meal workers and school meal programme supervisors. Other major work includes a large scale multi centric Phase III, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of live attenuated Bovine-Human Rotavirus Reassortant Pentavalent Vaccine (BRV-PV) against Severe Rotavirus Gastroenteritis in Healthy Indian Infants was initiated. NICED is a part of that.

Other divisional works include completion of assessment physical & mental health of 391 children (187 male and 204 female) residing in Paschim Medinipur and Purbo Medinipur districts of West Bengal of whom 60% are on anti-retroviral therapy (ART) and a cross sectional study among the health caregivers of under-5 children residing in slums of Kolkata to understand the patterns of diseases among under-5 children along with studying multi-component educational intervention designed to improve the knowledge of non-qualified allopathic practitioners of the urban slums.

Assessment of perceived health needs and available health care facilities of Malda

Investigator: K. Sarkar

An exploratory health research study was conducted at Malda District of West Bengal, which is considered to be one of the most backward districts within West Bengal. Objectives were to assess the perceived health needs of the community requiring visit to health facilities along with determining prevalence of Diabetes mellitus and Hypertension among the adults >18 yr. & Malnutrition among under-five year children. It was also aimed to study the problem of arsenic content of drinking water sources in vulnerable block primary schools causing ill health to the students. The study was done during October-13 to July-14. A total of 10,000 households (4000 from urban & 6000 from rural) were selected following selection of villages & municipal wards from the District list of villages & municipal wards by the probability proportionate to size (PPS) method. This community-based cross-sectional study was conducted through house to house visit by 6-teams of experienced field workers. A semi-structured field-tested questionnaire was introduced to collect the relevant information on socio-demography, perceived illness and health facilities visited by them. Following this, blood sugar was measured by SD Code Free Blood Sugar Monitoring System and blood pressure was measured using Rossmax – AW150 Blood Pressure Monitor**.** Both the instruments were standardized with regular accuracy checking. Same instruments were also used for a nation-wide District Level Household Survey-4th round in recent past.

The study revealed that the Respiratory tract illness (RTI) was the commonest morbidity, perceived by 16% of the studied population. This was followed by peptic ulcer syndrome (6%), followed by gastro-enteritis (4.7%). Regarding health facilities visited by them, about 65% of the rural & 48% of the urban sufferers took the services of quacks. This was followed by intake of services of private practitioners (urban-41%, rural-24%), followed by attending to government hospitals/health centers (11% for both rural & urban sufferers). This indicates that quacks play the most important role in day to day health care delivery services both in rural as well as urban areas. Overall prevalence of Diabetes was 5% (n=884); ranging from 2.8% in 18-40 yrs. to 11% among above 60 yr. age group. Diabetes was found to be significantly associated with older age, male sex, higher caste, Muslim religion, sedentary occupation and economically stronger population. There was no significant difference found between illiterate & literate subjects and people staying between rural and urban areas. Overall prevalence of hypertension was 25.7% that ranges from 14.6% in 18-40 yrs. to 58% in above 60 yrs. age-groups respectively. Hypertension was found to be significantly associated with older age, male sex, illiteracy, urban dwelling, sedentary working habit, higher caste, economically stronger population including owning houses. Unlike Diabetes, hypertension was not associated with religion. Arsenic study revealed that a total of 137 out of 300 water samples (45.6%) showed the presence of excess arsenic content of WHO permissible level for drinking water of more than 10 micro-gram/Liters. This study revealed that 61 schools were provided with arsenic filters and 31 of them had high arsenic content in their drinking water. Out of 137 schools with high arsenic content, 8 schools reported their students suffering from skin manifestations of arsenicosis in contrast to only one school reported the same, without having high arsenic content.

Assessment of nutritional status among primary & upper-primary school children of all districts of West Bengal

Principal Investigator: K. Sarkar

Co-Principal Investigator : S. Kanungo

A total of 24,111 school children were selected from 20 educational districts of West Bengal. Of them, half was taken from primary & rest was from upper primary classes, where Cooked Mid Day Meal Programme (CMDMP) was on operation. A total of 15 primary & 15 upper primary schools were selected in a district and within a school, 40 students were selected randomly (5 boys & 5 girls from each of class-I to Class VIII). So, 1200 students (600 primary & 600 upper-primary) participated in this study from each of 20 districts. A field-tested semi-structured questionnaire was introduced by the trained field workers under supervision of faculties of NICED to collect relevant information. Following this, anthropometric assessment was done using anthropometric rods & digital weighing machines with regular accuracy checks. This was followed by estimation of haemoglobin status of class-I & class-V students on the spot using Hemocue Hemoglobin Analyser. Data was transported to NICED for editing, entry & analysis. Malnutrition was assessed using guidelines of Indian Academy of Paediatrics.

The study revealed that state-wide prevalence of malnutrition was 26.1% & 19.6% in primary & upper-primary students respectively. Another 28.6% & 26.1% of the studied population were `At High Risk' of developing malnutrition (3rd to <10th percentile weight & height in respect to age & sex) among primary & upper-primary students (Fig 17).

State-wide Ideal Nutritional status was 17% & 23.5% among primary & upper-primary students. Prevalence of anaemia was 29% & 19% among primary & upper-primary students. Religion-wise, Muslim had a higher malnutrition than Hindus. Caste-wise, ST had a higher malnutrition than SC in primary but SC had higher malnutrition than ST among upper-primary students (Fig 18).

Other important observations indicated that malnutrition was higher in rural students than their urban counterpart and more in boys than girls. But prevalence of anaemia was higher among girls than boys. Problem of exclusive stunting was 6.9% & 5.7% and that of exclusive under-weight was 6.4% & 4.6% among primary and upper-primary students

respectively. Both under-weight and stunting was present in the tune of 12.8% & 9.3% in primary & upper-primary students respectively.

Malnutrition was higher among primary than upper-primary students. Within primary section, worst affected districts were Purulia, Jalpaiguri, Darjeeling Plain, Maldah, Murshidabad, Burdwan andCoochbehar – all had a malnutrition prevalence of more than 30% and may be considered as high prevalent districts. Alarmingly, malnutrition among rural primary students of 3 Districts (Jalpiguri, Purulia& Darjeeling Plain) had a prevalence of more than 40%, which must be considered as top priority. Medium prevalent Districts were Bankura, Birbhum, Uttar Dinajpur, DakshinDinajpur, South 24 Parganas, Midnapore-West, Hoogly, Midnapore-East & Nadia – all had a malnutrition prevalence of above 20% but less than 30%. Low prevalent Districts were North 24 Parganas, Howrah, Darjeeling Hill (DGHC) and Kolkata – all had a prevalence of more than 10% but less than 20%. Upper-primary students showed a slight lower prevalence of malnutrition in all districts. Most districts had a prevalence of anaemia of >20% except few. Worst affected district was South 24 Parganas. Districts such as South 24 Parganas, Darjeeling, Jalpaiguri, &Maldah – all had a prevalence of more than 40%.

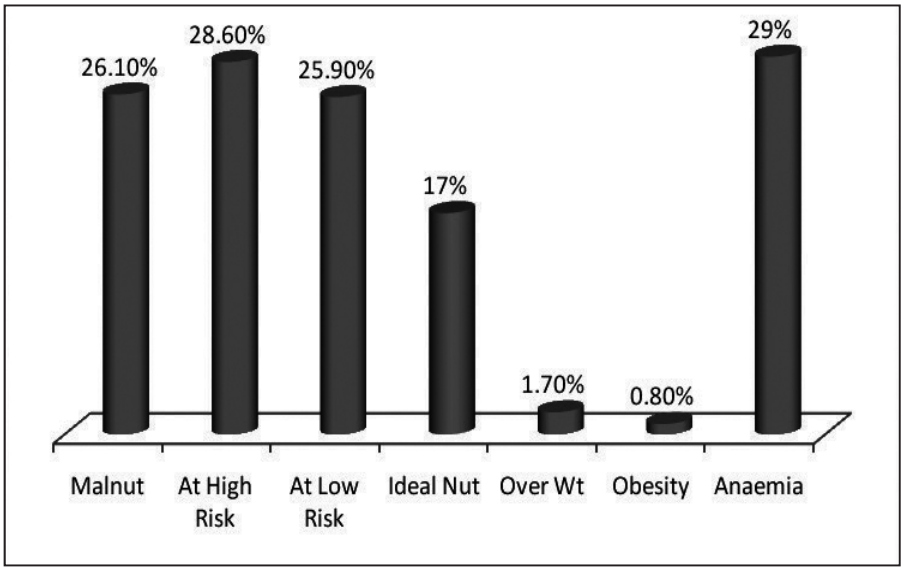


Fig 17. Prevalence of malnutrition, at high & low risk, ideal nutrition, obesity & anaemia in primary students

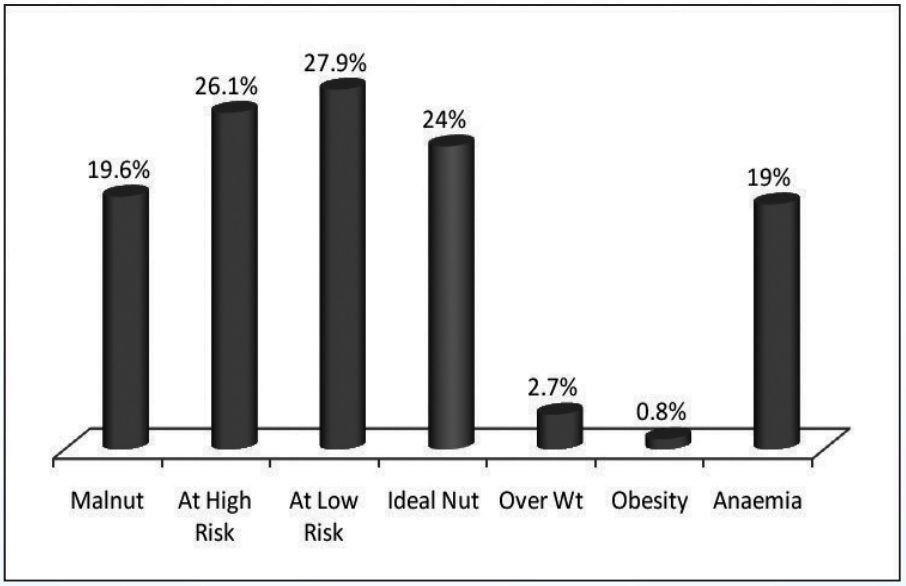


Fig 18. Prevalence of malnutrition, at high/low risk, obesity & anaemia in Upper-Primary students

Care needs assessment of children living with or affected by HIV in selected districts of West Bengal.

Investigators: S. Panda

A total of 287 children (133 male and 154 female) have been recruited from the district of Paschim Medinipur (mean age 11 years; SD±3 years; minimum 6 years and maximum 17 years); majority of them being Hindu (89%). Of the 287 participants, 108 (37%) are children living with HIV (CLWH) and the rest are affected (defined as one or both parents being HIV sero-reactive). Mothers constituted the largest proportion of interviewees (248/286; 86%) while we explored issues around main caregiver of a CLWH or an affected child (mean age 37 years; SD±3 years; minimum 23 years and maximum 75 years). About 93% of the care providers (266/287) reported undergoing HIV test and 84% of them reported being HIV sero-reactive. Among CLWH 63% (68/108) are on anti-retroviral therapy (ART). We have recruited 111 children (93% Hindu) from Purbo Medinipur (male:female 62:49); the age distribution being similar to that of children participating from Paschim Medinipur. About a third of the children recruited from Purbo Medinipur are living with HIV and the rest belongs to the affected group; 19% of CLWH are on ART. Mothers in this district constituted the largest proportion of interviewees as care givers (85%). Domestic dietary survey in both the districts are on-going.

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

Principal Investigator: A. K. Deb

Co-Investigators: S. Panda, A. Sinha (Asstt. Professor, Community Medicine, R.G. Kar Medical College & Hospital)

The objectives of this study was to understand the gaps and barriers in existing health communication in select rural areas of West Bengal and to identify potential solutions to improve the content and means of delivery of health communications. These issues were explored from the health care user as well as provider perspectives to enhance feasibility of development and implementation of an improved health communication technique. This study, conducted in two village panchayat areas in North 24-Parganas, involved (a) collection of relevant existing information through online and offline sources, and (b) qualitative research through in-depth interviews (IDI) and focus group discussions (FGD). The participants for the IDIs included ASHA workers, ANMs of the health sub-centres, public health nurses and health officials, whereas the FGDs were conducted among the mothers / primary caregivers of under-five children. More than fifty documents including research papers, reviews, reports and guidelines from various government and private agencies were accessed; they highlighted various health communication strategies adopted in different settings, their advantages and limitations, needs and scopes for modifications and cost implications for some strategies. The primary data, collected through the IDIs and FGDs, underlined current maternal and child health related information dissemination strategies followed by the government system. The major themes that emerged included – (a) a clear need for improvement of the current system in terms of extending the reach and making impacts; (b) delivering more focused information (on a few top priority topics), rather than diverse information at any given contact would be more useful; (c) provision of making audio-visual information dissemination wherever possible might add substantial benefits; and (d) during the dissemination process, presence and participation of a person who was locally popular and/or who had already adopted positive changes in behavior / practice, could have great impact. Participants also stressed on considering locally / culturally appropriate timing, duration, venue and language while disseminating health information.

An intervention to improve diarrhea related knowledge and practices among non-qualified practitioners in urban slum of Kolkata

Pricipal Investigator: S. Kanungo

As there was huge gap in diarrhea related knowledge and practice among non-qualified practitioners and a considerable proportion of diarrheal cases being attended by them in urban slums called for an urgent initiative to improve the knowledge among nonqualified practitioners to achieve a comprehensive goal of overall appropriate management of diarrhea in those vulnerable communities. This project aimed to assess the impact of a multi-component intervention in improving non-qualified practitioners' diarrhea related knowledge and practice to provide appropriate clinical management of diarrhea in urban slums of Kolkata.

A pre and post-test multi-component intervention study was conducted among 140 consenting pharmacists and nonqualified practitioners who participated in baseline study. The interventions constituted a supportive guidance to each of these nonqualified practitioners once a month over a period of 6 months.

Contents of the training module:

Training modules translated in local languages, were designed in a lucid language provided information on the following domains regarding: diarrheal diseases (signs and symptoms), their occurrence and transmission, causative organisms, management, prevention/control, cholera (disease as a whole) and oral rehydration solutions (ORS) (Fig19).

Fig 19. Training module for non-qualified practitioners.

Preliminary analysis showed that 4.84% subjects, do not have any specific clinic, 62.90% had specific clinics and 32.36% were pharmacists. While 71.77% were practicing for >10 years, only 2.42% were in practice for less than one year.73.17% prescribed antibiotics to all diarrhea and 52.03% prescribed antibiotic to all cholera patients.

Factors influencing antibiotic use in diarrhea :

The role of health system responsiveness in the interplay between perception and treatment seeking behavior among caregivers of under-5 children in urban slum of Kolkata, India

Although we can expect that patterns of childhood diseases, caregivers' perception regarding the severity of ailment of the kid and perceived healthcare responsiveness might be related to their treatment seeking behavior but the extent and direction of these associations are not entirely known. It is also important to know what other socio-demographic and economic factors are associated with these parameters and how. A cross sectional study was undertaken among the caregivers of under-5 children residing in slums of Kolkata to understand the patterns of diseases among the under-5 children, evaluation of caregivers' perception about common illnesses of under-5 children, Estimation of the related healthcare responsiveness as perceived by the caregivers, assessment of the treatment seeking behavior of caregivers regarding ailments of under-5 children and exploration of possible interrelationship among these estimates and their variations across the strata of socio-demographic aspects.

Initially a list of households having at least one under-5 child in the slum areas was prepared and each household was provided with a unique identification number (UID). Next, based on this UID, random sampling was done to get the required sample size. Information collection was planned based on door to door survey using structured and field-tested questionnaire from consenting caregivers in the selected households.

Qualitative component: Three focus group discussions have been conducted involving five eligible caregivers of under-5 children randomly selected from the initially visited 50 households for each focus group discussion. Based on the focused group discussions, the following themes that were identified to be relevant (based on initial analyses) were

1. Availability of the practitioners and cost of treatment (especially the fees of the physicians) were the most important factor for the selection of practitioners (whether qualified or non-qualified or pharmacists)
2. Non-qualified practitioners and pharmacists were more commonly chosen healthcare provider than qualified practitioners for the ailments of under-5 children in the study area
3. Severity of the ailment was the most common reason of visiting a qualified practitioner
4. Cost, waiting time and non-welcome behavior were the most common barriers for visiting the qualified practitioners for each ailment.
5. Lack of attention (self-perceived) and waiting time were the experienced problems in Governmental sector.

Analyses of the quantitative study will be conducted after the completion of the data collection.

Phase III, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Live Attenuated Bovine-Human Rotavirus Reassortant Pentavalent Vaccine (BRV-PV) Against Severe Rotavirus Gastroenteritis in Healthy Indian Infants

Principal Investigator: S. Kanungo

Worldwide, of all the rotavirus diarrheal cases, 88.5% are due to G1-G4 serotypes, which are considered common serotypes. The G9 strain is known to be one of the five important serotypes globally with a relative frequency of 4.1%, however, a high occurrence of G9 strains has recently been observed in several countries including India. The Serum Institute of India, developed the live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine for oral vaccination against human rotavirus gastroenteritis in healthy infants and proof of vaccine efficacy was planned.

Three doses of BRV-PV are administered concomitantly with UIP vaccines to healthy infants. BRV-PV contains = Log105.6 FFU/dose of each rotavirus serotype G1, G2, G3, G4 and G9.in the efficacy trial, in which infants in several regions of India representative of different demographic, climatic and sociocultural factors were enrolled. NICED is a part of this large multicentric phase III, randomized, double-blind, placebo-controlled trial, to evaluate the efficacy and safety of this live attenuated bovine-human rotavirus reassortant pentavalent vaccine (BRV-PV) strains against severe rotavirus gastroenteritis among 7500 healthy Indian Infants across the nation, approved by DCGI and Health Ministry Screening Committee (ICMR). In NICED, recruitment of 700 children completed in Mar 2015. The work was initiated in Aug 2014. Primary objective is to see the efficacy of this pentavalent vaccine Audio visual recording of the consent process was adopted in this study, as instructed by DCGI. The children recruited for the study are planned to be followed for two years of life. Surveillance for severe rotavirus gastro enteritis has started. The study in Kolkata are conducted in collaboration with Post Graduate Institute of Paediatric Sciences, wither active cooperation for EPI vaccination as well as intervention. Adverse event and serious adverse events are dealt with utmost care. Field clinics were set up in the community for recruitment, consenting as well as adverse events management (Fig 20).

- Total no of Acute Gastroenteritis / diarrhoeal episode till 30th April 2015 recorded are 512.

G1	G2	G3	G4	G5	G6	G7
329	128	36	13	3	2	1

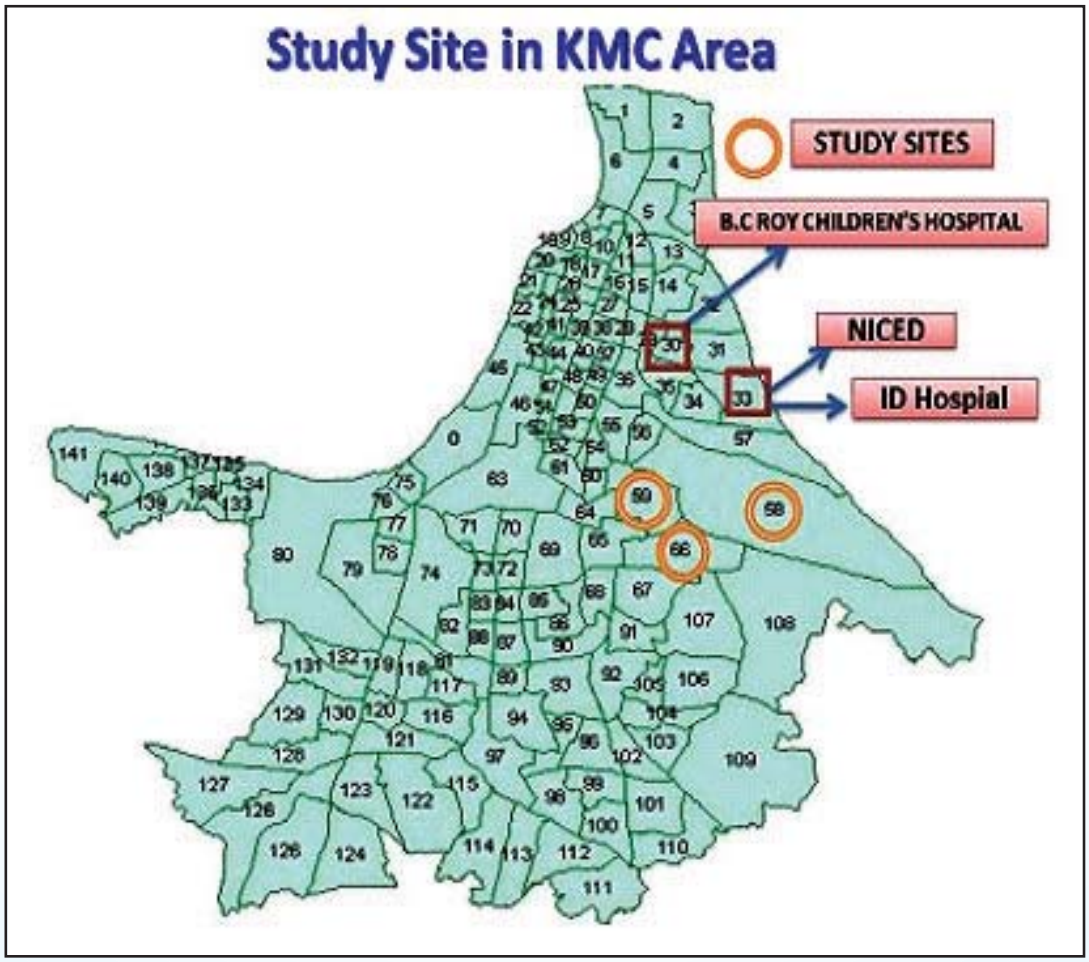


Fig 20. Study site for Rota-phase III study.

Awards/ Honours Received

S. Kanungo

- Awarded PhD from University of Kolkata in Jan 2015

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

K. Sarkar

- Attended International Public Health Congress, held at Kolkata, during February 2015
- Organised a training workshop on `Assessment of malnutrition among adolescent school children' was organised at NICED for field level workers & their supervisors during 28 – 30 June 2014. It was attended by 30 participants. They were trained to estimate anaemia using Haemoglobin Analyser & assessment of malnutrition at field, accuracy checks of instruments, filling out questionnaire and relevant do's & don't's .

S. Panda

- Attended 11th International Rotavirus Symposium held in Delhi during 3rd-4th September, 2014 as an invitee and contributed a rotavirus vaccine policy related paper in the journal titled Vaccine published on the eve of this event.
- Functioned as Scientific Co-Chair of the Organizing Committee of the 7th National Conference of AIDS Society of India- ASICON 2014 held during 12-14 December, 2014 in Kolkata.
- Organised a workshop focusing on "Exposure to Research carried out by NICED" at the behest of the National Institute of Homeopathy under the Department of Ayush in the Ministry of Health & Family Welfare, Government of India on 24 April, 2014.
- Acted as a resource person in the ICMR supported training titled 'Design and Statistical Analysis for Biomedical Research', organized by the Bio-Informatics Division of during 16-18 September, 2014.

A. K. Deb

- Attended the Joint Meeting of the Steering Group, Working Groups and Project Investigators for ICMR Task Force Project on Pneumonia Etiology in India held at ICMR Hq, New Delhi on 18 June, 2014.
- Attended as an expert in the NACO proposal development meeting held at PHFI in Gurgaon during 22-23 July, 2014.
- Participated in the Migrant Refresher ToT for IBBS organized by NACO/PHFI at New Delhi during 26-27 November, 2014.
- Participated as Observer / Chief Regional Trainer in the State-Level Training of IBBS for Assam and Meghalaya held at Guwahati during 3-6 May, 2014.
- Attended the protocol development and finalization workshop for the proposed pneumonia etiology study of ICMR held at NIE, Chennai during 5-6 August, 2014.

S. Kanungo

- Delivered an oral presentation on the topic "Problem of Diabetes and Hypertension in resource poor setting" at 15th Annual Conference on Indian Railway Public Health Association IRPHACON 2014 and 44th Annual Scientific Seminar & CME Programme of Indian Railway Medical Association on 16 Jan 2015.

- Delivered an oral presentation on the topic " Efficacy of bivalent whole cell oral cholera vaccine in endemic setting: a story from India on 13 Feb 2014 at the 14th World Congress on Public Health at Science City in Feb 11-15, 2015 in Kolkata, India
- Acted as faculty and delivered an oral presentation on Current "Epidemiology of Cholera & disease burden in Indian Subcontinent" at the 4th Meeting of the Initiative against Diarrhoeal and Enteric diseases in Asia (IDEA) at New Delhi India on 30 Mar -2 Apr, 2015
- Acted as the Lead Facilitator and faculty for the preconference workshop titled " Research in Public Health - research methodology" at International Student Meet on Public Health at the 14th World Congress on Public Health at Science City in 9-10 Feb, 2015 in Kolkata, India
- Participated in a workshop on "Systematic review on evidence and burden and strategies for control of typhoid fever in India" at Christian Medical College, Vellore, India, 10-12 Mar, 2015, supported by Indian Council of Medical Research.
- Gave an oral deliberation on the topic : Ebola Virus Disease (EVD)- Global Concern" on the occasion of organizing "Awareness program on the recent outbreak of Swine Flu virus and Ebola virus infection" at Lady Brabourne College, Kolkata, India on 28 Mar 2015

Immunology

This division has made a significant contribution by delineating the role of Toll-like receptor in (a) evolution of innate recognition of pathogenic structure; (b) abrogation of inflammatory bowel disease; (c) adjuvant-driven systemic B cell immunity and (d) innate memory-like CD8 T cell function (for sepsis).

Scientist:

Dr. T. Biswas, Scientist-F

Research Scientist:

Dr. R. Biswas

Staff:

S. K. Shaw, Technician-B

N. C. Mondal, M.T.S.

Awards

Subhadeep Mukherjee received Ph.D. degree, Jadavpur University.
Title of the Thesis: "Porin-induced Toll-like receptor signaling of colonic epithelial cells for chemokine and cytokine expression to facilitate cross-talk with immune cells"

Cytokine Regulation of Porin Stimulated B-1a Cell

Principal Investigator :T. Biswas

Porin treatment of B-1a cells up-regulated TLR2 indicating its role in the downstream signaling. Upon treatment with porin, the very early activation marker CD69 and the late activation marker CD40 were elevated on these cells within 6 and 24 h of culture respectively (Fig 21a). The adaptor molecule MyD88 present downstream of TLR2 was also upregulated by porin (Fig 21b). After 48 h of culture, the porin-treated cells were found to express the costimulatory molecule CD86 and MHC class II ($I\text{-}A^b$) (Fig 21c). B-1a cells failed to express the anti-inflammatory cytokine IL-10 but stimulated TNF-α and IL-12 under the influence of porin (Fig 22). This advocates the potential of porin to activate B-1a cells along with parallel induction of TLR2 so that the cells can receive help from neighboring B and T cells and amplify adaptive responses.

Generation of culture-differentiated innate memory CD8 cells with toll-like receptor expression and responsiveness to pathogen/danger-associated molecules

Principal Investigator : T. Biswas

Nonconventional innate memory $CD8^+$ T cells characteristically expressing CD44, CD122, eomesodermin (Eomes) and promyelocyticleukemia zinc finger (PLZF) were derived in culture from $CD4^+$ $CD8^+$ double positive (DP) thymocytes of normal BALB/c and C57BL/6 mice (Fig 23). These culture-differentiated cells constitutively express toll-like receptor (TLR)4 and release interferon (IFN)-γ and interleukin (IL)-10 (Fig 24).

Awards/ Honours Received

T. Biswas

- Served as invited reviewer of The Journal of Immunology; The Indian Journal of Medical Research

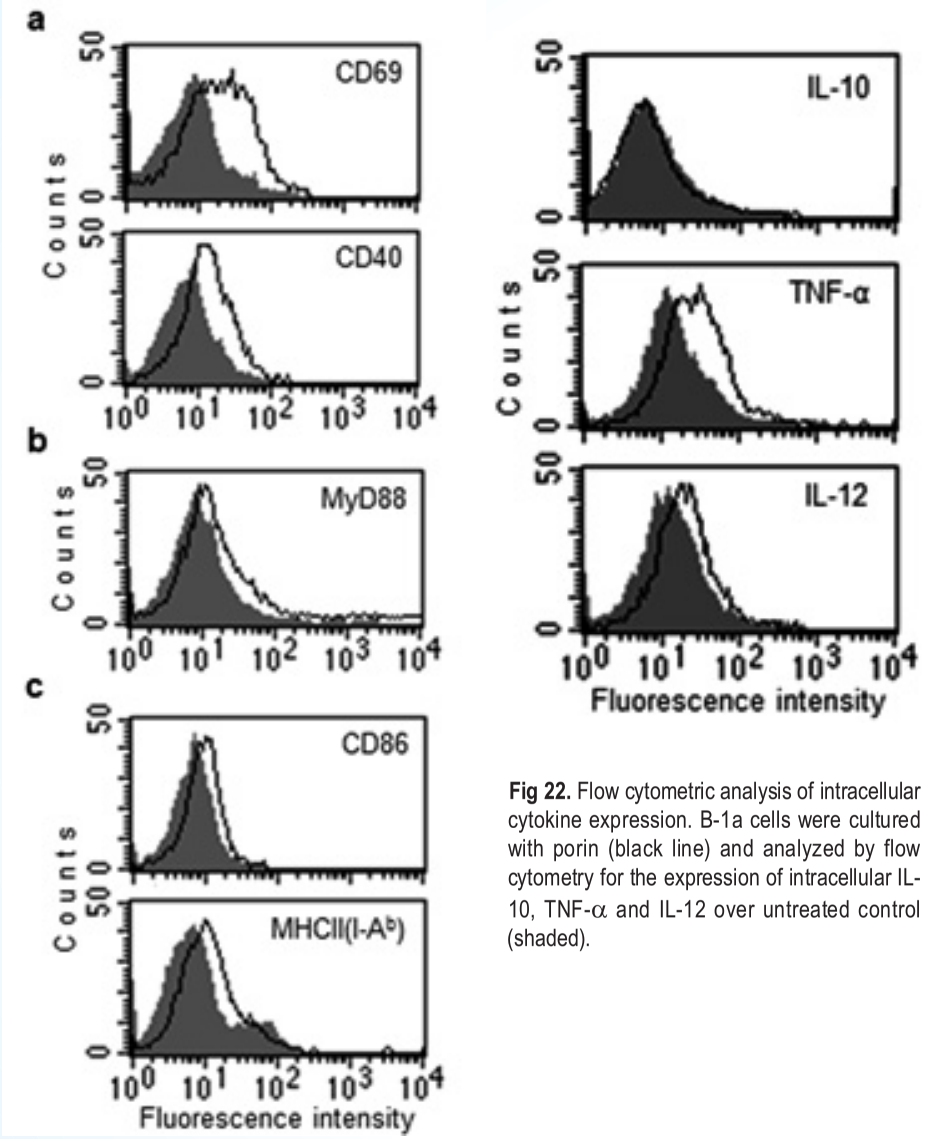


Fig 21. Effect of porin on the expression of activation and costimulatory molecules on B-1a cells. (a) B-1a cells were cultured with and without porin and analyzed by flow cytometry for the expression of CD69 and CD40 as indicated by untreated (shaded) and treated (black line) cells. (b) The flow cytometric analysis of porin-induced (black line) expression of MyD88 compared to untreated control (shaded). (c) The B-1a cells were analyzed by flow cytometry for the expression of CD86 and MHC class II (I-Ab) after culturing with either porin (black line) or complete medium alone (shaded).

Fig 22. Flow cytometric analysis of intracellular cytokine expression. B-1a cells were cultured with porin (black line) and analyzed by flow cytometry for the expression of intracellular IL-10, TNF-α and IL-12 over untreated control (shaded).

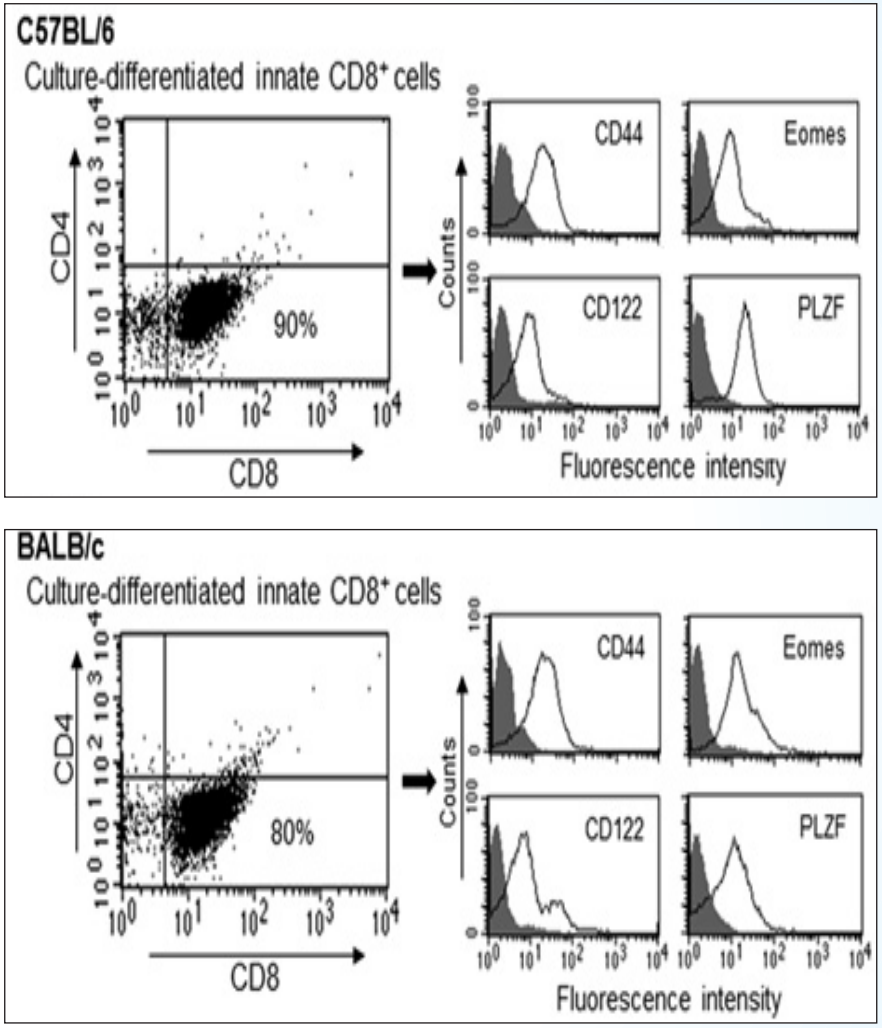


Fig 23. Culture-differentiated CD8+ cells display innate memory-like property. Flow cytometric analysis shows culturing of CD4+CD8+ DP thymocytes of C57BL/6 and BALB/c mice results in appearance of CD8+ T cells after 48 h of culture. The differentiated CD8+ cells were analyzed for expression of CD44 (black line), CD122, Eomes and PLZF over isotype-matched control (shaded) after 48 h of culture.

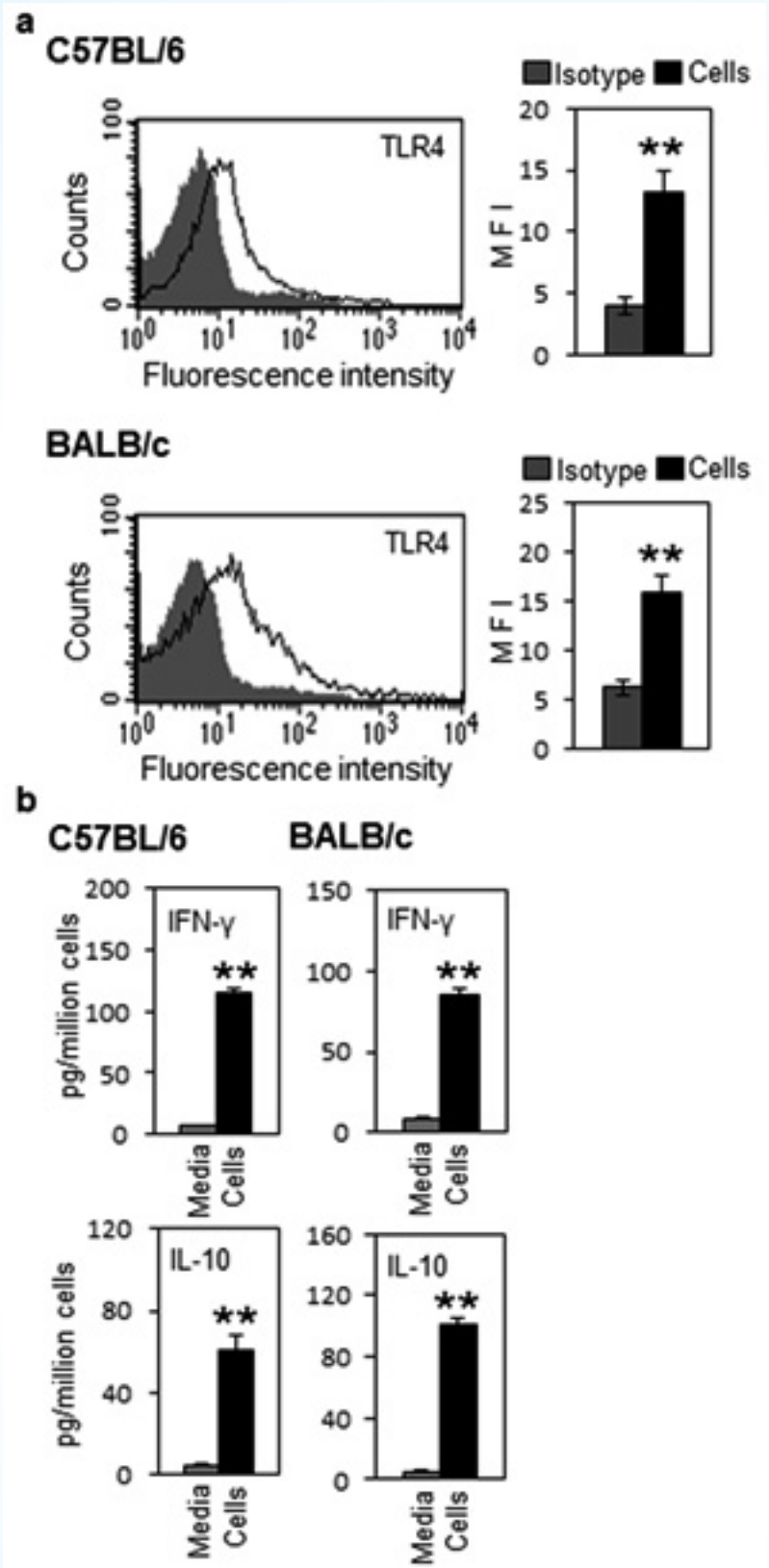


Fig 24. Analysis of TLR4 and quantification of IFN-γ and IL-10 release by innate CD8+ T cells. (a) Cells were assessed for TLR4 expression (black line) on differentiated CD8+ SP T cells from normal strains of mice at 48 h. Shaded profiles indicates isotype-matched controls. (b) Sorted DP cells from C57BL/6 and BALB/c mice were cultured for 72 h to assess the release of IFN-γ and IL-10 by ELISA. The bar diagrams show mean ± SEM of three separate experiments. **P<0.005.

Parasitology

The division of Parasitology currently emphasizes on the research to understand the biology of major diarrhea causing parasites at its transcriptomics, proteomics and metabolomics 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. Recently we have identified plant compound showing significant activity against the viability of Entamoeba. The major areas of our interest are to understand the stress management pathway during oxidative stress in *Giardia lamblia*. We have identified several genes which are differentially regulated during oxidative stress can be drug targeted. As it was known to us that *Giardia* does not secret any toxin but we have identified some proteins (metabolic enzymes) which are secret in the extracellular milieu and hypothesized that may be involved in the reactive oxygen species detoxification. For the first time we have reported that Giardia contains an antioxidant system by which it can survive under the environment of high PO_2. It helps to understand the effects of oxidative stress on microaerotolerant *Giardia* at its cellular, genomic and molecular level and its relation with basic signaling pathway in response to oxidative stress. We have been characterized the local isolates of Kolkata 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 involved with extensive surveillance study of enteric parasites.

During the calendar year 2014-15 the assessment of helminthic burden among different age groups in West Bengal with special emphasis on children of low socio economic class has been performed. Identification of new pathogenic strains of *Giardia* and *Cryptosporidium* in Kolkata, India has been carried out. 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. We have involved with projects to show small RNA-Protein interaction during ribosome biogenesis. This can easily be exploited as a target for rational drug designing in different systems. Studies on these aspects will be useful in future for fighting against very primitive but existing gastrointestinal disease like giardiasis, amoebiasis etc. we have developed a new high resolution genotyping of *Entamoeba histolytica* to assess its virulence and pathogenicity and identified a new Indian pathogenic isolates based on this genotyping. 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. A whole genomic DNA library was constructed and microarray has been performed to identify the differentially expressed genes which are involved in oxidative stress regulation in *Giardia*.

The study has identified various biological processes involved in the regulation of gene expression pertaining to oxidative stress management in *G. lamblia*. These processes required several components, such as regulatory elements, transcription factors and cofactors, chromatin modification proteins and proteins involved in post-transcriptional regulation. These components will continue to grow, as future studies identify examples of direct communications between regulatory proteins, and reveal how gene networks are regulated co-ordinately through these interactions during stress in *G. lamblia*. The transcriptomic data has indicated towards some very important genes on which further studies can be carried out. They can be used as drug targets as this parasite also faces similar types of oxidative stresses inside our gut that has been mimicked here in vitro. The results have opened some new directions for further investigations. Our other studies revealed that the modulation of the fate of pyruvate in one direction or the other can be important for homeostatic response of *Giardia* to oxidative stress. This could alter functioning of the antioxidant system and have protective effects against DNA damage induced by oxidative stress. Alterations of pyruvate metabolism are observed in *Giardia* due to high oxygen environment. This could be advantageous for *Giardia* trophozoites in such stressful

condition. Surveillance study revealed that Giardia still remained as the major parasite in the diarrhoegenic population of Kolkata.

Scientist:

Dr. S Ganguly, Scientist E

Staff:

Mr. S. L. P. Singh, Technician B

P. Chowdhury, Laboratory Assistant (Project Staff)

Post Doctoral Student:

Dr. Prasanta Saini

Pre Doctoral Students:

Avik Kumar Mukherjee

Koushik Das

Dibyendu Raj

Sumallya Karmakar

Awards

Arjun Ghosh received Ph.D. degree from Jadavpur University.
Title of the Thesis: Studies of small RNA-protein interaction in Giardia lamblia.

Esha Ghosh received Ph.D. degree from Calcutta University
Title of the Thesis: Studies on oxidative stress regulation in giardia lamblia at its transcriptomic, proteomic and cellular level.

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 has been performed on the samples collected from different parts of semi urban and rural western India. Recent technological advances of parasites and helminthes have improved our understanding of host-parasite interaction, 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 helminthes detection and PCR was adapted for other diarrheagenic parasites. After that, further confirmation was carried out by sequencing. 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.

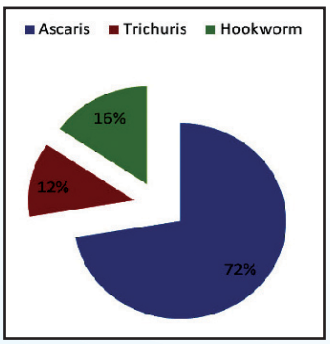


Fig. 25 : Percentages of each helminth out of 2195 positive cases.

The prevalence of Ascaris, Trichuris, and Hookworm is presented in the Table with estimated 95% confidence interval (Fig. 25). 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 estimated 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.

Differential pathogenesis of Giardia: Role of Giardia Virus.

Investigator: S. Ganguly

GLV Detection procedure and outcomes:

RNA isolation and PCR amplification: Viral RNA was isolated by using Viral RNA Minikit, Qiagen (Fig. 26). cDNA preparation and PCR amplification was performed by using Superscript-III One Step RT-PCR kit, Invitrogen (Fig. 27).

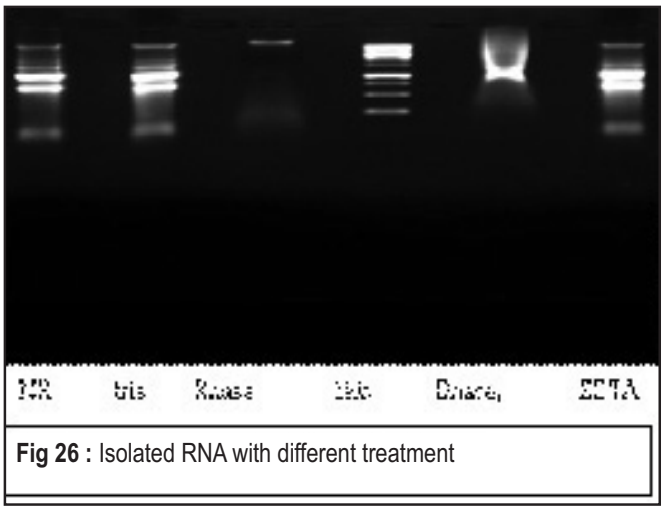


Fig 26 : Isolated RNA with different treatment

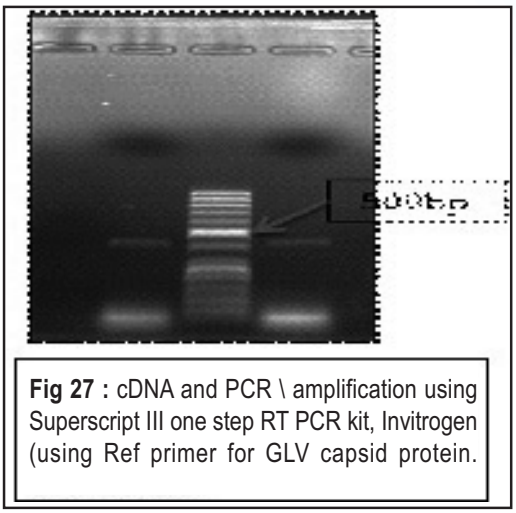


Fig 27 : cDNA and PCR \ amplification using Superscript III one step RT PCR kit, Invitrogen (using Ref primer for GLV capsid protein.

Protocol

- Various amounts (1–2 ng) of eluted RNA were subjected to RT-PCR using the SuperScriptIII One-Step RT-PCR kit (Invitrogen Cat no. 12574-030) and primers directed to the GLV-capsid protein sequence (GenBank L13218). [The forward primer sequence (GLV-CF) was 5'-GCCAGGATCTGGTAATTGCT-3' corresponding to nt 1251–1270; the reverse primer sequence (GLV-CR) was 5'-CTAGCGTCCTTTGAATACA-3' corresponding nt 1541–1569.]
- Viral RNA was denatured in the presence of GLV-CF and GLV-CR primers (0.4 mM) by heating at 94 C for 3 min, quick-chilled in wet ice, and subjected to RT-PCR following procedures provided by the manufacturer (Invitrogen).
- [RT-PCR consisted of 1 hr incubation at 53°C, followed by 3 min denaturation at 94°C, and then 40 cycles of 94°C for 30 sec, 53°C for 30 sec, and 68°C for 1 min, followed by a final extension at 68°C for 5 min]
- RT-PCR products were analysed by polyacrylamide gel electrophoresis followed by ETBR staining.

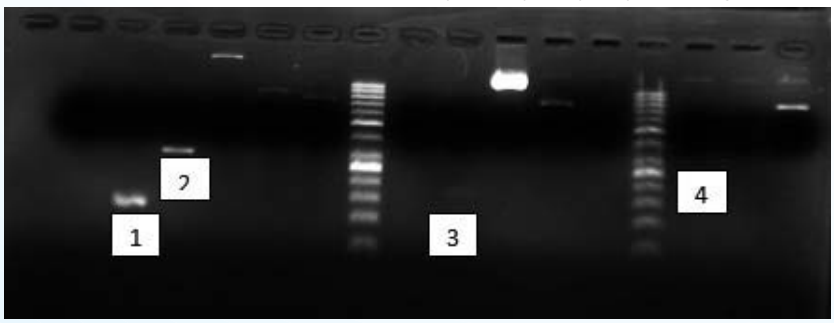


Fig. 28 : cDNA and PCR amplification using superscript III one step RT PCR kit, invitrogen (using new designed primer for GLV capsid protein.

Sequencing Result:

The sequence obtained has no significant identity with reported cds of GLV capsid sequence. (Alignment provided as FASTA file named GLV wg_cap_4F) New PCR primers were designed to target the conserved region of GLV capsid protein and PCR was done according to the reference protocol (Fig. 28).

PCR products (Fig. 28):

Findings:

Few DNA bands from the desired base pair were purified and sequenced with the specific primers (Table 5).

Conclusion:

- The Portland I strain of Giardia lacks GLV
- Local strains of Giardia also lacks GLV
- Thus, differential pathogenesis of Giardia is caused as per host system and genomic or better said transcriptomic and proteomic regulations.
- Identification of new genes in Giardia inside human GUT (Microarrary hybridization)

We have used different in vitro procedures for mimicking human GUT, like high oxygen tension etc. to find out what are the differentially regulated factors in Giardia that helps the parasite to live inside the human GUT even at very high oxygen tolerance level than they can withstand. We have used a genomic DNA microarrary for hybridization procedure for fishing out these particular candidate regulators.

Microarray analysis: The hybridized microarray slides were scanned and more than 200 clones have been identified that show 5 folds or higher times upregulation or downregulation than the control set. The scanned picture (Fig.29) and the analysed result (Fig.30) have been shown below.

a. **Sequencing result:** The result is shown in the following table 6.
b. Real time PCR validation: Some of the important genes found from the sequencing result have been checked by Real time PCR. PCR result of Hsp90, nitroreductase and Pyruvate ferredoxin oxidoreductase have been shown below (Fig 31):
c. **2D analysis:** Differential transcription of some genes due to oxidative stress were further analysed in differential protein expression level using 2D gel electrophoresis. The gel picture after silver staining has been shown below (Fig 32):

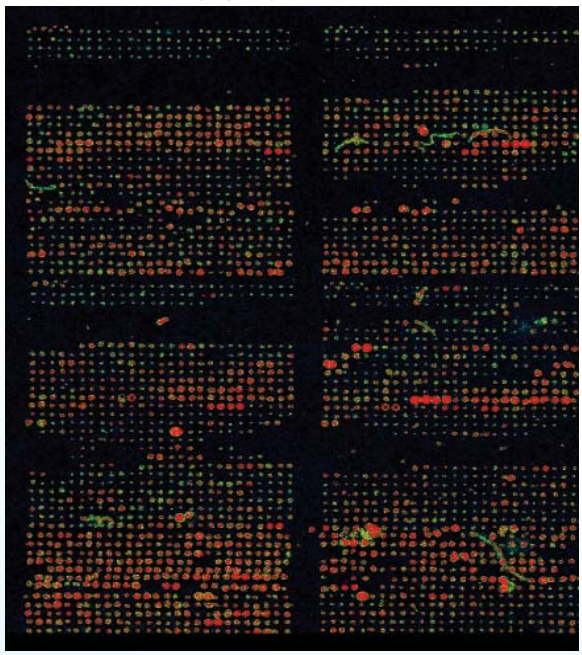
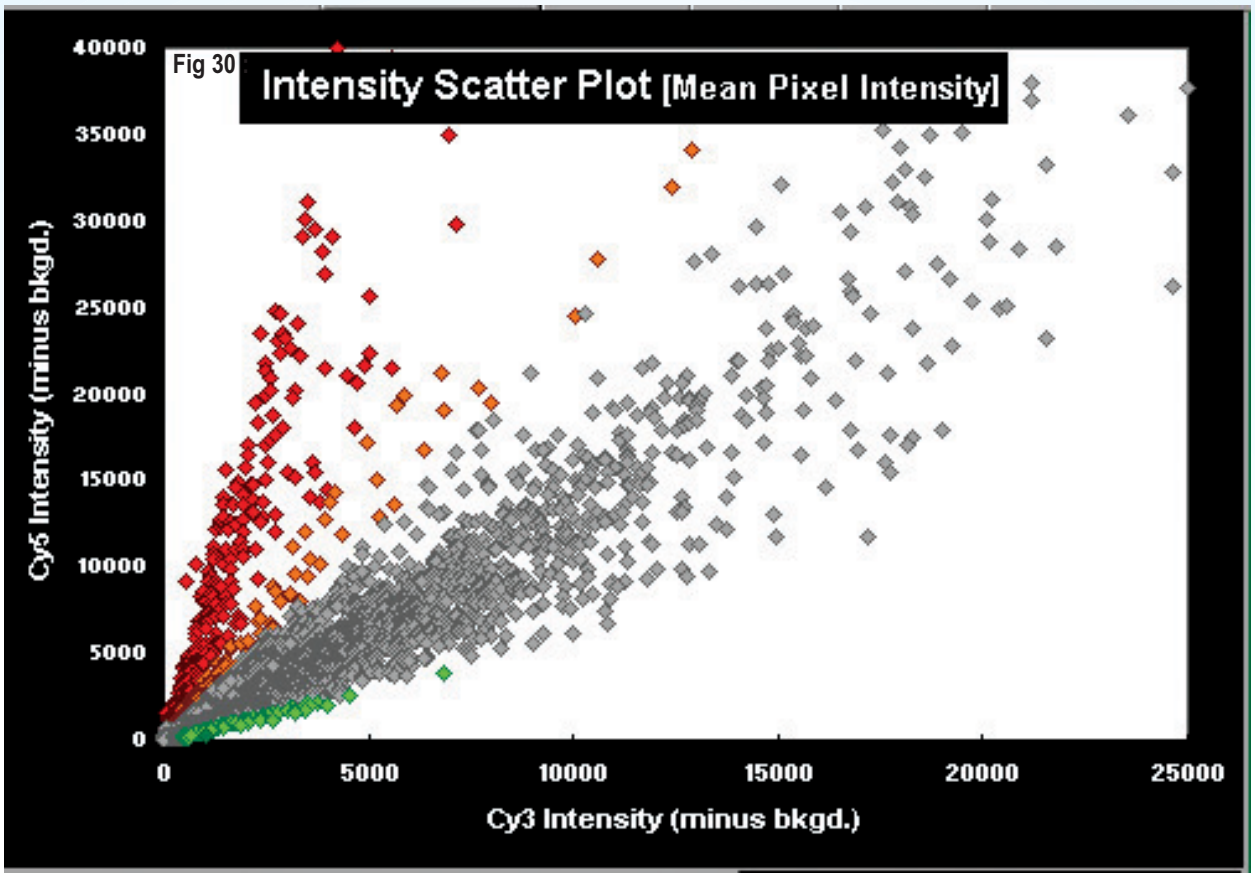
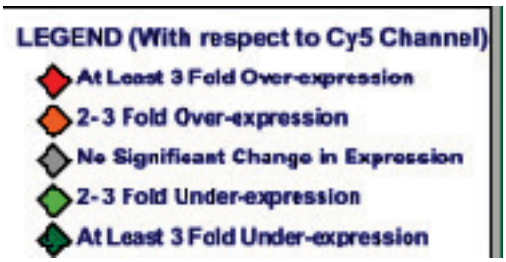


Fig 29 : DNA Array Hybridization with stressed cell lines : someUp-regulated (red) and down-regulated (green) spots (genes) are highlighted here.





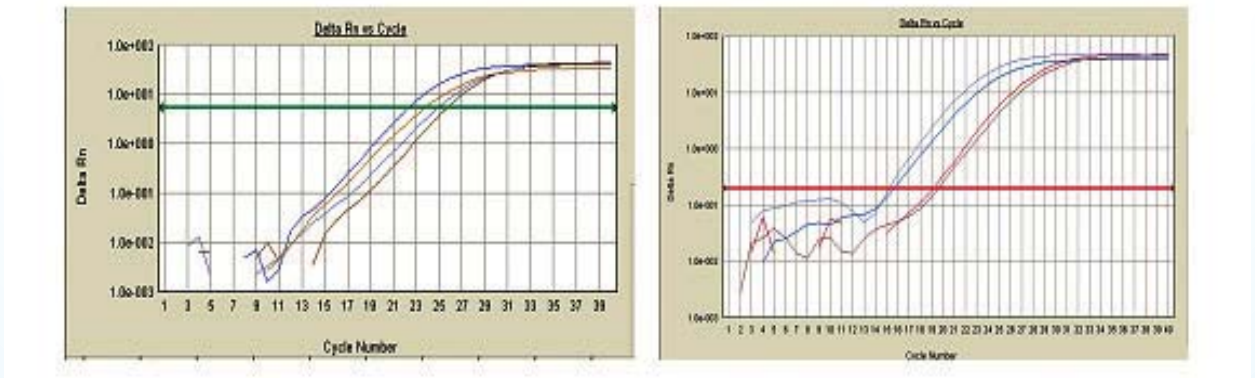


Fig. 31 : Differential expression of Hsp90, Nitroreductase (NR) and Pyruvate-ferredoxin oxidoreductase (PFOR) in control and stressed cells

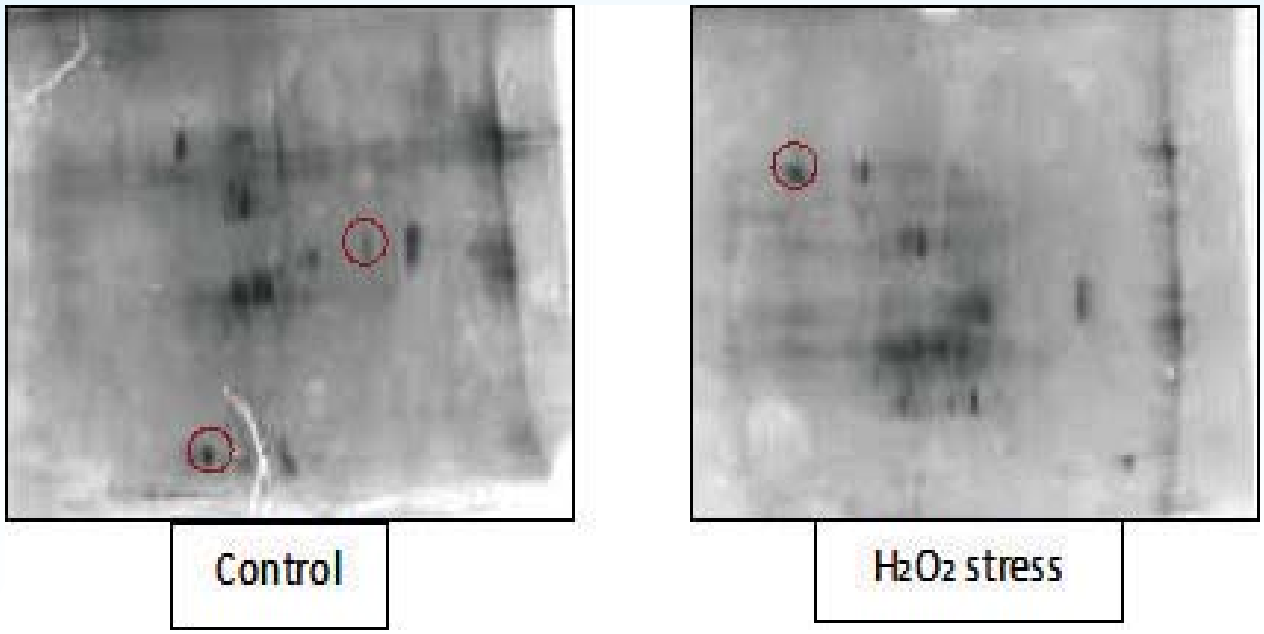


Fig 32. 2D gel electrophoresis. (a) Control set and (b) Oxidative stressed cell set.

Table 5 : Identification of genes by sequencing with specific primers

Primer Set	Identity with (Accession no.)	Score	E value	Query coverage
L21F	XM_001705748.1	141	9e-31	95%
L31F	XM_001707957.1	326	7e-86	91%
L45R	XM_001706447.1	463	5.5e-16	90%
L51F	XM_001706605.1	582	7e-163	99%
4F	XM_001706802.1	129	0.78	59%

Table 6 : Identification of different genes by microarray analysis

Names of the genes	**Gene_ID**
Metabolic enzymes coding genes	
NADH Oxidase	GL50803_9719
NADH Ferrredoxin Oxidoreductase	GL50803_17151
Pyruvate Ferredoxin Oxidoreductase	GL50803_114609
Thioredoxin Reductase	GL50803_9827
Nitroreductase	GL50803_15307
Arginine deiminase	GL50803_112103
Malate dehydrogenase	GL50803_3331
Alcohol dehydrogenase	GL50803_13350
Phoaphatase and kinase coding genes	
CAM Kinase	GL50803_16034
Serine threonine protein phosphatase	Gl50803_21498
Transcriptional/translational and cell divisional protein coding genes	
Small subunit rRNA	GL50803_r0019
Large subunit rRNA	GL50803_r0013
TAR RNA loop binding protein	GL50803_32741
Nuclear LIM interactor interacting factor-I	GL50803_14905
TMP55	GL50803_137641
Protein 21.1	GL50803_13590

Pathophysiology

The Division of Pathophysiology, NICED is actively undertaking research on various projects related to different diarrhogenic bacteria. This division has previously showed that 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. The mechanism of immunogenicity of *S. flexneri* 2a OmpA as vaccine antigen correlates with its ability to activate macrophages with the surface expression of MHCII, CD80 and CD40, which in turn, facilitates stimulation of adaptive immune response by activation of $CD4^+$T cells. Intranasal immunization of mice with OmpA induces antigen specific IgG and IgA production in both the systemic and mucosal compartments, demonstrating participation of B cells in OmpA-induced protective immune response in vivo. OmpA of *S. flexneri* has been identified as a novel molecule coordinating the innate and adaptive immune responses, hence proving itself as an optimal vaccine candidate.

Our division also works on microbial proteases from *V. cholerae* and *Escherichia coli*.Serine protease autotransporters of Enterobacteriaceae (SPATEs) are secreted proteins demonstrating diverse virulence functions. The distribution of SPATEs was studied among diarrheagenic and extraintestinal pathogenic *Escherichia coli*. We showed for the first time the association of SPATEs with neonatal septecemic *E. coli* (NSEC). The presence of SPATEs in the NSEC isolates may be considered as the most discriminatory trait studied here**.** We also report for the first time the presence of YghJ a secreted metalloprotease in NSEC which stimulates IL8 secretion in Int407cells.

The Division is actively engaged in intestinal ion-transport and barrier function relevant to the pathogenesis of secretory and inflammatory diarrhoea. We have explored a better understanding of the intestinal epithelial barrier function which is regulated by Epac (Exchange protein directly activated by cAMP) to foster new ideas for the development of therapies for diarrhea.

Scientists:

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. Irsad Ali.

R. Bhowmick.

A. Mondal.

P. Sarkar.

J. Aoun.

T. Saha.

Study of the 34kDa outer membrane protein of Shigella flexneri 2a induced B cell immune response

Investigator: M. K. Chakraborty

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 explored that outer membrane protein A (OmpA) of *Shigella flexneri* 2a possesses the essential characteristics of a potential vaccine antigen, which includes crossreactivity, surface exposed epitope and conservation among strains. The mechanism of immunogenicity of *S. flexneri* 2a OmpA as vaccine antigen correlates with its ability to activate macrophages with the surface expression of MHCII, CD80 and CD40, which in turn, facilitates stimulation of adaptive immune response by activation of $CD4^+$T cells. TLR2 has been recognized as an indispensible factor in OmpA-mediated coordination between the innate and adaptive arms of the immune response. Moreover, OmpA evokes strong protective immune response against the homologous virulent strain in mice without addition of exogenous adjuvants and that the immunity might involve synergy among the cellular and humoral immune responses. Intranasal immunization of mice with OmpA induces antigen specific IgG and IgA production in both the systemic and mucosal compartments, demonstrating participation of B cells in OmpA-induced protective immune response in vivo. However, the effect of *S. flexneri* 2a OmpA on B cells has not been delineated yet. Hence, the present study has been instigated to illuminate whether OmpA can directly activate B cells and identify the molecular mechanism behind it. We have shown that OmpA of *S. flexneri* 2a activates B cells to produce protective cytokines, IL-6 and IL-10 as well as facilitates their differentiation into antibody secreting cells (ASCs) (Fig 33). The immune stimulatory properties of OmpA are attributed to the increased surface expression of MHCII and CD86 on B cells in a TLR2 dependent manner(Fig 34). Hence OmpA of *S. flexneri* has been identified as a novel molecule coordinating the innate and adaptive immune responses, hence proving itself as an optimal vaccine candidate.

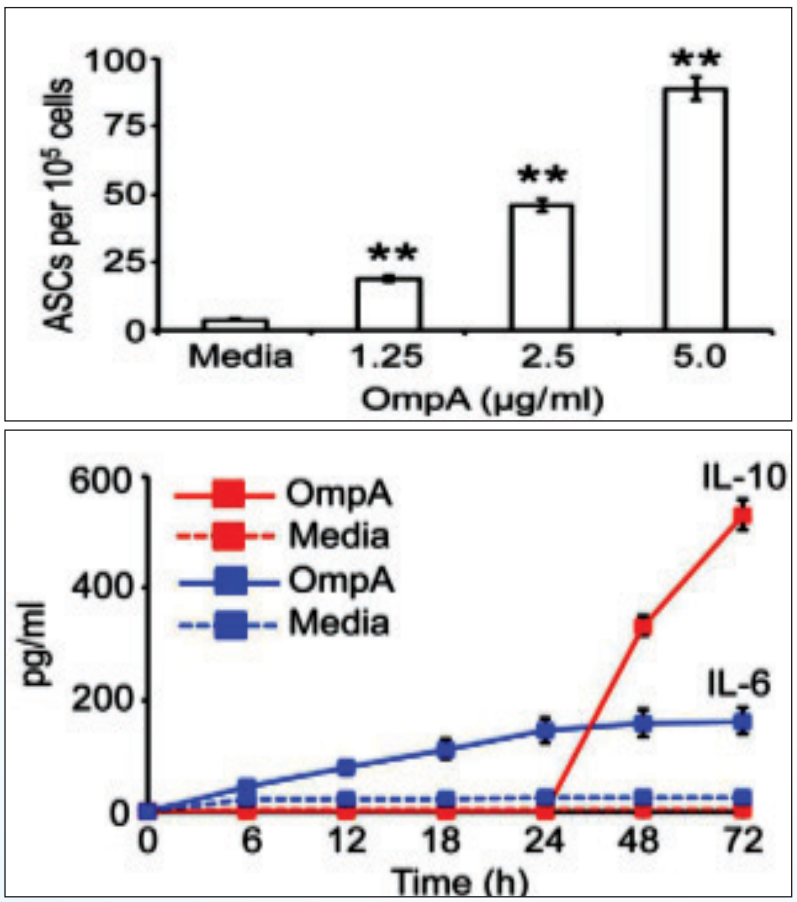


Fig 33. OmpA evokes proliferation and differentiation of splenic B cells as well as induces secretion of IL-10 and IL-6. (A) The total number of ASCs (IgM + IgG-secreting cells) was analyzed by ELISPOT at the end of a 72 h culture period. Results are expressed as numbers of ASCs/10^5 cells seeded and correspond to the mean 6 S.E.M of quadruplicate determinations. **, p,0.01, relative to the untreated (media alone) group. (B) Time kinetics of IL-6 and IL-10 production by B cells incubated without or with OmpA (5 mg/ml). The values are derived from IL-6 and IL-10 standard curves and represent the mean 6 S.E.M of three independent experiments performed. **, p,0.01, relative to the untreated (media alone) group

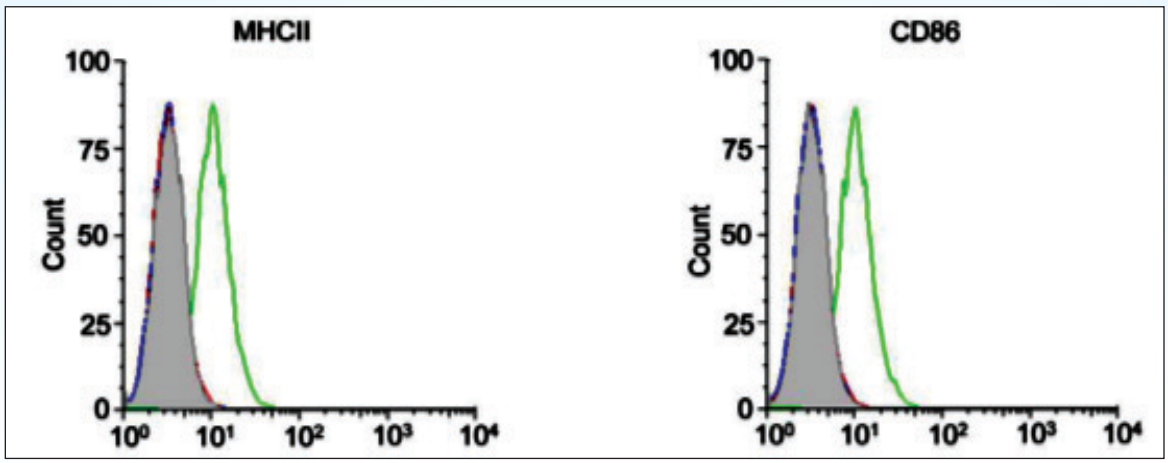


Fig 34. TLR2 is essential for OmpA-induced expression of MHCII and CD86 on B cells.B cells were incubated with anti-mouse TLR2 blocking antibody (100 ng/ml) for 1 h at 37^{O}C (red line). Cells were then cultured in the absence (blue line) and presence (green line) of OmpA (5 mg/ml) for 24 h. Cells were harvested and assayed for cell surface expression of MHCII and CD86. The shaded histograms denote the isotype control antibodies. Representative data from three independent experiments are shown.

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

Principal Investigator: A. Pal

Co-Investigator: S. Basu

Serine protease autotransporters of Enterobacteriaceae (SPATEs) are secreted proteins demonstrating diverse virulence functions. The distribution of SPATEs was studied among diarrheagenic and extraintestinal pathogenic *Escherichia coli*. We showed for the first time the association of SPATEs with neonatal septecemic *E. coli* (NSEC). The presence of SPATEs in the NSEC isolates may be considered as the most discriminatory trait studied here. **EJCMID, 2014.**

A 170 kDa secreted protease from a NSEC isolate was partially purified from culture supernatant after successive ion exchange and gel filtration chromatography. Mass spectrophotometric analysis of the putative protein band showed it to have homology with *E. coli* lipoprotein, *YghJ* having M60 metalloprotease domain. *YghJ* was cloned and expressed in *E. coli* TOPO10 cells. It was inhibited in presence of EDTA. It could degrade extracellular matrix proteins like fibrinonectin. It showed cell rounding effect on Int407 and HT 29 cells. It could trigger time and dose dependent IL8 response in Int407 cells. IL8 is an important inflammatory marker in sepsis. Distribution of *YghJ* gene showed it to be present in 78% of NSEC and 54% of E. coli from healthy neonates. Western blot analysis showed it is expressed in 80% of NSEC and in 33% *E. coli* from healthy neonates. We report for the first time the presence of *YghJ* a secreted metalloprotease in NSEC which stimulates IL8 secretion in Int407cells.

Epac-A new player in diarrheal diseases: role in intestinal ion transport and barrier function

Principal Investigator: M. H. Kazi

The overall summary of this proposal is to understand the contribution of Epac (Exchange protein directly activated by cAMP) protein and its underlying signaling events in epithelial ion transport and barrier function. The underpinning of this proposal is that there is a urgent need for new pharmacotherapy with diarrhea as well as cystic fibrosis (CF) and inflammatory disorders, this might be achieved by understanding how Cl^- secretion is being regulated both normally as part of normal digestive physiology and how this becomes abnormal in diarrheal diseases as well as determining

the way to stimulate an alternative signaling pathway of Cl^- secretion (in case of CF). We have also explored a better understanding of the intestinal epithelial barrier function which is regulated by Epac to foster new ideas for the development of therapies for diarrhea in patients with IBD (Inflammatory Bowel Diseases). Working on this project, we have demonstrated that cAMP regulates intestinal chloride secretion not only by Protein Kinase A as had been assumed but activation of Epac which acts by elevating intracellular calcium[J Gen Physiol. 2010 Jan;135(1):43-58]. This is a new concept for our cAMP works generally and is one of the first to apply this concept to intestinal epithelial cells. Moreover, we have demonstrated for the first time that an apical potassium channel called KCNN4c expression and activity are markedly decreased due to Epac1 depletion involving Rap1A-RhoA-ROCK signaling and provide a molecular framework for new understanding of the regulation of epithelial Cl- secretion [J Biol Chem. 2013 Jul 12;288(28):20404-15].

In this reporting period, we have explored the role of Epac1 protein in the regulation of tight junction (TJ) function in human colonic cell line, T84. Studying on this aspect, what we have seen that depletion of Epac1 caused reduction of Transepithelial Electrical Resistance (TER], and increment of paracellular flux of non-charged particle as shown in Fig. 35. We tentatively interpreted such decreased TER and increased paracellular permeability as subtle forms of junctional barrier disruption. Our study is in progress to know how Epac1 and its underlying signaling pathway participate in the formation of TJ or contribute to the regulation of paracellular function.

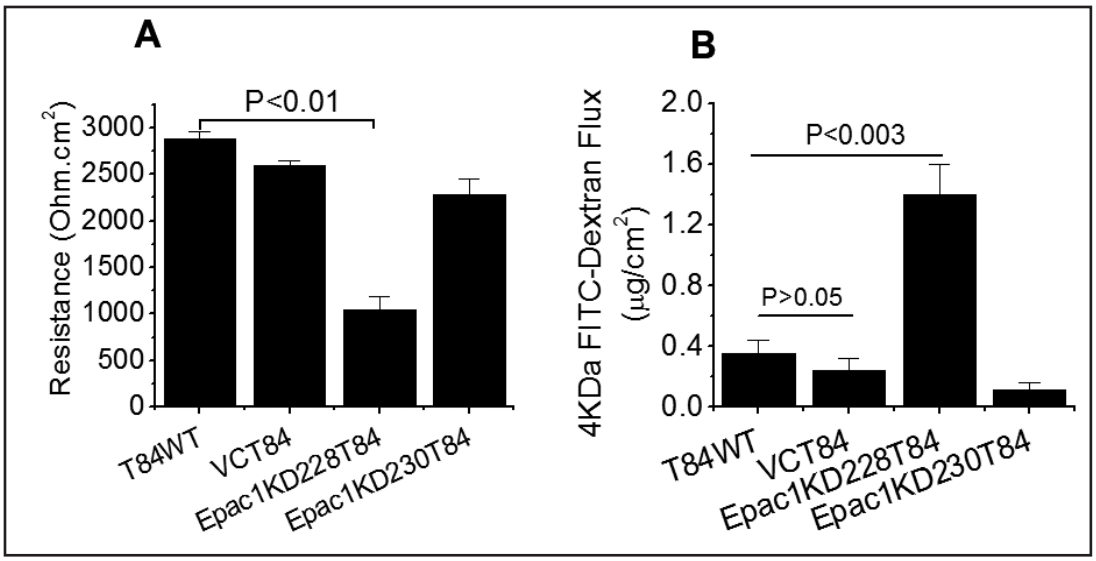


Fig 35. Depletion of Epac1 in T84 cells by lenti shRNA caused alteration of TER (A) and paracellular flux of 4KDa FITC-Dextran (B). Stable cell lines of T84 wild type with expression of Epac1 knock down was generated by infecting cells with human Epac1- specific lenti-shRNA particles, and positively transduced cells were selected with 10µg/ml puromycin-containing medium. Values are mean ± SEM; n = 6-10.

Anti-diarrheal mechanism of Zinc: Effect on epithelial ion transport and barrier function

Principal Investigator: M. H. Kazi

Co – Principal Investigator: M. K. Chakrabarti

There is insufficient physiological understanding of Zn's action on intestinal ion transport (transcellular vs. paracellular), which is a major parameters in improving diarrhea. Neither there are reports of whether Zn induces cation [(Na^+) absorption and/or inhibits anion (Cl^-) secretion because these properties are characteristic of most anti-diarrheal drugs. It remains to be clarified whether the effects of Zn may be beneficial in the modification of tight junction (TJ) proteins which are altered by various pathogens in infectious/inflammatory diarrhea (e.g. Shigellosis). During this reporting period,

we have made progress to demonstrate the role of Zn in electroneutral NaCl absorption by stimulation of NHE3 (sodium-hydrogen exchanger isoform 3). We hypothesized that Zn could stimulate intestinal sodium and water absorption via effects on NHE3, since NHE3 is the major way Na^+ is absorbed in the GI tract and is inhibited in all diarrheal disease in which NHE3 activity has been studied. We demonstrated that incubation of HA-NHE 3 expressing Caco-2 cells for 30 min at 37^{O}C with 100µM Zn increased NHE3 activity by 48 ± 3%. In this reported period, we have studied the mechanism by which NHE3 stimulation by Zn was occurred. We measured NHE3 activity in HA tagged NHE3 expressing Caco-2 cells flurometrically using pH sensitive dye BCECF. As shown in figure 36a Zn was not able to stimulate NHE3 activity in the presence of PLC inhibitor U73122 (10µM). This finding encouraged us to measure intracellular calcium [Ca^{2+}]i in HA-NHE3 expressing Caco-2 cells using Fura-2AM as a calcium sensitive fluorescence probe. Zn application increased [Ca^{2+}]i in these cells (352 ± 25nM). We then measured [Ca^{2+}]i in the presence or absence of PLC inhibitor U73122 to demonstrate that Zn mediated rise of [Ca^{2+}]i is a PLC dependent process. As shown in figure 7D, the PLC inhibitor U73122 abolished the elevation of [Ca2+]i. Having shown that Zn acutely stimulates NHE3 activity and increased [Ca2+]i in Caco-2 cell which was inhibited by PLC inhibitor U73122, we have determined whether Zn stimulation of NHE3 activity was happened through increased exocytotic insertion of NHE3 in epithelial cells. The change in the amount of HA-NHE3/Caco-2 cell surface was determined by surface biotinylation assay. The amount of NHE3 protein abundance was increased to the plasma membrane by 40% in the presence of Zn in HA-NHE3 expressing Caco-2 cells (Figure 36b). Taken together all these data, we conclude that i) Zn acutely stimulates NHE3 in polarized human intestinal cells, Caco-2 by increasing surface NHE3 amount. ii) Zn increases NHE3 activity via a PLC dependent pathway yet an undefined mechanism. We are further progressing this objective by measuring NHE3 activity thus Na^+ absorption, in an ex vivo model of mouse ileal tissue by using pH sensitive dyes SNARF-4F.

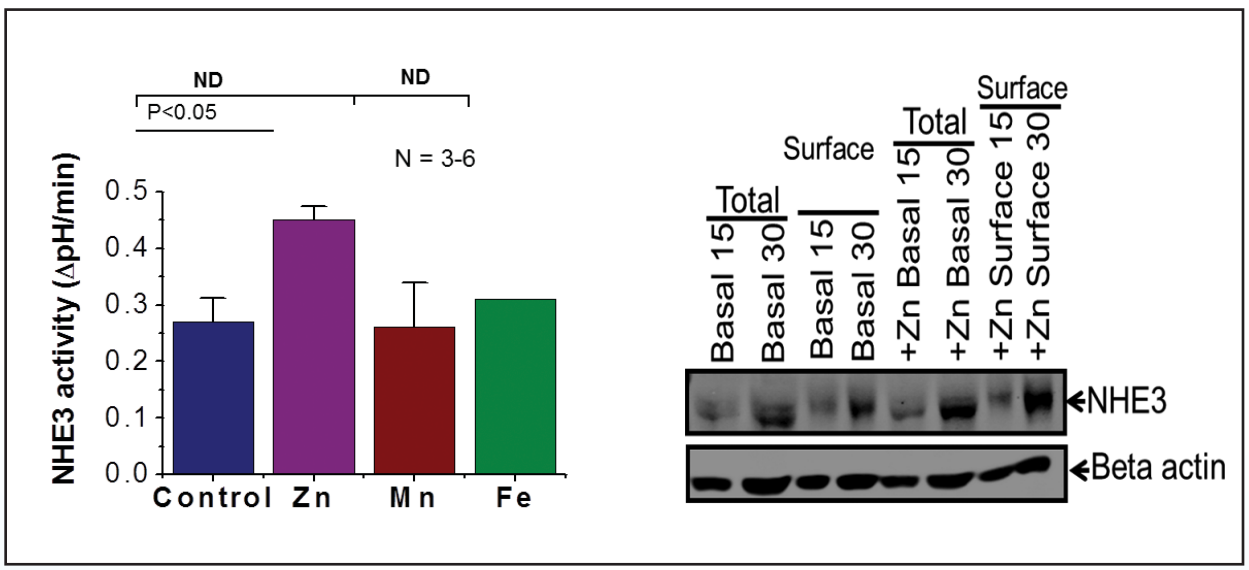


Fig 36. Zn stimulates NHE3 activity in a PLC dependent manner. NHE3 activity was measured in HA-NHE3 expressing Caco-2 cells fluorometrically in presence or absence(control) of Zn and/or PLC inhibitor U73122 (A). Apical surface protein (NHE3) after Zn stimulation in confluent Caco-2 transfectants on permeable membrane selectively biotinylated and retrieved from solubilised cell lysate by streptavidin-affinity precepitations. Biotinylated proteins (NHE3) were fractionated by SDS-PAGE, blotted onto nitrocellulose membrane and probed with antibodies specific for NHE3(B).

Awards/ Honours Received

M. K. Chakraborty

- Served as Fellow, National Academy of Science, Allahabad
- Served as Fellow, West Bengal Academy of Science & Technology
- 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.He has been elected again the convener, Medical and Veterinary Science Section and council member of West Bengal Academy of Science & Technology from 2014-2016.
- As Vice-President of The Physiological Society of India, 2010-2014 and 2014 onward 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 of "Everyman's Science".

A. Pal

- Board of Post-Graduate Studies in Physiology, Burdwan University.

M. H. Kazi

- American Physiological Society's Research Recognition Award presented at the APS GI & Liver Section of Experimental Biology 2014 meeting, 26-30 April in San Diego, CA, USA.
- Editorial Board Member of The Physiological Report - a sister Journal of American Physiological Society and UK Physiological Society.

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

M. K. Chakrabarti

- Participated and delivered a Keynote lecture on "Anti-cancer activities of bacterial toxins" at a national seminar on "Science and Technology for human development" on 8- 9 December, 2014 at Kanpur.
- Attended and delivered lectures at the section of Medical sciences (Including Physiology) on "Vaccine against Shigellosis" on 5January 2014. M.K. Chakrabarti also chaired a plenary session on Health & Disease
- Participated at inaugural session and delivered a lecture at a conference on Science and Technology for Human Development organized by Manipur University, Imphal held on 20-22 January, 2015.
- Participated and a delivered a lecture in the awareness program in a tribal area rural school-Bandh Nabagram Gandhi H.S. school, Birbhum organized by ISCA Kolkata Chapter during 7-8 February, 2015.
- Attended a seminar on "Science and Technology for human development" at Amravati University, on 9-11 March, 2015.

A. Pal

- Attended the Biologia 2015 at Indian Institute of Science Education and Research, Kalyani 21 March, 2015 and delivered a lecture on "Microbial proteases and their role in pathogenesis".

M. H. Kazi

- Paramita Sarkar, Irshad Ali Sheikh, Joydeep Aoyun, Tutul Saha, Subhra Chakraborty, Manoj K. Chakrabarti, Dhira Rani Saha and Mirajul H. Kazi. Zinc recovers altered intestinal ion-transport and barrier function caused by Shigella infection in T84 cells at the Experimental Biology Meeting, April 26-30, 2014 held in San Diego Convention Centre, San Diego, CA, USA.
- Irshad A. Sheikh, , Mikio HayashiTanaya Chatterjee, Dhira Rani Saha, Pinak Chakrabarti, Manoj K. Chakrabarti, Mirajul H. Kazi. Recombinant accessory cholera enterotoxin (Ace) of Vibrio cholera induces diarrhea by stimulation of a chloride channel and inhibition of the Na+/glucose co-transporter SGLT1 at the Digestive Diseases Week, May 3-6, 2014 in Chicago, Illinois, USA.

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 interaction 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 activities to include studies on influenza viruses and has organized a routine surveillance program in collaboration with NIV Pune 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.

NICED being a premier institute in the field of health research has been actively engaged in HIV/AID research and National AIDS Control program since mid-1980s. Currently, major projects including (i) HIV Surveillance (Sentinel as well as Integrated Biological and Behavioral Surveillance) for HIV estimation in different risk group populations, (ii) Molecular studies for HIV detection for babies born to HIV infected mothers, (iii) HIV viral load assay, (iv) HIV drug resistance mutations, (v) Understanding the molecular diversity of HIV in the country and (vi) HIV testing Quality Assurance are being carried out in the Virology Division.

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 ICMR, NACO, CDC Atlanta and Okayama University, Japan.

Scientist:

Dr. T. Krishnan, Scientist 'F

Dr. Malay Kumar Saha, Scientist 'E'

Dr. M. Chawla-Sarkar, Scientist 'E'

Staff:

Dr. S. 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, MTS

Project Scientist II

Dr. Mukti Kant Nayak
Dr. Subrata Biswas
Ms. Srijita Nandi
Dr. Mallika Ghosh

Pre-doctoral Fellows:

Satabdi Nandi
Shampa Chanda
Satarupa Mullick
Paulami Mandal
Upayan Patra
Arpita Mukherjee

Awards

Tapasi Roy received Ph.D. degree from Calcutta University

Title of the Thesis: Surveillance For Common Respiratory Viruses Among ARI Patients In Kolkata: Molecular Characterization And Analysis Of Potential Antivirals For Influenza Viruses.

Umesh Chandra Halder received Ph. D. Degree from Jadavpur University

Title of the Thesis: Role of Influenza A virus protein in the modulation of host cell signaling pathways : unrevealing versaillesque avenues of viral pathogenesis.

Detection of emerging virus among acute gastroenteritis cases in Kolkata, India

Principal Investigator: T. Krishnan

Co-Investigators: M. K. Bhattacharaya, P. Indwar

Screening for emerging virus was carried out in faecal specimens of hospitalized gastroenteritis cases in Infectious Diseases and Beliaghata General Hospital, Kolkata, India. The genomic RNA was extracted and electrophoresed in agarose gel and stained with ethidium bromide to screen for other viral etiological agents other than Group A rotavirus. Some of the viral pathogens associated till date with diarrhea cases were Group B rotavirus, Group C rotavirus, Norovirus, Sapovirus, Astrovirus, Picobirnavirus and Adenovirus. In course of the study a three segmented genome profile of a picotrirnavirus-like viral agent was observed in 122/1045 faecal specimens of different age groups (Fig 37). The clinical symptoms were watery diarrhea in most of the cases and accompanied by vomiting, abdominal pain and fever in some cases (Fig 38). The picotrirnavirus-like viral agent was the only detectable agent in 69/122 cases and showed mixed infection with other pathogens in the remaining positive cases (Fig 39).

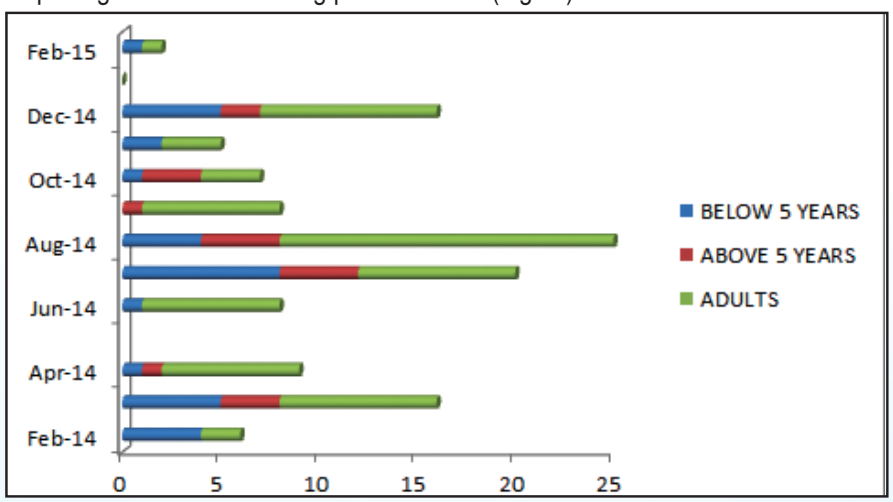


Fig 37. Detection of picotrirnavirus-like genome profile in faecal specimens from hospitalised cases aged below five years, older children above five years and adults,at Infectious Diseases and Beliaghata General Hospital.

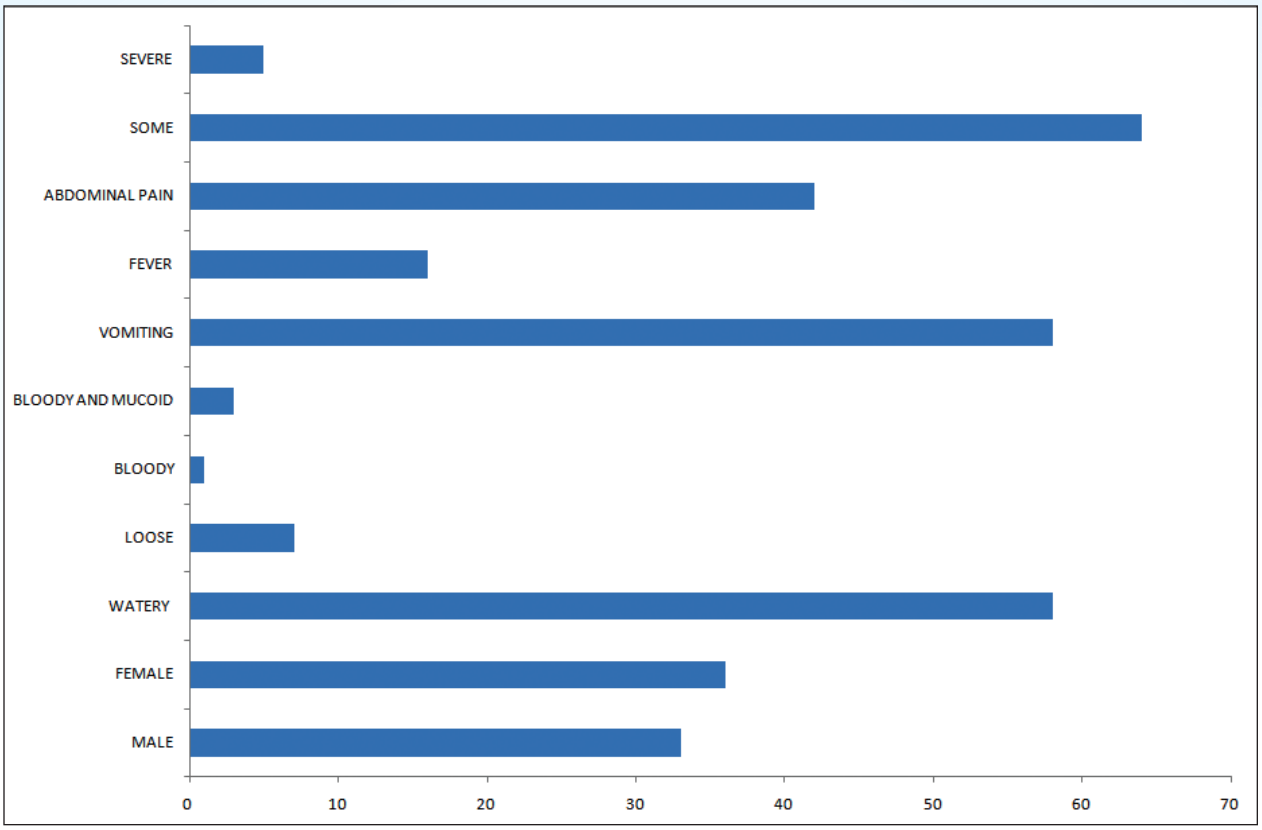


Fig 38. Clinical features of picotrirnavirus associated as sole agent in diarrhoea cases with reference to gender, nature of faeces and clinical symptoms viz vomiting, fever, abdominal pain and extent of dehydration.

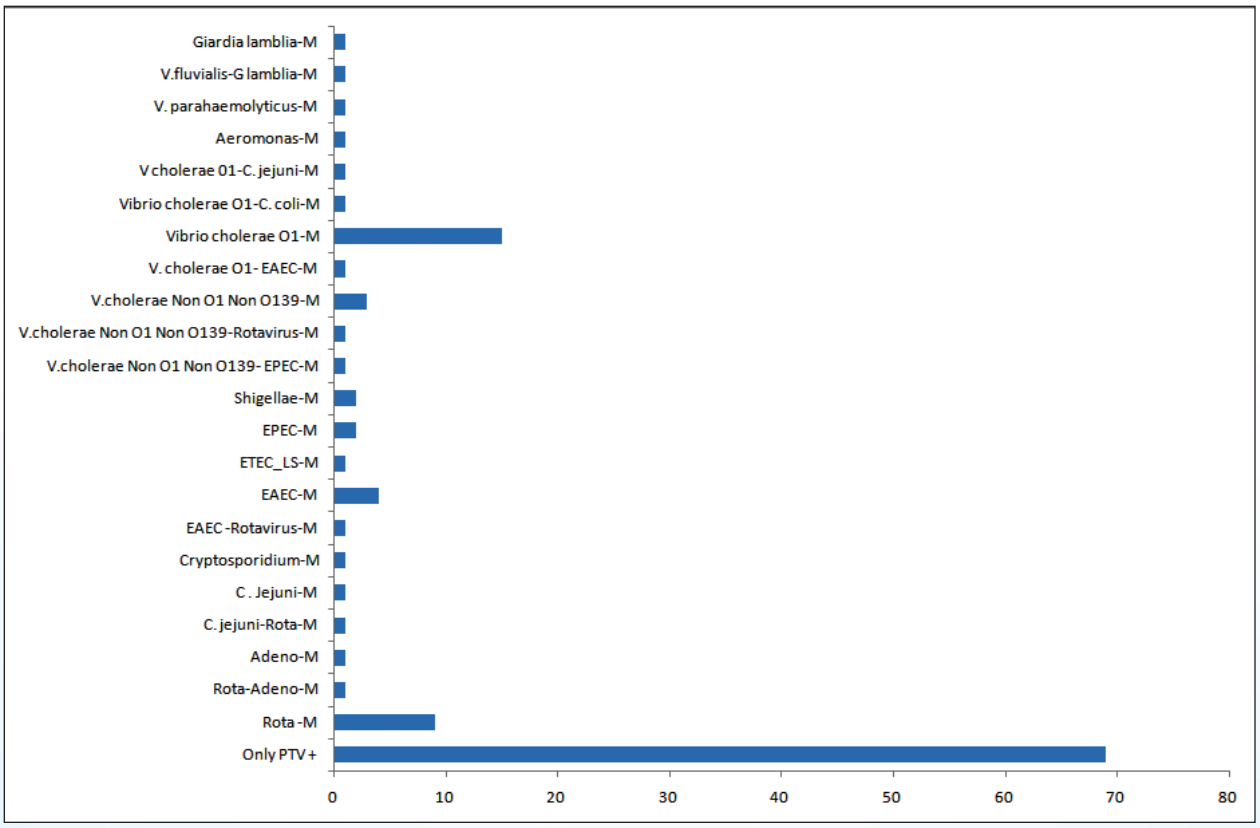


Fig 39. Detection of picotrirnavirus-like agent as sole pathogen and as co-infection with other viral, bacteria and other parasites.

HIV among infants born to HIV infected mothers: Molecular diagnosis using Dried Blood Spot (DBS) samples.

Principal Investigator : M. K. Saha

Mother to child transmission (MTCT) of HIV during pregnancy, delivery and breast feeding is a major challenge for prevention and control of HIV spread. Almost 2000 infants are infected with HIV every day through MTCT in resource poor countries and 370,000 infant infections worldwide each year. Diagnosis of HIV infection becomes difficult in babies less than 18 months due to presence of maternal antibodies. Conventional antibody tests are ineffective as it cannot differentiate maternal antibody and infant–derived antibody. Therefore, nucleic acid test become the exclusive tool for HIV diagnosis among infants born to HIV infected mothers. Molecular diagnosis of HIV 1 infection among new born using dry blood sample has been widely practiced due its several logistics advantages.

From states of West Bengal, Bihar, Mizoram and Chhattisgarh 3564 babies under 6 months age, born to HIV infected mothers and enrolled for molecular diagnosis of HIV were recruited. HIV was detected employing PCR amplification using Roche Amplicor HIV-1 DNA test, version 1.5 (Roche Diagnostics) following manufacturers instruction. Laboratory testing quality was assured by 100% concordance result in proficiency testing using DBS conducted by CDC, Atlanta, USA.

HIV positivity ranged from 5% to 15% among babies from different states. In spite of use of Nevirapine for mother and the newborn to prevent MTCT by reducing HIV viral load, even as high as 15 % babies were found to be HIV infected. Breast feeding increased the risk of HIV acquisition for the infants. These findings rationalizes changing of national program of single dose Nevirapine to lifelong ART for HIV infected mothers to achieve the national ambitious goal of zero HIV transmission from mother to child by 2015 . DBS seems a rational choice for resource poor setting for HIV molecular diagnostics.

Correlates of HIV Risk among Men Who Have Sex with Men in Chattisgarh and in Nagaland, India.

Principal Investigator: M. K. Saha

Dynamics of HIV epidemic are largely understudied among men having sex with men (MSM) in India, while their potentially critical role in HIV spread is evident. MSM, in India are mostly hidden due to stigma and discrimination and are hard to reach population. Rising HIV burden for Chhattisgarh with the distinction of highest HIV prevalence (15 %). among MSM in India warrants special attention to study. HIV epidemic in the high prevalent north-eastern state of Nagaland probably concentrated among MSM with 2nd highest (13.6 %) prevalence in the country. Dearth of information regarding the socio-behavioral correlates of HIV acquisition among MSM thus called for bivariate and multivariate analyses of HIV Sentinel Surveillance data.

In Chhattisgarh, Older age, unemployment and receiving money for sex with a man were associated with higher HIV risk. Among 227 participants most were young (mean age 26 years), school-level educated (52. %), urban residents (99.56%), doing service (46.3%), not involved in heterosexual activities (97.4%) or paid sex (68.7%). No participants reported injecting drug use and almost all (98.7%) were Kothi.

Among 243 MSM in Nagaland, increasing age, middle school or higher education, being Kothi, involved in both insertive and receptive roles and having paid and received money for sex with a man were strongly associated with higher HIV risk . Participant's mean age was 28.3 years, 46.1% were illiterate, 27.2% were unemployed, 57.0% identified them as Kothi, 14.8% were bisexual, and 19.6% exchanged money for sex with men

Some of the observed associations lacked statistical power due to sparse data, therefore, studies with larger MSM population required to find predictors of HIV risk. The epidemic is probably being concentrated among MSM and may

drive the epidemic in the central as well as the north-eastern India where HIV burden is alarmingly high.

Uncertainty of Measurement for ELISA.

Principal Investigator: M. K. Saha

An analytical result with estimation of Uncertainty of Measurement (MU) presents the confidence level of assay. Enzyme-linked immunosorbent assay (ELISA) is a routine test for infectious disease sero-diagnosis. MU for ELISA result is not reported since procedure of MU estimation is hardly available. International Organization for Standardization (ISO) provides the Guide to the Expression of Uncertainty in Measurement (GUM) with set of guidelines. However, no detailed procedures or instructions for evaluating specific measurement processes are described. This study tried to develop a step by step procedure for MU estimation in a serology laboratory by describing the potential sources of uncertainty during each step of ELISA (Fig 40).

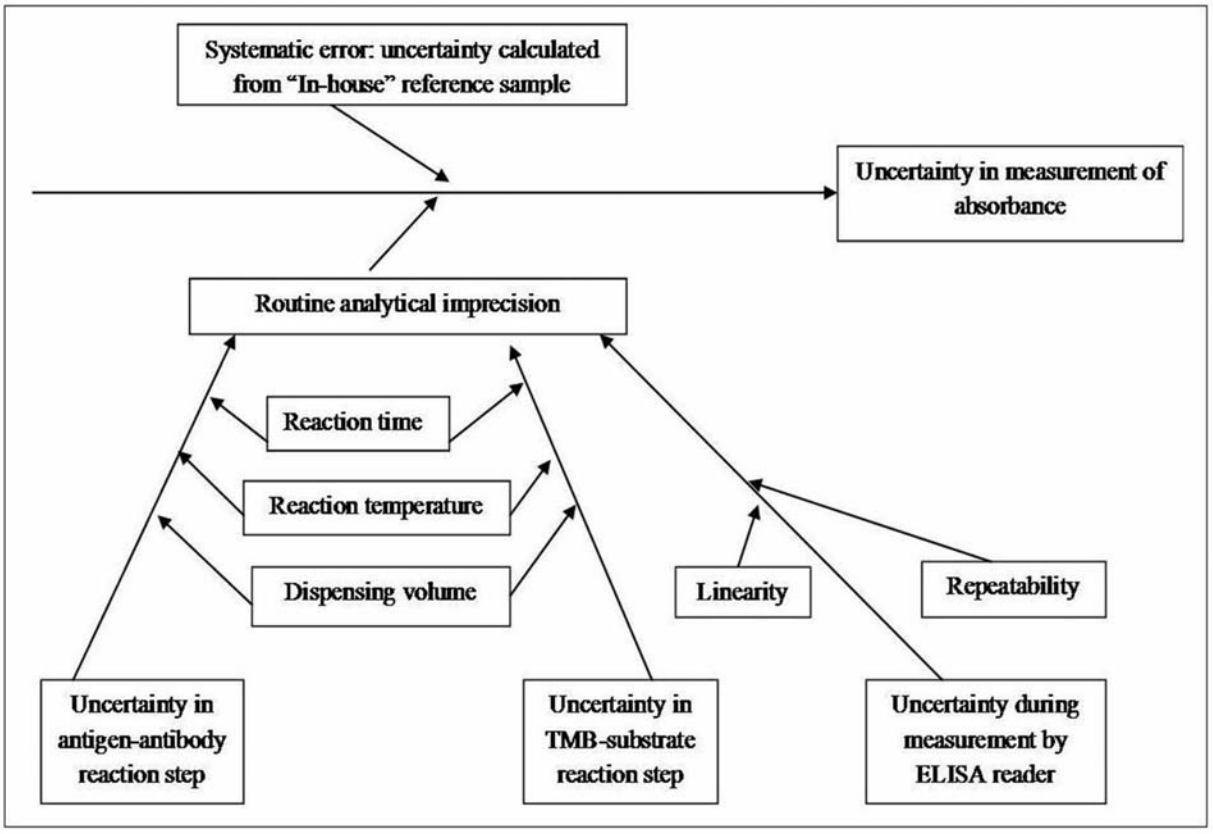


Fig 40. cause and effect

HIV infected blood sample was tested by commercial ELISA following routine procedure. Uncertainty was estimated by specification of measure and, identification of uncertainty sources, quantification of values attributed to the sources of uncertainty and calculation of the combined standard uncertainty following GUM by converting all the standard uncertainties of ELISA into dimensionless relative standard uncertainties.

To calculate the total uncertainty, the uncertainty propagation law was used for which there were only divisions and products. All individual components were converted into relative uncertainties for avoiding mixed units arising out of different sources of uncertainties. Relative standard uncertainty was expressed as relative standard deviation (RSD) which was calculated as standard deviation divided by mean of a set of results. The combined uncertainty of the

mathematical model, which was a product or quotient, could also be considered as the model equation, as following:

$$[u(y(x_1, x_2, \ldots))]^2 = \sum_{i=1}^{n} C_i^2 u(x_i)^2$$

where y(x1,x2,...) is the function of several parameters x1, x2,... and C_i is a sensitivity coefficient for all the cases. Each variable's contribution was measured as the square of the associated uncertainty expressed by the standard deviation multiplied with the square of the relevant sensitivity coefficients. Systematic error and routine analytical imprecision were considered for the calculation of MU, the combined uncertainty is calculated using the model:

$$RSD_{Combined}^{2} = RSD_{Systematic\ error}^{2} + RSD_{Routine\ analytical\ imprecision}^{2}$$

The combined standard uncertainty u_C was calculated using the propagation principle

$$\frac{u_C(OD)}{OD} = \sqrt{RSD_{Systematic\ error}^{2} + RSD_{Routine\ analytical\ imprecision}^{2}}$$

Systematic error and routine analytical imprecision are considered the sources of uncertainty contributing to the total MU of this routine qualitative diagnostic method and the relative expanded uncertainty U_{rel} is 24% with 95% confidence level (k=2) (Fig 41)

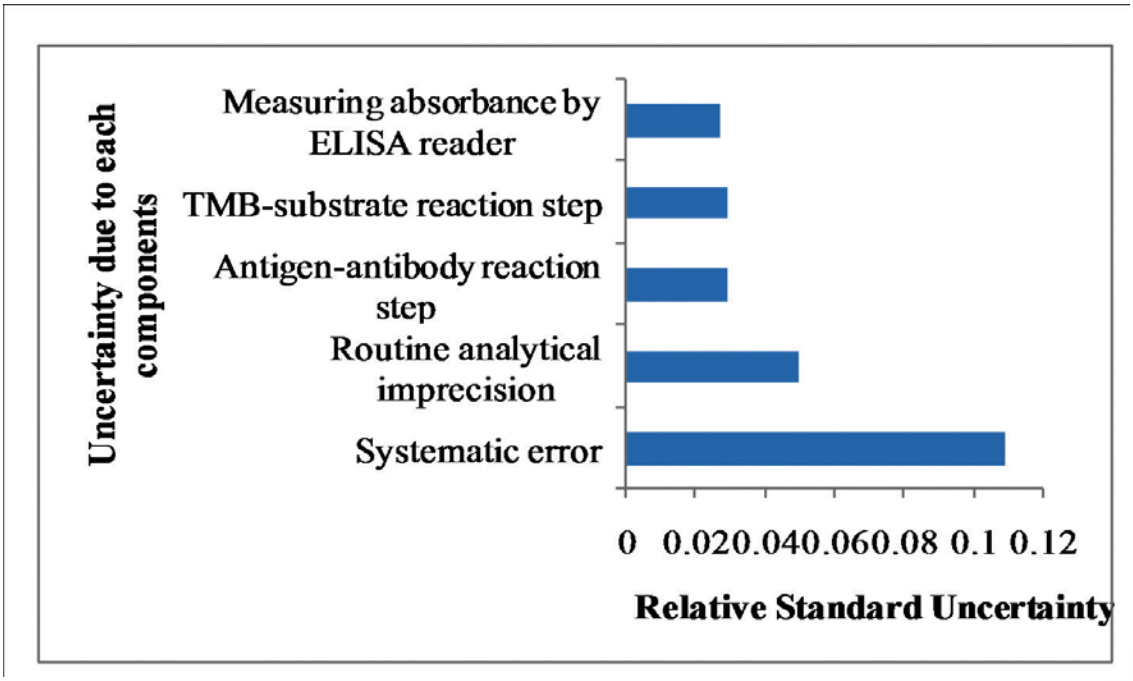


Fig 41. Uncertainty contributions

Quantification of uncertainty by combining systematic error and analytical imprecision seems a feasible method of MU for ELISA. The estimated MU reflects the extent of variation for the cut-off absorbance for equivocal outcome of the qualitative interpretation.

Structure-toxicity relationship of chemically modified chitosan as a candidate oral protein drug delivery carrier.

Principal Investigator: M. K. Saha

Chitosan (CS), a partial de-acetylation product of natural polymer chitin, available in different size (Mw) and degree of de-acetylation (DD) has gained increasing attention in the field of drug delivery system due to its favourable biological

properties such as non-toxicity, biodegradability, mucoadhesive properties and biocompatibility. However, solubility of CS only restricted in acidic solution limits its applications to bioactive agents such as gene delivery carriers, peptide carriers and drug carriers (Fig 42).

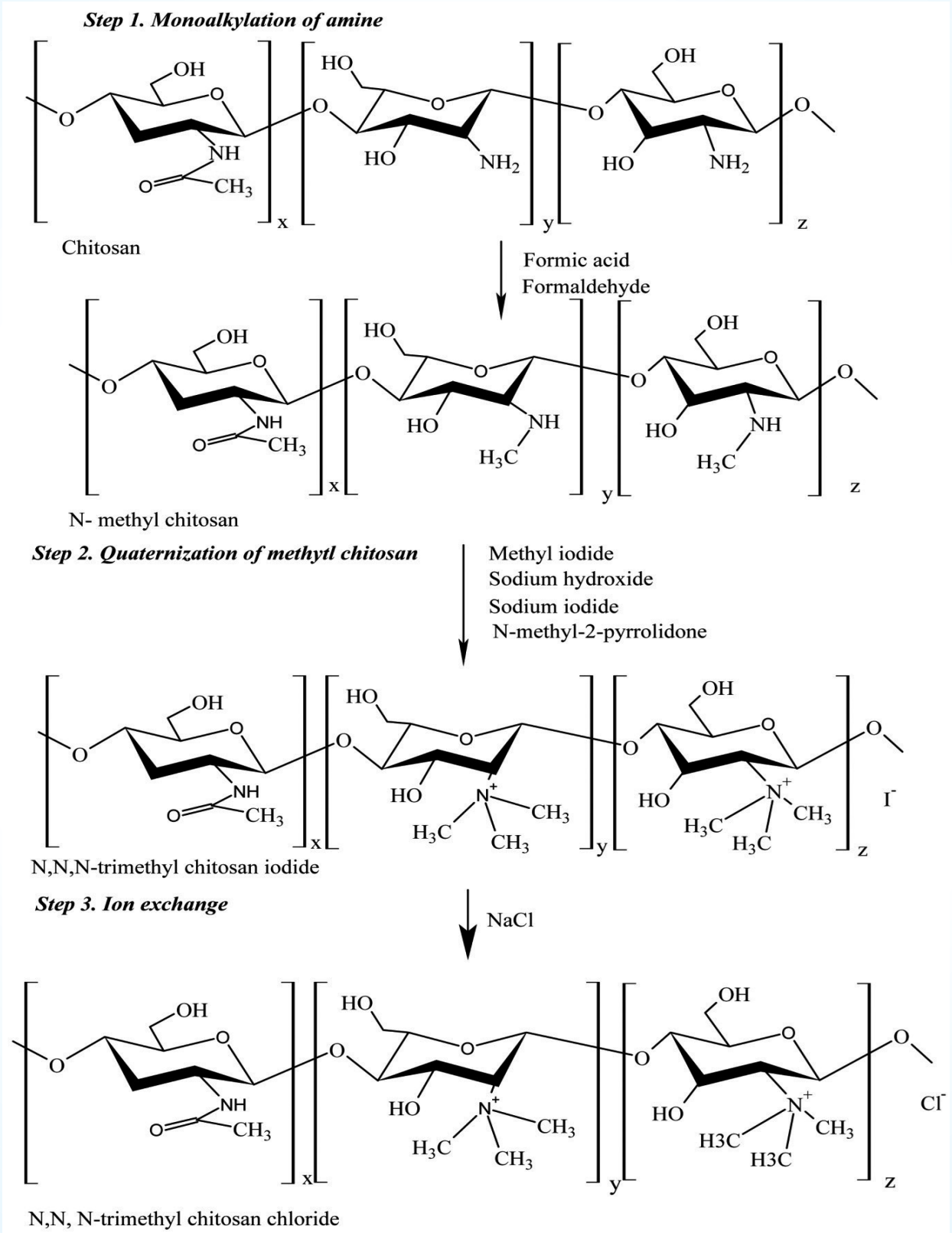


Fig 42. chemical structure & modifications

CS is a natural linear polysaccharide consisting of β1>4-D-glucosamine and β1>4-N-acetyl D -glucosamine units. Partial quaternization of CS's primary amine groups has been used to obtain a CS derivative that is soluble at physiological conditions. N,N,N-Trimethylated chitosan (CS-TM) have muco-adhesive properties and penetration enhancing properties making it a suitable carrier for delivery of hydrophilic peptide and protein macromolecules. This enhancing capacity is due to opening the tight junctions between adjacent epithelial cells through interactions between the protonated (positively charged) amino groups on the C-2 position and the negatively charged sites on the cell membrane and/or in the tight junctions. N,N,N-Trimethylated chitosan with varying degree of quaternization (DQ) was evaluated employing MTT and LDH assay for cytotoxicity, erythrocyte aggregation and haemolysis for hematotoxicity and histopathology of liver, kidney and spleen for sub-acute toxicity. Higher concentration of chitosan with 22% DQ and native chitosan resulting insignificant abnormalities among experimental group of mice, but chitosan with higher DQ as 50% and 61% may lead to concentration dependent cytotoxicity, hematotoxicity and increased renal and hepatotoxicity (Fig 43).

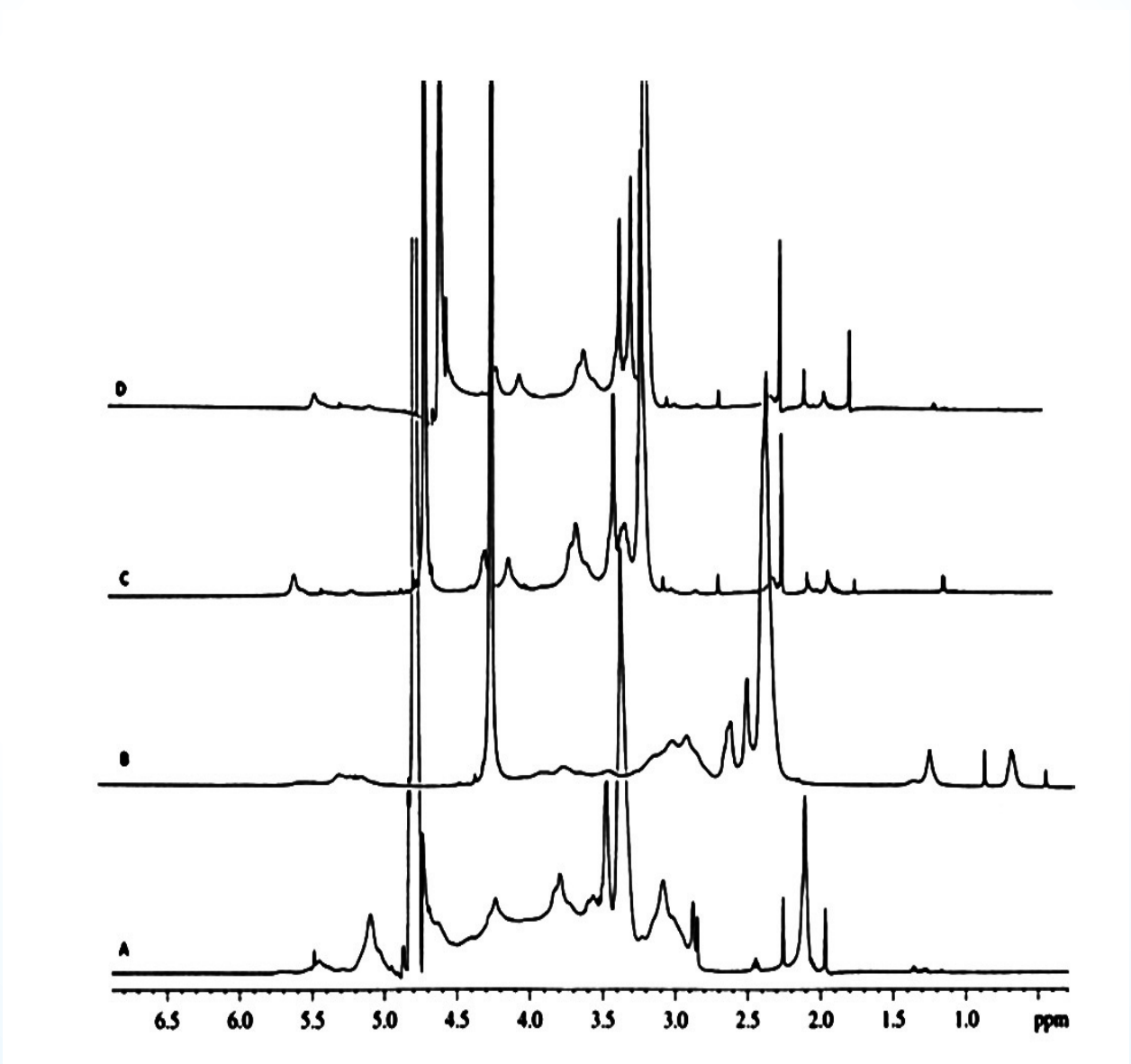


Fig 43. NMR

In vitro cytotoxicity and hematoxicity and in vivo toxicity results were correlated. A linear relationship was observed

between cationic charge density and toxicity. Degree of toxicity was not affected significantly due to difference of Mw of CS (Fig 44)

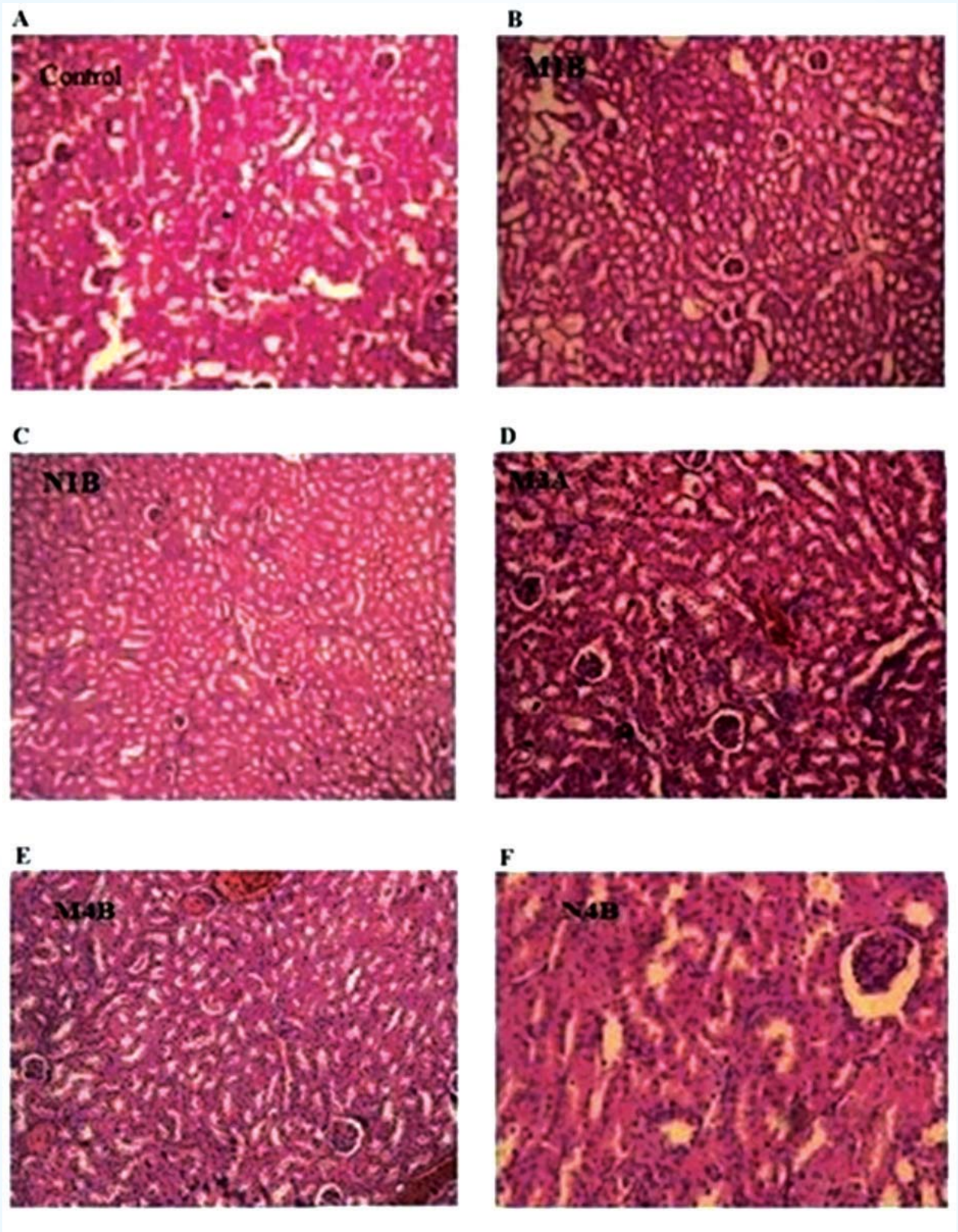


Fig 44. Kidney histogram

Development of alginate coated low molecular weight chitosan nanoparticles as new carriers for oral vaccine delivery.

Principal Investigator: M. K. Saha

Oral vaccine delivery systems offer lower cost, ease of administration, higher patient compliance, reducing the need for trained personnel, averting vaccine-related infections correlated to the disposal and reuse of needles in systemic delivery as well as higher capacity for mass immunizations.

Chitosan (CS) nanoparticles as oral vaccination delivery carriers are beneficial, but their limited ability to control the release of encapsulated antigens and easy solubility in acidic medium is an obstacle. Coating antigen loaded particles with an acid-resistant polymer like sodium alginate helps to overcome the obstacles. CS nanoparticles are constructed by ionic gelation process linking sodium tri-polyphosphate as a negatively charged molecule and CS by mixing of two aqueous solutions at ambient temperature while stirring without using sonication or organic sol-vents. Proteins with low isoelectric point, such as BSA and measles antigen, were better associated with the nanoparticles when dissolved in the alkaline sodium TPP solution. (Fig-45)

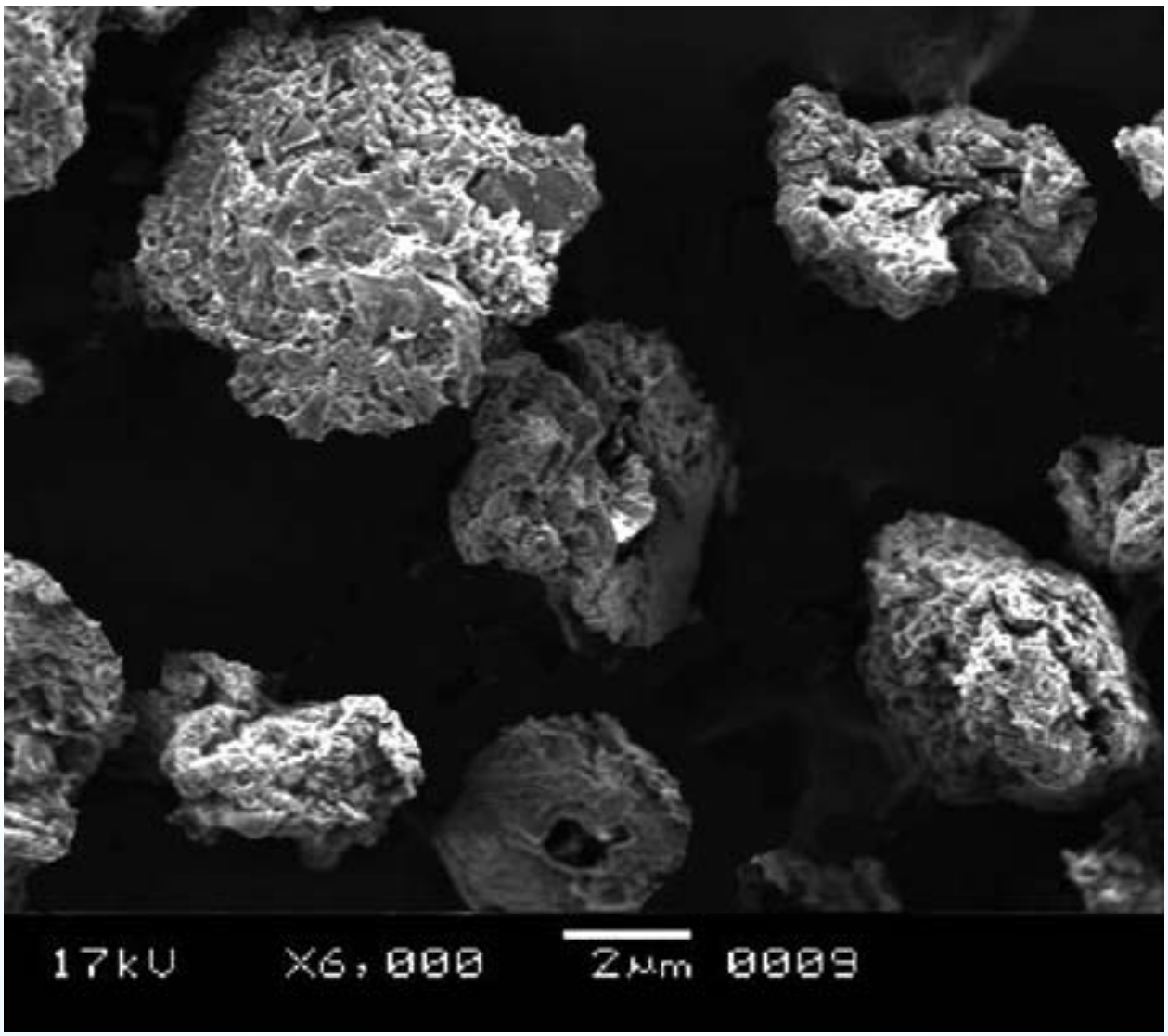


Fig 45. Electron Micrograph

Measles antigen entrapped in low MW CS nanoparticles coated with sodium alginate was formulated. The size and

surface properties of the nanoparticle were manipulated with different MW of CS. In vitro release studies showed initial burst release followed by extended release, best fitted in the Makoid–Banakar model (R2> 0.98). SDS-PAGE assay revealed that alginate coating could effectively protect antigen in acidic condition for at least 2 h. Cell viability was assessed using MTT assay into HT 29 cell line. Formulations were orally administered to mice and immunological responses were evaluated using ELISA method. Obtained results showed that measles antigen-loaded CS nanoparticles induced strong immune response and significant correlation was observed between the immune response with CS MW. Protecting ability of antigen in gastric environment, sustained release kinetics, systemic and mucosal immune responses and low cytotoxicity observed for the alginate coated nanoparticles demonstrated that LMW CS could be promising platform for oral vaccine delivery (Fig 46).

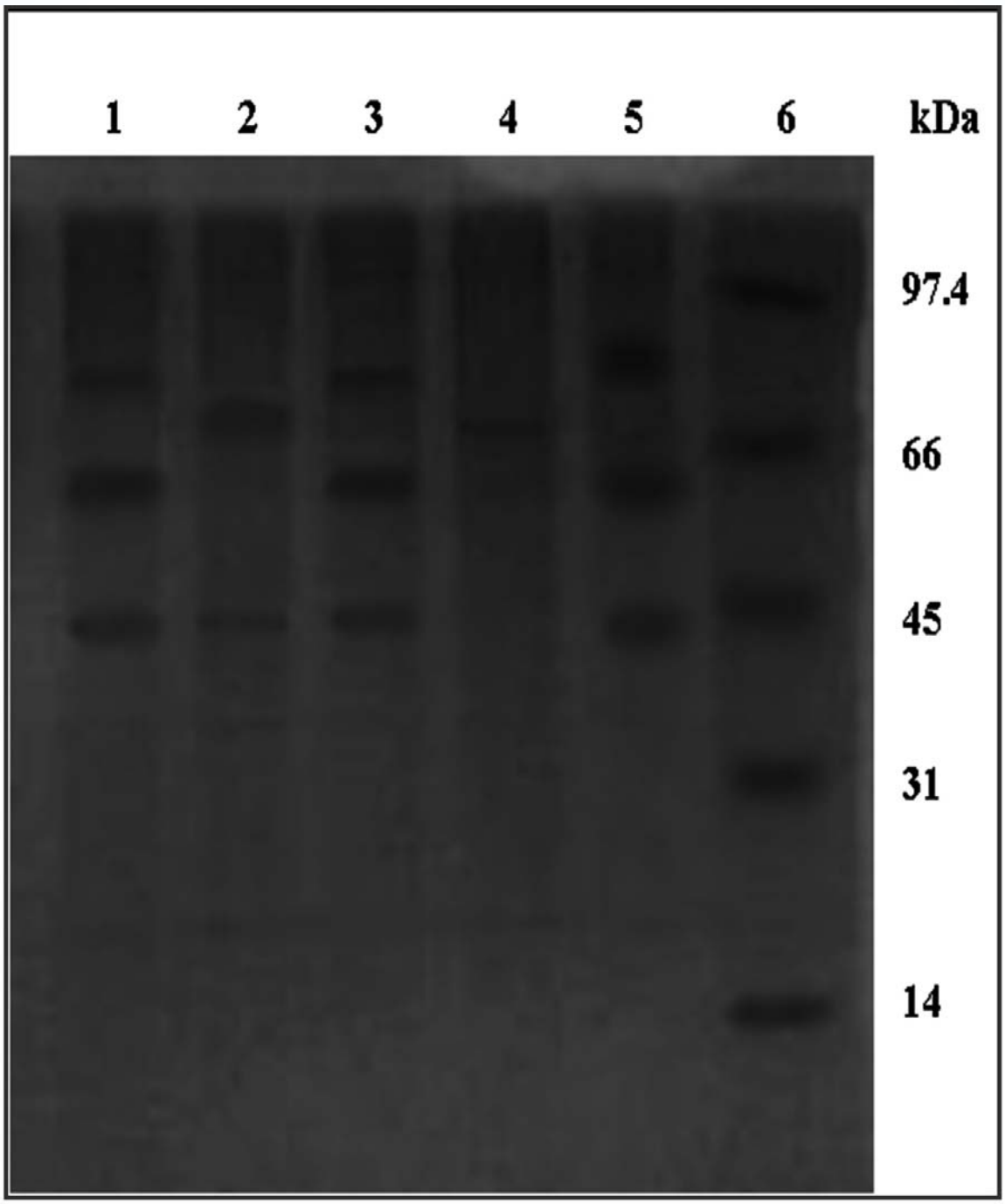


Fig 46. Gel electrophoresis data

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

Principal Investigator (PI): M. Chawla Sarkar

Co-PI s: T. Ramamurthy, M. K. Bhattacharya, K. Rajendran

A total number of 914 stool samples from hospitalized diarrhoeal patients (<5 yrs old) from Medinipur and Kolkata were screened for rotavirus during April 2014 to Feb 2015. The stool samples were screened for rotavirus using Rota-ELISA kit detecting the VP6 antigen. Among 914 total samples, 487 samples were detected as rotavirus positive (53%). 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. G1P[8] was the most common type during this period. Unusual zoonotic strains were detected at low frequency (<2%).

Analysis of rotaviruses and their interactions with the host: NSP1 attenuates MAVS protein to Antagonize RIG-I/MAVS Signaling Pathway

PI: M. Chawla Sarkar

In response to viral infection, the cytosolic RIG-I like RNA helicases interact with viral RNA to activate the Mitochondrial Antiviral Signaling protein. This adaptor protein, also known as Cardiff/VISA/IPS-1, is the key regulator of cellular interferon and apoptosis pathway due its capability to activate IRF3 and NFkB. Rotaviral non-structural protein 1, a 55 KD protein is involved in antagonizing the host interferon response by down regulation of IRF1, IRF3 and IRF7. Structurally NSP1 contains one cysteine-rich region near its amino terminus which forms one or two zinc fingers, involved in virus function whereas the carboxyl half of the protein which has a RING-E3 ubiquitin ligase domain has a pathogenic role In this study we observed that NSP1 is competent in down regulating MAVS protein resulting in a total shutdown of RIG-I/MDA-5-MAVS signalling pathway in a strain independent manner.

To analyze role of MAVS *in vitro* cell culture and virus infection, overexpression of proteins, quantitative real-time RT-PCR, gel electrophoresis, immunoblot analyses, transient transfections and reporter gene assays. For identifying protein-protein interaction and protein aggregation, immunoprecipitation and SDD-AGE were used respectively. RV protein NSP1 down regulates MAVS protein proteosomally during RV infection when host PRR mediated IFN-â activation is critical. Importantly it was found that the degradation was RV strain independent in nature unlike IRF3 degradation. NSP1 overexpression in absence of other viral protein can also degrade MAVS protein. Mutagenesis studies suggested that CARD domain and TM domain of MAVS are sufficient to interact with NSP1 and inhibit IFN pathway, however, full length NSP1 is required for degradation of MAVS. In addition to MAVS degradation, NSP1 also inhibited detergent resistant MAVS aggregates which can activate IRF3 in cytosol.

During RV infection there is a potential redundancy in the functions of RIG-I and MDA-5, therefore abrogating MAVS results in a complete shutdown of the host RNA sensory machinery and thus, inhibiting IFN-I induction (Fig 47). Over all study highlights the multistep control of host innate immunity by a viral protein.

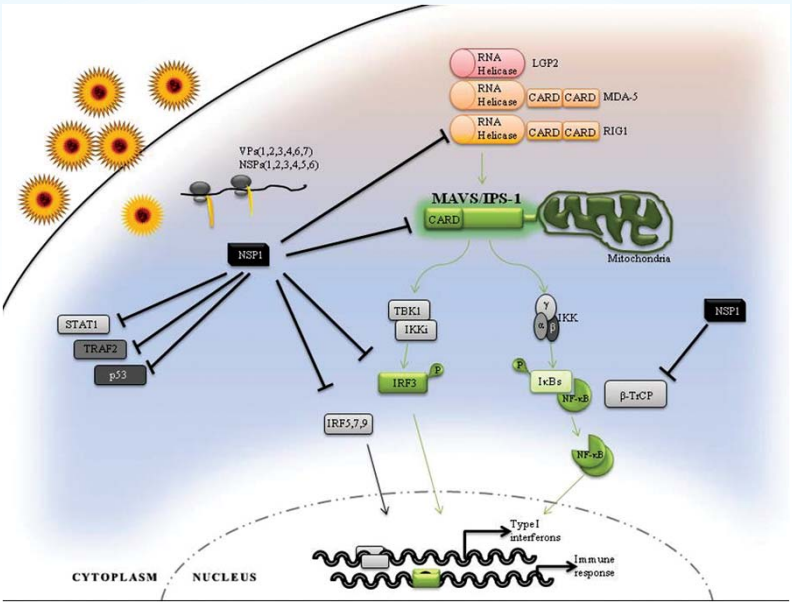


Fig 47. Mechanistic model for rotavirus NSP1-mediated attenuation of cellular proteins for improved infection. The model shows the critical role of MAVS in RLR mediated activation of type I IFN and immune response. By degrading MAVS, NSP1 can directly inhibit IRF3 and NF-κBsignaling. Other cellular proteins like IRFs and β-TrCP are targeted selectively but MAVS degradation was observed in all RV strains with functional NSP1.

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

PI: M. Chawla Sarkar

Co-PI: S. Kanungo

Nasal or throat swabs are collected from symptomatic patients (fever >37.5, running nose, cough/sore throat, body ache etc) from Three hospitals in Kolkata (BC Roy childrens hospital, NRS hospital and National Medical College) after obtaining informed consent form from the guardian/parent. Initially samples were screened by real time PCR for Influenza A and B. Positive samples were inoculated in MDCK cell line for virus propagation. A total of 678 samples were screened during regular surveillance of which 51 (7.5%) were positive for Inf A/B. Of 51 samples 30 were typed as Inf A and 21 as Inf 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.

A total of 1007 samples from admitted cases belonging to category Bii or C were tested from Jan 2015 onwards. Of which 296 (30%) were positive for pandemic H1N1 in West Bengal of which 20 patients died due to underlying complications. In addition, the scientist evaluated microbiology laboratories of private hospitals for their capacity to run InfA/H1N1 tests and submitted reports to the state health department for necessary approvals.

Awards/ Honours Received

T. Krishnan

- Editorial Board member of World Journal of Clinical Infectious Diseases (WJCID)
- Editorial Board member of ISRN Microbiology
- Editorial board member of OA Publishing London for OA Infectious Diseases.
- Editorial Board Member [Senior Editor] of Research Journal of Infectious

- Editorial Board Member of International Research Journal of Bacteriology [HOAJ]
- Selected as member for Asia Pacific Journal of Tropical Disease expert database
- Member of the editorial board of Journal of Immunology and Vaccine Technology.
- Editorial Board member of Epidemiology Reports by Herbert Open Access Journals.
- Editorial board member/peer reviewer of American Journal of Life Sciences, SciencePG.
- Invited to deliver the Prof. A. K. Chandra Memorial lecture titled Insights into the genetic diversity of novel gastroenteritis viruses in Kolkata, India on 4 April 2014 during the one day National Conference on "Current Research in Microbiology" under the auspices of ***Centenary Celebration of*** Department of Botany, University of Calcutta in collaboration with Prof. A. K. Chandra Memorial Committee and Prof. R. P. Purkayastha Memorial Committee.
- Resource person in Division of Virology, NICED for Laboratory Exposure to Final Year BHMS Students on 24 April 2014.
- Invited as External Reviewer for evaluation of Research Proposal from Research Grants Council (RGC) of Hong Kong in May 2014.
- Invited to participate and propose a Frontiers Research Topic in Section of Epidemiology of the journal Frontiers in Public Health, an open access publishing partner of Nature Publishing Group in 2014.
- Awarded FULL FINANCIAL SUPPORT by Okayama University to attend and present our research work at XVI International Congress of Virology held simultaneously with XIV International Congress of Bacteriology and XV International Congress of Mycology at Palace de Congress Convention Center in Montreal Canada during27 July – 1 August 2014.
- Invited as external examiner to conduct the Ph.D viva voce examination for a student of Central Forensic Science Laboratory, Directorate of Forensic Science Services, Ministry of Home Affairs, Govt. of India, held on 26 September 2014 in Dept. of Life Science & Biotechnology, Jadavpur University.
- Assigned as Reviewer of Insight Virology and Insight Infectious Diseases since October 2014.
- Invited to participate as Resource person in Seminar held on 20 December 2014 in Department of Botany, Scottish Church College, Kolkata.
- Invited to participate in the Obaid Siddiqi Memorial Oration Lecture 2015 delivered by Prof C N R Rao titled "Celebration of Science" on 7 January 2015 at College of Medicine & Jawaharlal Nehru Memorial Hospital, organized by National Institute of Biomedical Genomics, Kalyani.
- Invited to deliver a NICED PhD Course work lecture titled 'Virology' on 13 January 2015.
- Invited to deliver a lecture at the 2nd International Conference on Frontiers in Biological Sciences [InCoFIBS], held at National Institute of Technology, Rourkela from 22 – 24 January 2015.
- Invited as Resource person for the Clinical Development Services Agency workshop on Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness) held at B M Birla Heart Centre from 26 – 27 February 2015.

M. K. Saha

- Expert Member for National Program in other organization:
  - Principal Member, Sectional Committee Immuno-Biological Diagnostic Kits, Bureau of Indian Standards. New Delhi, Govt of India since 2010.
  - Member, Expert Committee, Strengthening of Quality Control Testing Procedure of Immuno Diagnostic

Kit Laboratory (IKDL), National Institute of Biologicals, New Delhi, Govt of India, since 2014.

- Special Invitee in the Technical Resource Group on HIV Surveillance and Estimation, NACO, New Delhi, Govt of India, since 2012
- West Bengal SACS has nominated Dr. M. K. Saha as Technical Expert for HIV Rapid Test Kit, since 10-02-2015.

- Accreditation and Excellence:
  - Untiring efforts of Dr. M K Saha with support for Institute management NICED lab achieved the ISO-15189: 2012 standard and through rigorous process of assessment by the Assessors, the Lab was accredited by NABL for the QUALITY & COMPLTENCE.
  - On the journey of excellence in performance, the HIV Molecular lab has been receiving appreciation from different National and International Organizations.
  - Division of Global HIV/AIDS, CDC, USA, recognized NICED lab for outstanding performance for the Qualitative HIV 1 DNA Testing Using Dry Blood Sample (DBS) Consecutively for 3 years (2012, 2013 & 2014)
  - All the labs involved in National AIDS Control Program received 100% scoring in proficiency testing conducted by outside agencies.
- National Guidelines and Technical Standard Development.
  - National Guidelines on Quality System for HIV Testing Laboratory for National AIDS Control Organization (NACO)
  - National Standards for Immuno-Biological Diagnostic Kits for Bureau of Indian Standards (BIS).

Conferences/ Seminars/ Workshops/Trainings Attended/ Organised

T. Krishnan

- Triveni Krishnan. Insights into the genetic diversity of novel gastroenteritis viruses in Kolkata, India. Presented at the one day National Conference on "Current Research in Microbiology" on 4th April, 2014 during the *Centenary Celebration of* Department of Botany, University of Calcutta in collaboration with Prof. A. K. Chandra Memorial Committee and Prof. R. P. Purkayastha Memorial Committee.
- Triveni Krishnan and SumioShinoda.Emerging viruses associated with gastroenteritis cases in Kolkata, India. Poster presentation at the XVI International Congress of Virology.[ICV], held at Palace de Congress Convention Center in Montreal, Canada between 27 July to 1 August 2014. VIR-PT2002 in Page 181 of Programme book on IUMS 2014 [XVI ICV].
- Triveni Krishnan. Viral gastroenteritis and approaches to give protection with available vaccines. Presented at the 2nd International Conference on Frontiers in Biological Sciences, held at National Institute of Technology Rourkela from 22 -24 January 2015
- Triveni Krishnan. History of evolution of ethical guidelines and clinical research regulations. Presented at Clinical Development Services Agency workshop on Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness) held at B M Birla Heart Centre from 26 – 27 February 2015

M. K. Saha

- Participated, as a panelist, in the "NACO-National Meet on Strengthening HIV Laboratories in India: Journey of Three Decades" at New Delhi during 4-5 December, 2014.
- Regional Training of Trainers for IBBS was organized by RI and held at NICED during 31 March-06 April

2014. SST members, SACS representatives, regional and state FRA team, MAHITI, NACO, PMU, consultant trainers for IBBS, RI team participated the training.

- RI Project Management Training was held at Delhi during 18-19 April 2014. RI team members attended the training.
- Three batches field level training were conducted by GFK MODE for West Bengal & Sikkim was held from 24 April-7 May, 14 -27 May at Seva Kendra, Kolkata and 26 May-8 June at ICMARD, Kolkata. RI team members and SST members attended as resource person.
- Field Training for Nagaland was held at Kohima during 19 May-1 June 2014. RI team members and SST members attended as resource person.
- Field Training for Assam & Meghalaya was organized in two batches on 28 April-11 May 2014 and 19 May-1 June 2014 at Guwahati. RI team members and SST members attended as resource person.
- Training on Testing DBS samples under IBBS 2013-14 was held during 3-4 June 2014 at National AIDS Research Institute, Pune. NRL In-Charge and Technical Officer Participated from NICED.
- EQAS Workshop and NABL Accreditation for SRLs was held during 30-31 October, 2014 at NICED, Kolkata. Participants were Lab In-charges and Technical Officers from State Reference Laboratories of Assam, A&N Islands, Meghalaya, Jharkhand and Odisha.
- National Pre Surveillance Meeting was held on 20-21 November 2014 at NIHFW, New Delhi. RI Focal person, Project Coordinator & Research Officer attended the meeting.
- Regional Training of Trainers for eastern region was organized by RI and held at NICED during 24-25 November 2014. National HSS Program Officer, RI officers, SST members and SACS personnel participated in the training.
- Panel Sera Distribution Workshop was held at National AIDS Research Institute, Pune on November 24th, 2014. Technical Officer from NICED attended the workshop.
- Refresher TOT at National level for Migrant typology held in New Delhi on 26-27 November 2014. Research officer from RI attended the training.
- Refresher field level training for migrant typology held at Bhubneshwar during 18-22 December 2014. Research officer from RI attended the training as resource person.
- State level training of site personnel of Meghalaya was organized in two batches. One during 16-17 December 2014 and the other during 19-20 December 2014 at Shillong. RI team member and SST members attended the training program as resource person.
- State level training of site personnel of West Bengal was organized in two batches. One in Kolkata on 16-17 December 2014 and one in Siliguri on 22-23 December 2014. RI team members along with SST members attended both the training programs as resource person.
- State level training for Andaman & Nicobar Islands was organized in two batches 23-24 December and 25-26 December 2014 at Port Blair. SST member attended both the training program as resource person.
- State level training of site personnel of Chhattisgarh was held on 29-30 December 2014 at Raipur. RI team attended the training program as resource person.
- Site personnel training for Nagaland was held during 6-9 January 2015. RI project coordinator and SST members from Nagaland attended the training as resource person.
- State level training of site personnel of Sikkim was held on 12-13 January 2015 at Gangtok. SST members

attended the training program as resource person.

- NACO-National Meet on Strengthening HIV Laboratories in India was held at National AIDS Control Organization (NACO), New Delhi during 4-5 December, 2014. NRL In-Charge and Technical Officer Participated from NICED.
- GCLP, NABL Accreditation and EQAS workshop for SRLs was held during 12-13 March, 2015 at NICED, Kolkata. Technical Officers and Lab. Technicians from State Reference Laboratories of Assam, A& N Islands, Meghalaya, Jharkhand, Odisha participated in the workshop.
- Workshop on Early Infant Diagnosis was held at National AIDS Control Organization (NACO), New Delhi on 16 March 2015. In Charge of EID Lab & Technical Officer from NICED attended the workshop.
- Workshop on Tool Kit for Technical Assistance (TA) providers for the HIV testing Reference Laboratory Network was held on 17-18 March, 2015 at National AIDS Control Organization (NACO), New Delhi. NRL In-Charge and Technical Officer from NICED attended the workshop.
- Training Program on Medical Laboratories Quality Management System and Internal Audit as per IS/ISO 15189 BUREAU OF INDIAN STANDARDS (BIS) was held at Bureau of Indian Standard(BIS), Eastern Regional Office, Kolkata during 17-20 March, 2015. Research Officer and Technical Officer from NICED attended the training Program.

M. Chawla Sarkar

- Eleventh International Rotvirus Symposium 3-5 Sept 2014, New Delhi. Poster: "Hospital Based surveillance and genetic characterization of rotavirus strains in children (< 5years) with acute gastroenteritis in Kolkata revealed resurgence of G9 and G2 genotypes during 2011-2013". S Mullick, P Mandal, MK Nayak, K. Rajendran, MK Bhattacharya, T Ramamurthy, M Chawla Sarkar.
- Eleventh International Rotavirus Symposium 3-5 Sept 2014, New Delhi. Poster: "NSP1 attenuates MAVs proteins to Antagonize RIG1/MAVS Signaling Pathway" S Nandi, S. Chanda and M. Chawla Sarkar.
- International workshop on Epidemiology and Control of Influenza, VPCI and APAC, Delhi during 7-8 Nov 2014. Oral presentation: "Surveillance and genetic Analysis of Influenza virus strains in eastern India".
- 17th International Conference on Emerging Infectious Diseases (EID) meeting at Taipei, Taiwan 25-29 Jan 2015. Oral Presentation: "Calmodulin Positively Regulates Rotavirus Infection by Modulating Host Cell Cycle Progression".
- 17th International Conference on Emerging Infectious Diseases (EID) meeting at Taipei, Taiwan 25-29 Jan 2015. Oral Presentation. "Rotavirus modulates activity of antiviral protein Viperin to combat host induced IFN responses during infection". Satabdi Nandi, Shampa Chanda and Mamta Chawla-Sarkar.
- Participated in the Advanced Training for "Sequence Characterization of Untypable Rotavirus Strains" during 7-11 April 2014 at CDC, Atlanta, USA being deputed by ICMR.
- Organised a workshop and Training on "Surveillance and Laboratory Techniques for Indian National Rotavirus Surveillance Network:Eastern Region" during 28-30 May 2014 at NICED Kolkata

SERVICES

S. Dutta

- Confirmed the identification and serotyping of Salmonella & Shigella isolates received at NICED from various Medical colleges and other academic institutions of India. Timely feedbacks were sent to the concerned organizations.
- Delivered talk and imparted training to Homeopathy students.

A. Palit

- During the epidemic outbreaks (2014-15) 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 of the undersigned.
- Water samples had been received from different PHCs of N. 24 Pargana, Nadia, Howrah and Hooghly 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, 29 samples had been received from various sources of which 20 had been found to be positive for faecal coliforms and 4 for presence of *V.cholerae* O1 (Table 7).

Sl No.	District	No. of samples received	Source					Culture Positive	PCR positive
			Tap	Tube well	Drinking water	Pond	Others		
1.	Howrah	4	4	-	_	-	-	3	2
2.	North 24 Parganas	9	4	4	-	-	1	-	-
3.	Nadia	7	-	3	1	-	3	2	1
4.	Hooghly	9	-	2	-	1	6	4	1
	Total	29	8	9	1	1	10	9	4

B. L. Sarkar

- Phage typing study initiated at NICED since its inception and today, this study is one of the mandates recognized by WHO. NICED operates as a WHO collaborating centre for diarrheal diseases research and training. The strains of V. cholerae isolated from clinical, environmental and outbreak sources from different endemic regions of the country are sent for confirmation, biotyping, serotyping and phage typing. The results of these strains are dispatched to respective counterparts from time to time.

M. K. Bhattacharya

- Associated with the setting up of the Diarrhoeal Treatment & Training Unit (DTU) at the Infectious Diseases Hospital, Kolkata and we are running the outdoor of Diarrhoea treatment unit at B. C. Roy Children

Hospital.The objectives of the DTU are as follows:

a) To treat the children and infants with ORS suffering from some dehydrating diarrhoea.

b) To educate the mother how to treat a diarrhoeal child with ORS or other home available fluid at home to prevent dehydration and also health care.

c) To educate the community about the use of ORS in diarrhoea and impact of health care through the mother's who are getting education at the DTU.

d) To increase the awareness for using ORS in the community through mother's who are getting education at the DTU.

- Started surveillance programme for Diarrhoeal Diseases at the Infectious Diseases Hospital, Kolkata, for the first time in West Bengal with the following objectives:

a) To monitor changes in disease patterns including drug sensitivity (particularly for cholera and shigellosis).

b) To create database on diarrhoeal diseases and to generate preliminary information for researchers to design new research protocols.

c) To develop an early warning system for forecasting an epidemic.

d) To furnish information to be applied for improvement in patient care and better preventive measures.

- Help the Health Department, Govt. of India and Govt. of West Bengal supplying the report as suggested by the institutional head.
- Actively involved in routine teaching of internees from different medical colleges of Kolkata at the DTU ward of I.D. Hospital.
- Responsible for investigation of any outbreak/epidemic occurs due to diarrhoeal illness or any other illness.

K. Sarkar

- Informed Govt. of West Bengal about the problem of arsenic contaminated drinking water at various block primary schools of Malda. Also informed about the weakness of health care delivery services at Malda as a backward district & its utilisation by people.
- Provided the Department of School Education, Govt. of West Bengal, information on malnutrition & anaemia among school students of its 20 educational districts. The detail of this study was presented before Chief-Secretary, Govt. of West Bengal, at his conference room at `Nabanna' on 18 February 2015. The chief secretary instructed the authorities of School Education Dept. to follow the suggestions given by NICED.

S. Panda

- Children experiencing psychological distress and having other care needs due to HIV related issues were addressed by networking with the Child Psychiatrist located at Tata Medical Centre, Kolkata. Collaboration was also established with community based organizations in order to assist psychosocial development of children living with or affected by HIV.

M. K. Saha

HIV Testing Quality Assurance:

NICED National HIV Reference Lab has been implementing HIV testing Quality Assurance program for the State Reference Labs of A&N Islands, Assam, Jharkhand, Meghalaya and Odisha since 2002 under the External Quality Assurance Scheme of National Reference Laboratory funded by National AIDS control Organization (NACO) , Government of India.

Figure 1: EQAS training: Discussion on Lab Quality Issues

Figure 2: HIV Western Blot

Conducting Proficiency Testing by Panel Sera preparation for State Ref Labs.

- Testing laboratory for HIV Sentinel Surveillance (ANC) 2014. Quality Assurance for HIV Sentinel Surveillance Lab result (Retesting of all positive and 5% negative)
- Referral service for confirmation of HIV testing results of SRLs and other organizations.
- Training for Medical Officers, Lab/Program Supervisors and Medical Lab Technologists for HIV testing as and when requested by different organizations.
- Testing Dry Blood Spot (DBS) for Integrated Biological Behavioral Surveillance (IBBS)
- Verifying results for all samples referred by several organizations. The samples tested, result communicated within the turnaround time 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.

Table 1: 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	40	37
2.	Woodlands Multispeciality Hospital Limited	6	5

Table 2: Integrated Biological and Behavioral Surveillance (IBBS) 2014-2015: Testing Center data, NACO-NRL, NICED, Kolkata

State	No. of Sample received	No. of Sample rejected	No. of Sample tested
Assam	2827	00	2827
Chhattisgarh	3894	19	3875
Nagaland	2008	03	2005
TOTAL SAMPLE TESTED = 8707			

Table 3: HIV Sentinel Surveillance 2014-15 (ANC): Quality Assurance for SRLs under NACO NRL, NICED, Kolkata (sample received from April 2014 to March 2015).

Sl. No	Name of SRL/Testing Centre	Samples sent by SRL		Samples re-jected by NRL	Confirmed Result at NRL		No. of Dis-cordant
		HIV -ve	HIV +ve		HIV -ve	HIV +ve	
1.	Rajendra Institute of Medical Science, Ranchi, Jharkhand	219	09	00	219	09	Nil
2.	Patuliputra Medical College, Dhanbad, Jharkhand	129	04	00	41	04	Nil
3.	SCB Medical College, Cuttack, Orissa	110	01	00	110	01	Nil
4.	VSS Medical College, Burla, Orissa	96	06	00	96	06	Nil
5.	MKCG Medical College, Beharampur, Orissa	33	01	00	33	01	Nil
6.	MGM Medical College, Jam-shedpur, Jharkhand	95	04	00	95	02	02

Kit Evaluation by NICED Consortium Labs (NARI, NIMHANS, NICED & NCDC)

Evaluation of HIV, HBV & HCV kits for quality for procurement by National and State agencies for nationwide supply in AIDS Control Program and in blood banks to prevent blood borne infections and ensuring safe blood transfusion.

Figure 3: Panel discussion on Evaluation of Diagnostic Kits

Table 4: Diagnostic Kits Evaluated by NICED Lab

Type of Kit	*No of Batch/ Lot evaluated*
HIV ELISA	04
HIV Rapid	10
HBsAg ELISA	00
HBsAg Rapid	03
HCV ELISA	08
HCV Rapid	06
Total	31

NICED as a member of "Consortium of National Reference Laboratories for Kit Quality" evaluated performance of HIV, HBV and HCV kits for all the National procurements for AIDS Control Program (NACP III and NACP IV) for ensuring quality kits. Request for evaluation is routed through the consortium secretariat, NARI, and all the labs are assigned the task for evaluation in a predefined rotational basis to avoid any bias.

Integrated Counseling & Testing at ICTC, NICED

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

- Conducting HIV diagnostic tests.
- Providing psychological support
- Link people with other HIV prevention, care and treatment services.

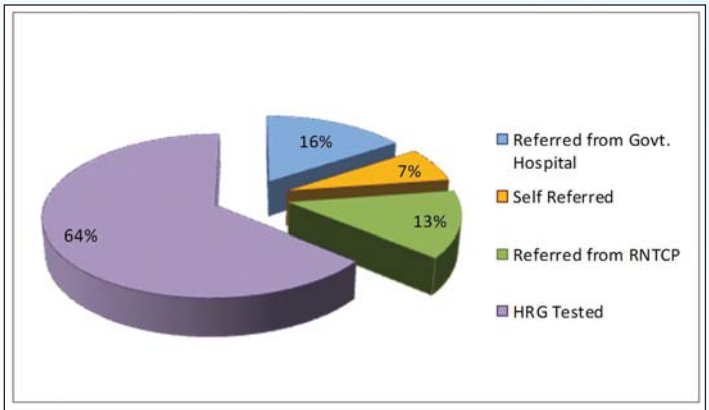


Figure 4: Client referral to ICTC

Table 5: ICTC Data from April 2014—March 2015

Total Tested	Positive	Positivity	Referred from RNTCP	HIV-TB Co-infection
1063	20	1.88%	142	7

Early Infant Diagnosis

Molecular Diagnosis of HIV among babies (< 18 months) born to HIV infected mothers using DBS employing state of art molecular assay for 14 states of East and North eastern India. This is to ensure early initiation of ART for the infected babies and also to monitor effectiveness of current practice of PPTCT (Prevention of parent to child transmission).

Table 6. Status of DBS and Whole Blood Samples received at NICED from April 2014 to March 2015.

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	566	559	39	14	14	14
Orissa	142	141	13	3	3	3
Chhattisgarh	0	0	0	0	0	0
Bihar	270	268	31	6	6	6
Jharkhand	71	71	4	2	2	2
Mizoram	125	125	3	0	0	0
Assam	34	34	0	0	0	0
Manipur	47	44	1	0	0	0
Nagaland	79	79	5	4	4	4
Meghalaya	0	0	0	0	0	0
Arunachal Pradesh	0	0	0	0	0	0
Sikkim	0	0	0	0	0	0
Tripura	0	0	0	0	0	0
A & N Islands	0	0	0	0	0	0
TOTAL	1334	1321	96	29	29	29

EID program generated evidence rationalized change of National ART regimen for preventing mother to child HIV transmission as well as use of only DBS samples (removing whole blood sample test for confirmation) for molecular diagnosis of HIV. 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 <18 months. NICED Molecular HIV Lab started EID in 2010 initially with three states, WB, Orissa & Chhattisgarh. With the gradual success of the program, other states (Jharkhand, Bihar, Assam, Manipur, Mizoram, Nagaland, Meghalaya, Arunachal, A&N, Sikkim, and Tripura) were also included under NICED. Presently, 116 ICTCs are involved in collection of DBS samples in 14 states under NICED-RRL and 30 linked ART centres are collecting Blood Samples. A total of 1334 DBS and 29 Whole Blood Samples received at NICED during 01.04.2014 to 31.03.2015. All DBS DNA PCR reactive specimens are further confirmed by 2nd HIV-1 DNA PCR test performed with Whole Blood samples.

Regional Institute (East)-HIV Surveillance

HIV surveillance for east and north eastern states of India (as Regional Institute-East) for estimation of HIV & monitoring effectiveness of national program for intervention to prevent HIV infection. (6 Regional Institutes; PGI, AIIMS, RIMS, NICED, NIE, NARI)

Regional Institute (East), NICED, implemented HIV Sentinel Surveillance for the East and N-E states with the aims 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 Regional Institute (East) is also to focus on different aspects of IBBS implementation starting from Pre surveillance Assessment, Pre testing of tools for IBBS, regional level & field level trainings, coordination meetings with NACO, SACS and Field Research Agency (FRA), monitoring & supervision of rapid field assessment, sampling frame development and field survey in assigned domains. 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) tablet based application.

Spectrum of Activity

- Technical support & guidance to SACS in overall planning & implementation of HSS and IBBS activities in eastern Indian states, facilitating smooth implementation of surveillance activities by liaising with concerned state authorities addressing specific problems at sentinel sites/ testing labs/ FRA.

Figure 5: Regional ToT for IBBS at NICED

- Technical validation & approval of new site 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 & lab personnel.
- Monitoring & Supervision during HSS through site visits by RI team members.
- Constitution of State Surveillance Teams (SST) and coordination of all their activities including Monitoring & Supervision by SST members.
- Ensuring timely reporting & corrective action at sites/testing labs during the round.
- Data Entry, matching, modifying, freezing & cleaning through SIMS.
- Concurrent data monitoring and initiation of corrective action, as required.
- Coordination with PMU/NACO/FRA about software related bug.
- Sampling and Cluster selection for survey.
- Giving inputs to improve the software program.
- Analyze the data during survey period for better field work.
- 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 & 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.

Figure 6: Dried Blood Samples

Table 7 : No. of samples allotted for HSS-ANC round

States	No. of Sites	Samples Allotted	No. of Testing lab
A & N Islands	4	1600	1
Chhattisgarh	20	8000	3
Meghalaya	8	3200	2
Nagaland	13	5200	2
Sikkim	4	1600	1
West Bengal	23	9200	4

- Monitoring and supervision for IBBS field recruitment at FRA, field training and submission of feedback through IIMS.
- 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.

Monitoring and Supervision

- Sample Frame Development of IBBS started from June 2014. RI team members visited 24 Pargans (S) and Burdwan domain for FSW typology; 24 Pgs (S) and Hooghly domain for MSM typology; Kolkata and East Midnapore domain for MIG typology; East Midnapore domain for CMW typology and Kolkata domain for IDU & MIG typology

Table 8 : RI. Data management status till February 2015

States	Samples Allotted	Data forms received	Primary Entry done	Secondary Entry done	Match-ing done
A & N Is-lands	1600	830	340	288	288
Chhattisgarh	8000	4476	2883	2273	2273
Meghalaya	3200	1919	1304	999	999
Nagaland	5200	639	0	0	0
Sikkim	1600	1156	696	470	470
West Bengal	9200	6588	4842	1618	1618
Total	28800	15608	10065	5648	5648

- Uttar Dinajpur for CMW typology, Darjeeling domain for MSM, IDU and TG typology and South Sikkim domain for IDU typology. RI (E) team also supervised SFD field activities at Wokha domain for IDU typology, Dimapur domain for IDU, MSM & FSW typology of Nagaland and Tinsukia domain for IDU typology & Dibrugarh domain for IDU typology of Assam and Kolkata domain of West Bengal for TG typology.
- RI (E) team members supervised main survey field work at Darjeeling, Sikkim, 24 Parganas (S), Kolkata and East Khasi Hills domain for IDU/ MSM typology; Jalpaiguri, Burdwan, 24 Parganas (S) domain of West Bengal and Jaintia Hills domain of Meghalaya for FSW typology.
- Pilot testing of 'screening tools' for migrant typology was conducted in Kolkata domain by RI team on 12 February 2015. Data was collected from randomly selected 127 migrant populations from five different clusters at Burrabazar among gold artisan/goldsmith, puller and porters.

Domain update based on SFD findings

- Domains Dropped: 3: IDU Typology: (Wokha in Nagaland & Dibrugarh in Assam); MIG Typology: Dakshin Dinajpur in WB
- Domain Merged: 1: MSM Typology: Kamrup Urban and Barpeta MSM domains are merged and a new domain (Assam_West_MSM) created.
- Take All Domains: 2: TG Typology: (Dakshin Dinajpur); FSW Typology: (Jalpaiguri)

Plasma Viral Load Assay for HIV

- HIV Viral load assay for East & N-E for ensuring efficacy of ART and taking evidence based decision for initiation of further treatment.
- NICED Molecular HIV lab restarted HIV viral load assay for the patients under ART for monitoring effectiveness of ongoing treatment as per national guidelines and also to assist in HIV drug resistance mutation assay.

Organizing advanced trainings

- Conduction of advanced training and assisting in conducting training (by providing training materials & as resource persons) for remote states like Chattisgarh, A&N, Nagaland, Assam, Meghalaya, Jharkhand & Sikkim for National AIDS Control Program.
- 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, HSS, IBBS and Molecular Diagnosis of HIV employing DBS.

S. S. Das

- The Biomedical Informatics Center under the Division of Clinical Medicine assisted the scientists and research scholars from NICED, other research institutes, regional medical colleges and universities in the analysis of microbial genomes, three dimensional structure of proteins as well as statistical analysis.

A. K. 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.
- Acted as reviewer of research proposals and project works of undergraduate medical students for Short Term Studentships (STS) under the Indian Council of Medical Research.
- Served as Associate Member, Drinks and Drinking Water Sectional Committee, FAD 14, Bureau of Indian Standards, Govt. of India.
- Acted as Chief Trainer, Eastern Region, Integrated Biological & Behavioral Surveillance (IBBS), National AIDS Control Program – Phase IV, NACO, Govt. of India.
- Served as Mentor, National Data Analysis Plan (NDAP) under the National AIDS Control Program – Phase IV, for Assam and Tripura.
- Acted as a member of the committee for the institutional initiative called “Health and Hygiene Campaign with a Focus on Diarrhoea and Enteric Diseases” targeted at school children in and around Kolkata.

S. Ganguly

- Provided 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.
- Provided QC and QA support facility in eastern India for parasitic detection under Indo-US joint program.

M. Chawla Sarkar

- The virology lab provided laboratory diagnosis for Influenza A/H1N1/2009 virus for referred cases from Hospitals in Kolkata for effective patient management during the outbreak in Jan-March 2015.

H. Koley

- Taught Physiology, Zoology and Microbiology to the M. Sc. students of the Vidyasagar University, Calcutta University, as honorary teacher.

Outbreak Investigation:

- A visit was made to STNM Hospital, Gangtok on 23 Nov 2014 by the following two members Central Team to inspect the allocated isolation facilities for Ebola Virus Disease (EVD): (Dr. Satyajit Sen, Regional Director Kolkata, Regional office of HealthÊ& FW, GOI and Dr. Shanta Dutta, Scientist F, NICEDÊ(ICMR). As per Govt. Order D.28015/4/2014/EMR/Pt from M/o Health and Family Welfare, Govt. of India,ÊDGHS, EMR dated 21 Nov 2014, the team members started their onward journey to Gangtok, Sikkim on 23 Nov 2014. Immediately after arrival the members started visiting the different units of the Hospital e,g, Emergency, Outdoor, laboratory etc. They also inspected the under construction isolation facility of the hospital for checking the level of preparedness for EVD. The major points have been covered in the completed checklist provided by the EMR, DGHS, M/O Health and FW. Briefly, STNM Hospital has allocated one area for the isolation facilities, which may not be the ideal for construction of such unit. As per Medical Superintendent, STNM Hospital, there is acute shortage of space/ land to build such unit. The current unit has been located at least 50 meters higher up than the general hospital, and stone curved stairs need to be used for transportation of EVD patients. The unit is within the hospital campus, but attached to many other Dept. of the hospital like Cancer Registry Unit, IDD cell and Diet Stores.
- Assessment of the Post-Flood Public Health Situation in Baramulla District, Kashmir during October, 2014 by NICED Scientists

  As instructed by the Ministry of Health & Family Welfare, Directorate General of Health Services (Emergency Medical Relief), Govt. of India, Dr. A. K. Deb and Dr. A. K. Mukhopadhyay was deployed as a Public Health Specialist in Baramulla district of Kashmir during October 08 – 22, 2014 to offer expertise on post-flood surveillance, implementation of public health measures and outbreak investigations, if any.
  The district Baramulla, located on the north-west of Srinagar, was affected by the flood later - mostly due to overflown water that submerged and created havoc to Srinagar, and in some areas due to flashfloods from heavy rainfall. The flood water also lasted only for a few days without stagnation in the affected areas.

  About 16% of the villages in the district were affected by the flood. However, most of these villages were affected mild-to-moderately; some villages in a few blocks were severely affected and required special attention. These flood hit areas were already visited by one or the other EMR teams and the district health system also responded very quickly and efficiently so that the health situations even in the hard-hit areas were never beyond control.

  Except for an increase in number of dermatitis cases (following contacts with polluted flood water) in some areas and an increase of road traffic accident cases noted in the district hospital, there have been no outbreak of diseases so far in the district following the flood and at present there does not seem to have an imminent threat of any major outbreak in the flood-hit areas of the district.

  As far as diarrheal diseases are concerned, there was no increase in such cases since the flood – rather the number of cases in most areas showed a gradual decrease. This was possibly due to the effect of several factors – no stagnation of water, persistent use of tanker water, boiled water or water treated with chlorine tablets at household levels, as well as ensuing winter season that could negatively affect occurrence of diarrheal diseases. The other major water source to the affected areas that were being

supplied through the PHE department was recently checked for adequacy of chlorination and coliform count (MPN), and as per CMO of the district, was found suitable for human consumption. Thus, there was no report of any diarrheal outbreak in any part of the district and there seemed to be no threat for any imminent outbreak of such diseases either. However, one sporadic diarrhoeal sample collected from Archanderhama of Pattan was positive for multidrug resistant Entero pathogenic *E. coli.* So, continuous vigil in this regard should be maintained.

Temporary shelters for homeless people in village Bala, Kashmir

EXTRAMURAL PROJECTS

Title	Studies on molecular typing of *Salmonella* Typhi isolates from Kolkata: its relevance in controlling the transmission of drug resistant organisms.
PI	**Dr. S. Dutta**
Funding Agency	DST West Bengal
Duration	2014-17

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	2012-15

Title	Development of a bacteriophage-based biocontroltechnology for the treatment of cholera.
PI	**Dr. B. L. Sarkar**
Funding Agency	Indo-UK, DST
Duration	2014-16

Title	Exploration of the Biological Basis of Under performance of Oral Polio and Rota Virus Vaccines in India
PI	**Dr. R. K. Nandy & Dr. S. Kanungo**
Funding Agency	International Vaccine Institute, Seoul, Korea
Duration	2012-15

Title	Gastro Intestinal Tract Pathogen Repository, (GTPR)
PI	**Dr. R. K Nandy** (w.e.f. Feb 2014)
Funding Agency	ICMR, Govt. of India
Duration	2011-16

Title	Comparative analysis of the *Helicobacter pylori* strains isolated from North East India with other parts ofIndia in causing gastro-duodenal diseases
PI	**Dr. A. K. Mukhopadhyay**
Funding Agency	DBT, Govt. of India
Duration	2012-14

Title	Evolution of CTX prophages of *V. cholerae* O1 and O139 strains in Asia and Africa
PI	**Dr. A. K. Mukhopadhyay**
Funding Agency	MEXT-Okayama University Project, Govt. of Japan
Duration	2010-2015

Title	Studies on blood group antigen binding adhesin (babA) gene in relation to *Helicobacter pylori* mediated diseases outcome in India.
PI	**Dr. A. K. Mukhopadhyay**
Funding Agency	CSIR, Govt. of India
Duration	2015-17

Title	Acquired mechanisms of quinolone resistance in carbapenem–resistant Enterobacteriacae: relevance in neonatal healthcare.
PI	**Dr. S. Basu**
Funding Agency	DST, West Bengal
Duration	2015-2017

Title	Hospital based surveillance system for diarrhoeal diseases (IM/NICED/Surv./1)
PI	**Dr. M. K. Bhattacharya**
Funding Agency	Okayama University, Japan
Duration	Running since 2007

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, Japan
Duration	2015-2019

Title	Development of a universal Shigella vaccine based on virulence gene expression
PI	**Dr. H. Koley**
Funding Agency	National Institute of Infectious Diseases, Japan
Duration	2011-2015

Title	Biomedical Informatics Center of ICMR, 2nd Phase of Task-force Project
PI	**Dr. S. S. Das**
Funding Agency	Indian Council of Medical Research
Duration	2013-2018

Title	Development and pre-clinical studies on safety and immunogenicity of novel candidate vaccines against *Salmonella enterica* serovarTyphi and Paratyphi
PI	**Dr. S. S. Das**
Funding Agency	Department of Biotechnology
Duration	2012-2015

Title	Studies on immune responses elicited by candidate peptide vaccines and polysaccharide-peptide conjugate vaccines against *Salmonella enterica* serovarsTyphi and Paratyphi infections
PI	**Dr. S. S. Das**
Funding Agency	Council of Scientific and Industrial Research
Duration	2015-2018

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

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

Title	Study of mechanism of probiotic action in persistent diarrhea in children caused by enteroaggregative *E.coli* – using a mouse model
PI	**Dr. S. S. Das**
Funding Agency	Department of Health Research
Duration	2014-17

Title	Assessment of perceived health needs and available health care facilities of Malda District
PI	**Dr. K. Sarkar**
Funding Agency	ICMR
Duration	October 2013 to August 2014

Title	Assessment of nutrition status among primary & upper-primary school children of all districts of West Bengal
PI	**Dr. K. Sarkar**
Funding Agency	Dept. of School Education, Govt. of West Bengal
Duration	April 2014 to March 2015

Title	Phase III, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Live Attenuated Bovine-Human Rotavirus Reassortant Pentavalent Vaccine (BRV-PV) Against Severe Rotavirus Gastroenteritis in Healthy Indian Infants
PI	**Dr. S. Kanungo**
Funding Agency	PATH Vaccine Solutions, USA
Duration	2014- ongoing (3 years)

Title	Generation of Culture-differentiated Innate Memory CD8 Cells with Toll-like Receptor Expression and Responsiveness to Pathogen/Danger-associated Molecules
PI	**Dr. T. Biswas**
Funding Agency	DBT
Duration	2014-17

Title	Studies on burden of parasitic infections among different communities in Western part of India to support health impact evaluation of Total Sanitation Campaign
PI	**Dr. S. Ganguly**
Funding Agency	GFK Mode
Duration	2012-15

Title	Differential pathogenesis of Giardia: Role of Giardia Virus.
PI	**Dr. S. Ganguly**
Funding Agency	NIID, Japan
Duration	2012-15

Title	Study the molecular mechanism of anti-cancer and anti-tumor effect of bacterial protease
PI	**Dr. A. Pal**
Funding Agency	ICMR
Duration	2014-17
Title	National Rotavirus Surveillance Network - Referral Lab Eastern India
PI	**Dr. M. Chawla Sarkar**
Funding Agency	ICMR
Duration	2013-2017
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	2010-15
Title	Multisite Monitoring of Influenza Virus Strains in India Phase II
PI	**Dr. M. Chawla Sarkar**
Funding Agency	ICMR and DHHS USA
Duration	2009-14
Title	External Quality Assurance for HIV testing
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation
Duration	2012-2017
Title	HIV Sentinel Surveillance
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation
Duration	2012-2017
Title	Evaluation of diagnostic kits for HIV, HBV and HCV
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation/ Self Sustaining
Duration	2015-2020
Title	Molecular detection of HIV in infants and children under the age of 18 months
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation
Duration	2012-2017
Title	Counseling and testing for HIV, blood borne infections and STIs
PI	**Dr. M. K. Saha**
Funding Agency	WBSAP&CS
Duration	2012-2017
Title	Molecular assay for HIV-1 plasma viral load
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation
Duration	2015-2017
Title	Molecular characterization of HIV for drug resistance mutations among infant using dried blood spot sample
PI	**Dr. M. K. Saha**
Funding Agency	ICMR (DHR Translational Project)
Duration	2015-2018
Title	Utility of prevention of mother-to-child HIV transmission programme data for HIV surveillance
PI	**Dr. M. K. Saha**
Funding Agency	National AIDS Control Organisation
Duration	2013-2016

PUBLICATIONS

Publications

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Book Chapter

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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 (records are being maintained manually and computerized) 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 and maintain records in addition to process towards final settlement of Pension/Family Pension, DCRG, Commutation, Leave Encashment etc.
- 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 at NICED
- To maintain Staff Canteen
- To control deployed Security and House Keeping Staff
- To maintain and uploading and sharing information in ICMR Intranet e Portal.
- To maintain Grievance Procedure
- 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 97 extramural projects including Okayama project of Okayama University and VRDL Project of DHR. 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. The Load of work for Extramural projects (97 projects) has also tremendously expanded. The total workload is carried out by the existing staff.

(Subodh Karmakar)

Administrative Officer

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Ex. Professor, PSM, IMS, BHU
& Retd. NPO, WHO-India
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Former Dean
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Member : Prof. Pradeep Seth
Former Prof., AIIMS
President, Seth Research Foundation
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Director of Medical Education & Ex-Officio Secretary
Dept. of Hlth & FW, Govt. of W.B.
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Former Dean
Faculty of Medicine
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Prof. & Head, Dept. of Medicine
College of Medicine & J.N.M. Hospital
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Chief Reporter
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Member : Mrs. Debolina Sarkar
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Former Emeritus Medical Scientist (ICMR)
NICED, Kolkata

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Regional Laboratory Burdwan, West Bengal

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Scientist-F & Head, Division of Pathophysiology,
National Institute of Cholera and Enteric Diseases
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Member : Dr. Tapas Biswas
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Division of Pathophsiology,
National Institute of Cholera and Enteric Diseases
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Smt. Renu Jaiswal, B.A., L.D. Clerk
Shri J. Malakar, M.T.S.
Shri KH. Ibomcha Singh, M.T.S.
Shri Omkar Lal, M.T.S.

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Shri T. K. Chanda, B.Sc., Accounts Officer (Ad-hoc) – (till 31.10.2014)
Shri T. K. Saha, Section Officer
Shri P. K. Bose, B.Com, LL. B, Assistant
Shri G. C. Das, B.Sc., Assistant
Shri D. K. Gayen, B.Com., U.D. Clerk

Cash Section
Shri C. K. Naskar, B.Com., Assistant (Ad-hoc)
Shri R. Biswas, U.D. Clerk
Shri P. N. Jha, Sr. Technician-B (till 31.03.2014)

Store Section
Shri P. Bhadra, B.Com., Section Officer (Ad-hoc)
Shri S. Sen, B.Com., Personal Assistant
Shri S. Banerjee, B.Sc., Assistant
Shri R. Chowdhury, B.Com., Assistant
Shri P. Guha, B.Com., U.D. Clerk w.e.f. 27.11.2014
Shri A. Banerjee, B.Com., Telephone Operator
Shri T. K. Pal, M.T.S.

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Shri S. Ghosh, B.Com., Assistant
Shri K. Sharma, B.Com, U.D. Clerk (Ad-hoc) w.e.f 27.11.2014

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Shri A. Chandra, B.Com., U.D. Clerk
Smt M. Bhattacharya, M.T.S.

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Shri T. Pal, M.Sc., B.Lib.Sc., Library Information Assistant
Shri M. Chakraborty, Technical Assistant

Training & Extension
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Shri A. Jana, Technician-B
Shri A. K. Roy, Technician - B
Shri S. Adhikari, M.T.S.

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Shri A. R. Das, B.A., Care-taker
Shri S. Parui, Technician-C (Eng. Support)
Shri A. Sarkar, Technician-C, (Eng. Support)
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Shri S. Maiti, M.T.S.
Shri V. K. Singh, M.T.S.
Shri B. Das, M.T.S.
Shri M. L. Dosad, M.T.S.
Shri S. Mullick, M.T.S.
Shri S. Hazra, M.T.S.
Shri A. Das, M.T.S.
Shri B. Moshi, M.T.S.
Shri D. Turi, M.T.S.
Shri B. Mandi, M.T.S.
Shri S. K. Routh, M.T.S.
Smt B. Hela, M.T.S.
Shri R. Hela, M.T.S.
Shri A. Paramanik, M.T.S.
Shri A. Seal, M.T.S.

Media Section
Shri R. B. Bose, B.Com., Technical Officer (A) – till 30.06.2014
Shri N. K. Sikder, Technical Officer (A) – till 31.01.2015
Shri A. K. Saha, Technician-B
Shri S. Mondal, M.T.S.
Shri K. Roy, Technicial-B
Shri K. Ghoshal, M.T.S.

Animal Section
Shri K. C. Paramanik, M.Sc. (ME), Technical Officer (A)
Shri K. C. Tudu, Technician-C

Shri S.R. Balmiki, M.T.S.
Shri P. Turi, M.T.S.
Sh Shri N. C. Mondal, M.T.S.
Sh. S. Hari, Technician-B
Shri Rabi Hazra, M.T.S.

Vehicle Section
Shri D. Saha, Driver (Spl. Grade)
Shri S. Das, Driver (Grade I)
Shri D. K. Chowdhury, Driver (Grade I)
Shri H. P. Das, Driver (Grade I)
Shri G. Mehboob, Technician-C (Eng. Supp.)
Shri M. Ali Khan, Technician-C (Eng. Supp.)
Shri C. Nayak, Driver (Grade II) – till 11.01.2015
Shri A. K. Dutta, Driver (Grade II)
Shri S. Das, Driver (Grade II)
Shri S. Ghosh, Driver (Ordinary Grade)
Shri D. Dey, Driver (Ordinary Grade)

Staff list-as addendum

Division of Electron Microscopy

Staff
Arpita Sarbajna, Technical Officer A
Sushil Kumar, Technician B
Bivash Ranjan Mallick, MTS (Technical)

Predoctoral Fellow
Sayani Das CSIR JRF

Division of Epidemiology

Scientists
Dr Kamalesh Sarkar, Scientist F
Dr Samiran Panda, Scientist F
Dr Alok Kumar Deb, Scientist E
Dr Suman Kanungo, Scientist C
Dr Falguni Debnath, Scientist B

Staff
Swapna Manna, Technical Officer A
Ratan Lal Saha, Technical Officer A
Subrata Shil, Technical Officer A
Chandan Mandal, Technical Assistant
Avijit Chakraborty, Technician C

Predoctoral Fellows
Dr Kalyan Bhowmick, SRF Medical
Dr Tanmay Mahapatra, SRF Medical

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