वार्षिक रिपोर्ट
ANNUAL REPORT

2016-17

ICMR-NICED

भारतीय आयुर्विज्ञान अनुसंधान परिषद्
राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

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

WHO Collaborating Centre for Research and Training on Diarrhoeal Diseases

The Annual Report was compiled by the following editorial team

Dr Shanta Dutta
Scientist G and Director
Dr Triveni Krishnan
Scientist F, Division of Virology
Dr Alok Kumar Deb
Scientist E, Division of Epidemiology
Dr Pallavi Indwar
Scientist B, Division of Clinical Medicine
Smt. Saheli Samanta
Assistant Library & Information Officer, Library

Crafted & Impressed by
Semaphore Technologies Pvt. Ltd.
3, Gokul Baral Street, 1st Floor
Kolkata - 700012, West Bengal, India
E-mail: stpl@semaphoreindia.com
www.semaphoreindia.com

From the Director's Desk:

Indian Council of Medical Research-National Institute of Cholera and Enteric Diseases (ICMR-NICED) is recognized both nationally and internationally for its important contributions in the field of diarrheal disease research. Millions of lives, including those of young children, have been saved through the introduction and implementation of Oral Rehydration Therapy (ORT), which has immensely influenced the Diarrheal Disease Control program in the country. ICMR-NICED has played a pivotal role in this exercise by increasing awareness about ORT among the common people. Besides high quality research in basic sciences, the institute also undertakes applied research of translational importance and generate evidence to inform program development.

Capacity building and development of skilled human resources at national and sub-national level are other focus areas of ICMR-NICED. Several training programs and workshops are conducted throughout the year, which are attended by participants from academic and research organizations from all over the country. The scientists of the institute also supervise doctoral and post-doctoral students and serve as mentors to students pursuing Master's degree. Such arrangement enables the students of different universities to execute short term projects and learning state-of-the-art laboratory techniques.

Over the years, the institute has widened its portfolio including research on emergence of multidrug resistance, environmental surveillance of enteric pathogens, role of changing climate on waterborne diseases, surveillance of viral respiratory infections as well as studies on HIV disease. The financial and technical support received from several national as well as international agencies viz. Department of Health Research, Department of Science & Technology (Govt of India and West Bengal Govt), Department of Biotechnology, Ministry of Earth Sciences, Council of Scientific & Industrial Research, Indian Council of Medical Research, National AIDS Control Organization, West Bengal State AIDS Prevention & Control Society, Centers for Disease Control and Prevention USA, Japan Agency for Medical Research and Development, International Vaccine Institute South Korea, National Institute of Infectious Diseases Japan, Bill and Melinda Gates Foundation, AMED Okayama University Japan, Cardiff University, PATH Vaccine Solutions USA, are gratefully acknowledged. The State health authority of West Bengal, receives regular inputs from the ICMR-NICED following investigation of increased number of diarrhea, dengue fever and seasonal influenza (H1N1) cases. Recently ICMR-NICED has enhanced its capacity to offer laboratory investigation support in outbreak situations in the Eastern and North Eastern States through establishment of a Regional Centre of Viral Research and Diagnostic Laboratory (VRDL).

Research findings, generated by the scientists of ICMR-NICED, are regularly disseminated through peer-reviewed publications in reputed high impact national and international journals. Such scientific information is also presented in scientific meetings, forums, conferences and seminars. As part of public health advocacy, newspapers and television channels are kept informed about the research findings generated by the institute.

During the last one year, ICMR-NICED has participated in a number of important public health events including participation in community based fairs and outreach programs. The foundation day of the institute was celebrated with great enthusiasm. On this occasion Dr. Soumya Swaminathan, the Director General, ICMR and Secretary, Department of Health Research delivered the prestigious Dr. S.C. Pal Oration. Other popular lectures were also organized on 'Science Day', 'Doctor's Day', 'World AIDS Day' and on similar occasions. As part of 'Swachh Bharat Campaign', activities are planned and executed such as voluntary cleaning program, tree plantation

and boosting up of garbage disposal system within the institution premise as well as communities. Furthermore, lecture competition was organized to observe 'Vigilance awareness' week. The institute, in collaboration with ICMR-Headquarter (ICMR-HQ), actively participated as judges and mentors in the illustrious scientific event of 'Hackathon 2017'.

The relentless efforts of all the scientists, technical personnel and administrative staff deserve special mention. I convey my deepest appreciation to one and all for working as a great team to uphold the commitment of this institute towards the national interests. Scientists received accolades like Sentinels of Science Award for peer review in science and research, Dr. Y. S. Narayana Rao Oration Award of ICMR 2014, elected as Fellow of the West Bengal Academy of Science & Technology (WAST) in the year 2016.

Finally, I acknowledge the continued support received from ICMR-HQ, without which it would not have been possible to carry out all these activities of ICMR-NICED.

Dr. Shanta Dutta
Director

CONTENTS

RESEARCH ACTIVITIES

BACTERIOLOGY

Global emergence of fluoroquinolone (FQ) resistance in *Salmonella* Typhi (*S.* Typhi) has complicated the treatment of typhoid fever since the last decade. Widespread usage of FQs has major influences in reshaping the current *S.* Typhi population. Isolations of ciprofloxacin resistant and decreased ciprofloxacin susceptibility (DCS) *S.* Typhi isolates varied from 16.7% to 25.8% and 69.3% to 82.1% over the last three years in ICMR-NICED, Kolkata respectively. Overall the non-susceptibility to ciprofloxacin was found to be about 98%. FQ resistance was mediated by mutations in quinolone resistance-determining regions (QRDRs) of DNA gyrase and topoisomerase IV genes. Gradual increase in genetic diversity of ciprofloxacin resistant *S.* Typhi isolates was also noticed by pulsed-field gel electrophoresis (PFGE) and majority of the isolates belonged to haplotype H58. Increased occurrence of FQ especially ciprofloxacin resistance in *S.* Typhi Kolkata isolates may indicate complete withdrawal of the drug from typhoid treatment to stop treatment failure cases of typhoid fever. Information on circulating pulsotypes of ciprofloxacin resistant *S.* Typhi with prevalent H58 haplotype is important in containment of this deadly organism.

Ongoing environmental investigations in the Gangetic riverine estuarine system including the Sunderbans mangrove has established that biofilm production of *V. cholerae* and its survival in a micro environment created within the biofilm facilitates horizontal gene transfer (HGT). The study on Seasonal dynamics of enteropathogenic bacteria in Gulf of Khambat, Gujarat as primarily established the preponderance and dominance of *V. alginolyticus* in the aquatic milieu of the Western coast of the country. Survival and distribution of the diarrhoeagenic Vibrios of the riverine systems are influenced by the *in-situ* saline conditions.

V. cholerae, the dreaded pathogen to cause cholera in human, is enormously evolved to utilize multiple metabolic pathways that aid environmental persistence and intestinal survival. Carbon source dependent preferential involvement of specific metabolic pathway is a new area of research interest. It has been shown that dysfunctional Entner-Doudoroff (ED) pathway and/ or glyoxylate cycle caused severe virulence attenuation in *V. cholerae*. Use of inhibitors to these pathways by simple sugars also resulted in virulence attenuation.

Vibrio cholerae O1 has been causing outbreaks in Haiti. Numerous mutations have been reported in *V. cholerae* O1 strains associated with the Haitian outbreak. Retrospective analysis of *V. cholerae* O1 strains in Delhi, India showed that all the tested strains carried Haitian type tcpA (tcpACIRS) and variant gyrA indicating their first appearance before 2004 in Delhi. The Haitian variant rtxA and ctxB7 were first detected in Delhi during 2004 and 2006. The sequential accumulation of Haitian-like genetic traits among *V. cholerae* O1 strains in Delhi at different time points prior to the Haitian cholera outbreak has been demonstrated.

Macrolide resistance in Campylobacter occurs due to point mutation in V-region of 23S rRNA. PCR based assay has been developed to detect the azithromycin resistant and sensitive Campylobacter strains utilizing mutation responsible for the phenotype. This study describes a simple and rapid method for detection of mutation conferring macrolide resistance with additional feature of identification of sensitive strains.

Recent study indicated that presence of jhp0945, jhp0947 and jhp0949 within the plasticity regions are significantly associated with symptomatic expressions along with the increased virulence of *Helicobacter pylori* during in vitro study, whereas jhp0940 seems to be negatively associated with the disease in India. These results suggest that jhp0945, jhp0947 and jhp0949 could be useful prognostic markers for the development of duodenal ulcer in India.

The mode of transmission of New Delhi-metallo-β-lactamase-1 (NDM-1) both via mobile genetic elements and outer membrane vesicles (OMVs) was studied. The *A. baumannii* strain (ST 1462) released vesicles (25-100nm) during *in vitro* growth at late log phase. The study was the first experimental evidence of intra and inter-species transfer of plasmid-harbouring bla_{NDM-1} gene in *A. baumannii* via OMVs with high transformation frequency. In Enterobacteriacae bla_{NDM-1} showed its association with at least a remnant of ISAba125 at its 5'-end and *ble*MBL at its 3'-end.

Outer membrane vesicles (OMVs) offer immune responses and protection against enteric organisms.

Antimicrobial resistance among pathogens, causing nosocomial and community-acquired infections are an alarming aspect of clinical microbiology. As pathogens are becoming resistant to conventional therapy, attention is focussed on the development of vaccines for long and protective immune responses. Chicken is the most common bio-reservoir of *Salmonellae* and are affecting humans. Non-conventional antibiotics enhances the chance of MDR pathogens. We are working on the formulation of novel OMVs based immunogen against poultry salmonellosis and campylobacteriosis. Oral immunization with combination OMVs of circulating *Salmonella* and *Campylobacter* strains could help the poultry become free from the infection. This will benefit society in maintaining the health of the poultry bird (industrial aspect) and infection free poultry products and will reduce the chance of *Salmonella* and *Campylobacter* infections to the human (clinical aspect).

Scientists:

Dr. S. Dutta, Scientist G & Director
Dr. A. Palit, Scientist-F
Dr. B. L. Sarkar, Scientist-F
Dr. R. K. Nandy, Scientist-E
Dr. A. K. Mukhopadhyay, Scientist-E
Dr. S. Basu, Scientist-E
Dr. H. Koley, Scientist-D

Staff:

Mr. J. Kharwar, Technical Officer-A
Mr. S. K. Bhowmick, Technical Officer –A
Mr. A. K. Mondal, Technical Officer-A
Mr. S. R. Ghosh, Technical Officer –A
Mr. A. Ganai, Technical Officer –A
Mr. T. Barman, Technical Assistant
Mr. S. De, Technical Assistant
Ms. M. Das, Technician-C
Mr. R. Balmiki, Technician-C
Mr. S. C. Saha, Technician-C
Mr. M. L. Gupta, Technician-B
Mr. P. Samanta, Technician-B
Mr. B. Roy, Technician-B
Mr. S. Dey, MTS

Post-doctoral Fellows:

Dr. Goutam Chowdhury, (OUP)
Dr. Abhijit Sarkar,
Dr. Subhasree Roy, – Research Associate (ICMR)
Dr. Soma Mitra, (ICMR)
Dr. Raghwan, (NASI)

Pre-doctoral Fellows

Mr. Sambit Roy, CSIR (SRF)
Ms. Taniya Golder, ICMR (SRF)
Mr. Arindam Naha, ICMR (SRF)
Ms. Piyali Mukherjee, ICMR (SRF)
Ms. Saswati Datta, ICMR (SRF)
Ms. Somdutta Chatterjee, ICMR (SRF)
Ms. Shravani Mitra, CSIR (SRF)
Mr. Subham Mookherjee, (CSIR SRF)
Ms. Priyadarshini Mukherjee, UGC (SRF)
Mr. Bipul Chandra Karmakar, DST (JRF)
Mr. Prasenjit Samanta, CSIR (JRF)
Ms Sriparna Samajpati, DST (JRF)
Mr. Suhrid Maiti, NIID, (JRF)
Mr. Debaki Ranjan Howlader, CSIR (JRF)
Ms. Ushasi Bhaumik, DST (JRF)
Mrs. Madhumanti Halder, UGC (JRF)
Ms. Sharmi Naha, DST (JRF)
Mr. Sounak Sarkar, DST (JRF)
Mr. Punnag Saha, NIID (JRF)
Mr. Subhankar Mukherjeee, ICMR (JRF)
Mr. Vivek Mandal, CSIR (JRF)
Mr. Rudra Narayan Saha, ICMR (JRF)
Ms. Sangita Paul, (BMGF)
Mr. Suvajit Saha, MoES (JRF)

Ph.D. Awarded:

Dr. Dhrubajyoti Nag received Ph.D. from Jadavpur University

Title of the thesis: Studies on host immune responses against different forms of shigella immunogens in animal model

Dr. Ritam Sinha received Ph.D. from Jadavpur University

Title of the thesis: Studies on Immunogenicity and Protective Efficacy of Multi-serotype *Vibrio cholerae* Outer Membrane Vesicles in Animal Model

Dr. Prachetash Ghosh received Ph.D. From University of Calcutta

Title of the thesis: Role of blood group antigen binding adhesion (*bab*A) gene and R-M system genes in pathogenesis of *Helicobacter pylori* in India.

Title: Studies on mechanism of antimicrobial resistance especially fluoroquinolone resistance among *Salmonella* Typhi Kolkata isolates

Investigators: S. Dutta, S. Das

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S.* Typhi), remains an unresolved public health problem in India. Emergence of antimicrobial resistant strains poses a great concern for typhoid treatment and influences reshaping of current *S.* Typhi population. A total of 349 *S.* Typhi strains isolated from Kolkata during 2009-2016 were included in this study. Multidrug resistance (resistance to chloramphenicol, ampicillin, co-trimoxazole) was decreasing in *S.* Typhi (27/349, 7.7%) with increased resistance to fluoroquinolone (FQ). Interestingly 3.4% (12/349) *S.* Typhi Kolkata isolates showed high level resistance to amoxycillin/clavulanic acid (MIC ≥256 µg/ml). Single non-conjugative 180 kb non-IncHI1 plasmid was detected in 77.8% (21/27) MDR *S.* Typhi. Remaining 22.2% (n=6) MDR *S.* Typhi had no plasmid. MDR strains with or without plasmid shared the same set of resistance genes (bla_{TEM-1}, *cat*A1, *sul*1, *sul*2, *str*A and *str*B) and class 1 integron possessing dfrA7 gene cassette (Table 1). Two S. *T*yphi strains harbored 50kb transferrable plasmids carrying *dfr*A15 and *aad*A1 gene cassettes in class 1 integron. Of the 25 MDR isolates, 24 (95.5%) showed integration of the MDR locus between the chromosomal genes STY3618 and STY3619 (near *cya*A gene). Interestingly, the MDR locus was integrated into the chromosome of both plasmid-borne (non-IncHI1) and plasmid-less MDR isolates. In one MDR isolates, the MDR locus was found to be inserted at fbp gene on chromosome. Study isolates (n=97) harbored 10 different types of quinolone resistance-determining regions (QRDRs) mutation(s) (Table 2) among which single or double mutation in *gyr*A gene played a central role in mediating FQ resistance. None of them harbored plasmid-mediated quinolone-resistance (PMQR) genes. *Salmonella* Typhi isolates (n=25) with varying levels of ciprofloxacin resistance showed phenotypic activity of efflux pump in the presence of efflux pump inhibitor PAβN (40µg/ml), which did not corroborate with the genetic expressions of AcrAB-TolC efflux pump genes at transcription levels. In addition, no mutations were detected in the regulatory regions of this efflux system. Results of this study generated information useful for better understanding of the antimicrobial resistance especially FQ resistance.

Table 1 . Characterization of plasmids, resistance genes and class 1 integrons in *S.* Typhi Kolkata strains (n=29) during 2009-2016

Resistance profile (n)	Plasmid type (size) [a]	Presence of resistance genes [b]									VR size (gene cassettes) of class 1 integron [c]
		bla_{TEM-1}	*catA1*	*sul1*	*sul2*	*tetA*	*tetB*	*aadA1*	*dfrA7*	*strAB*	
AMP-CHL-SXT-STR-NAL (17)	Non-IncHI1 (180kb)	+	+	+	+	−	−	−	+	+	750bp (*dfrA7*)
AMP-CHL-SXT-STR-NAL (6)	NP	+	+	+	+	−	−	−	+	+	750bp (*dfrA7*)
CHL-SXT-NAL (4)	Non-IncHI1 (180kb)	−	+	+	−	−	−	−	+	−	750bp (*dfrA7*)
TET-SXT-STR-NAL-CIP-OFX-LVX(2)	IncN (50kb)	−	−	+	−	+	−	+	−	−	1.6kb (*dfrA15,aadA1*)

Table 2. QRDR mutations in *S.* Typhi Kolkata isolates (n=97) having different fluoroquinolone MICs

QRDR type(n)[#]	QRDR mutation(s)				MIC range (µg/ml)			
	GyrA	GyrB	ParC	ParE	NAL	CIP	OFX	LVX
Q1 (2)	WT	WT	WT	WT	2.0	0.016-0.064	0.032-0.064	0.016-0.032
Q2 (1)	Asp87Asn	WT	WT	WT	256	0.25	0.5	0.25
Q3 (2)	Asp87Tyr	WT	WT	WT	256	0.25	0.5	0.25
Q4 (32)	Ser83Tyr	WT	WT	WT	32->256	0.25-0.5	0.5-1.0	0.25-0.5
Q5 (19)	Ser83Phe	WT	WT	WT	>256	0.12-0.5	0.5-1.0	0.25-0.5
Q6 (1)	Ser83Phe	WT	WT	Leu502Phe	>256	0.5	1.0	0.5
Q7 (1)	Ser83Phe	WT	Glu84Lys	WT	>256	1.0	1.0	0.5
Q8 (4)	Ser83Phe	WT	Glu84Gly	WT	>256	1.0	1.0	0.5
Q9 (1)	Ser83Phe; Asp87Gly	WT	Ser80Ile	WT	>256	16	8.0	4.0
Q10 (34)	Ser83Phe; Asp87Asn	WT	Ser80Ile	WT	>256	16-≥32	16-≥32	4.0-32

Title: Studies on Molecular typing of *Salmonella* Typhi isolates from Kolkata: its relevance in controlling the transmission of drug resistant organisms

Investigators: S. Dutta, S. Samajpati

Typhoid fever is an acute systemic infection caused by *S.* Typhi. Antimicrobial therapy is the mainstay of treatment but emergence of antimicrobial resistance (AMR) has become a major global problem. Subtyping of *S.* Typhi isolates is essential in discriminating the isolates for improved molecular epidemiological investigations. A total of 260 *S.* Typhi isolates were collected from various hospitals in Kolkata, India during 2014-16. Those isolates were tested for AMR profiles, mechanism of AMR & their molecular subtypes (Table 3). Majority of the isolates were resistant to nalidixic acid NALR (97.6%) followed by ciprofloxacin (23.8%). Isolation rates of MDR (resistance to ampicillin, chloramphenicol, cotrimoxazole with or without tetracycline resistance) *S.* Typhi and only tetracycline and cotrimoxazole resistant (nonMDR) *S.* Typhi were 4.2% and 0.76%. AMR markers (*bla*TEM-1, *cat*A, *sul*1, *sul*2, *dfr*A15, *str*A-*str*B, class 1 integron with dfrA7 & dfrA15) genes were detected in both MDR & non MDR isolates by PCR. A single non-conjugative plasmid of 180 kb and IncH1 plasmid (230kb) was found in 81.8% (9/11, ACQ) and 9% (1/11, ACTQ) MDR isolates (Table 4). One MDR isolate was without any plasmid. A Single conjugative 50kb, IncN type plasmids were found in two non MDR isolates. 65.7%, 171/260 of *S.* Typhi belong to H58 haplotype. 100 representative *S.* Typhi strains of different AMR profiles were analyzed by Pulse field gel electrophoresis (PFGE) (Fig 1) and clustered regularly intersparced short palindromic repeats (CRISPR). The isolates were subtyped into four distinct clusters with 81% similarity and twenty-five (P1 to P25) pulsotypes by PFGE and three different sequence types by CRISPR typing. MLVA analysis was done for 63 representative *S.* Typhi isolates belonging to different clusters of PFGE profile. Nine clusters and fifty nine MLVA types (M1 to M59) were formed using six VNTR loci. The clonal MDR, NAL^R and NAL^R-CIP^R *S.* Typhi isolates in PFGE Profile could be differentiated by using MLVA. The study reiterates the importance of monitoring of AMR profiles and molecular subtypes of locally circulating *S.* Typhi isolates for better understanding of the epidemiology, transmission and treatment modalities of the disease.

Table 3: Antimicrobial resistance profiles of *S.* Typhi Kolkata isolates 2014-16

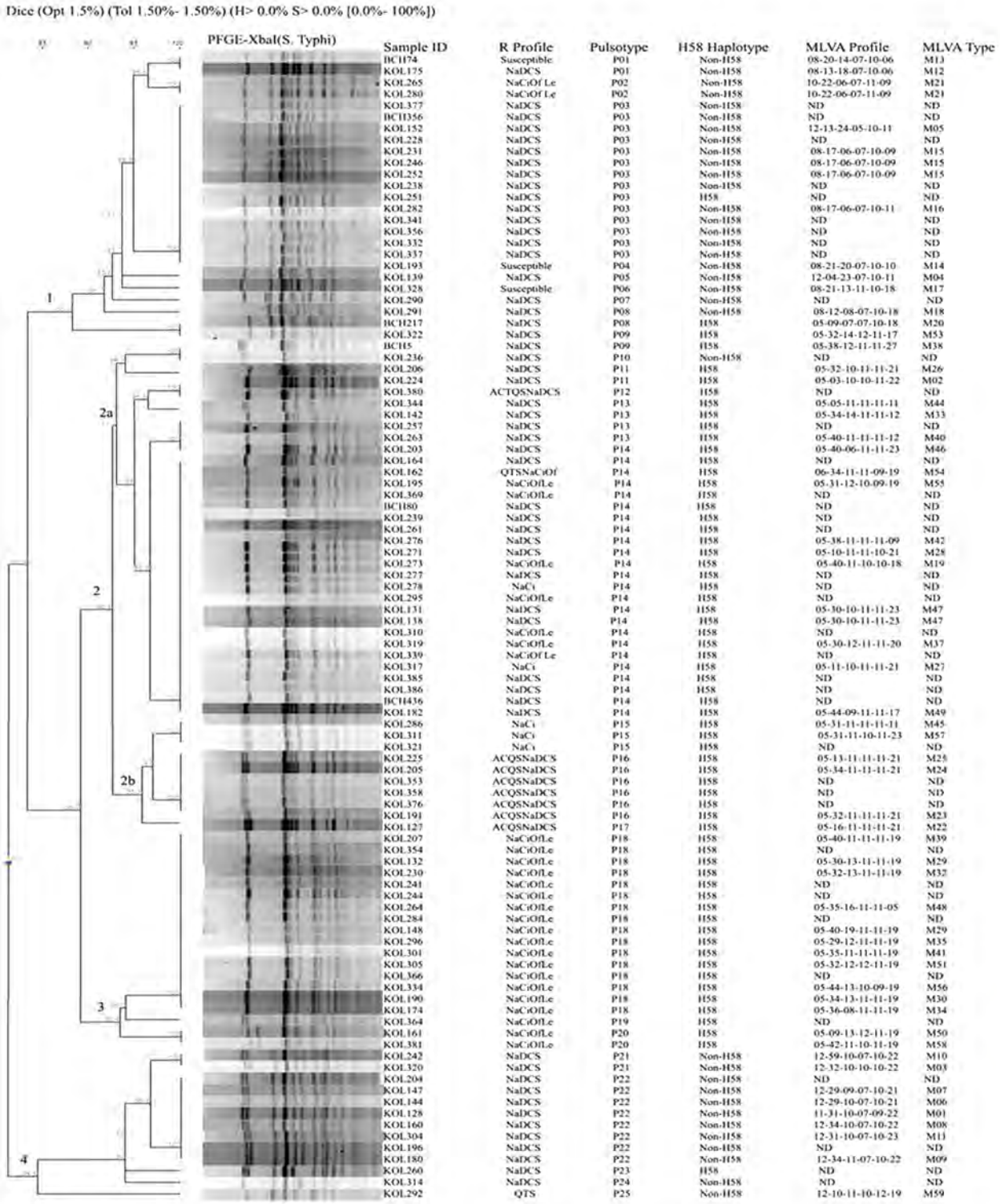
Resistance profile	*S.*Typhi isolates(n= 260)
Na^RDCS	180/260 (69.2%)
$NaCi^R$	9/260 (3.4%)
$NaCiOfLe^R$	52/260 (20%)
$QTSNaCiLe^R$	1/260 (0.38%)
QTS^R	1/260 (0.38%)
$ACQSNa^R$	10/260 (3.8%)
$ACTQSNa^R$	1/260 (0.38%)

Na^R Nalidixic acid resistance
$NaCi^R$ Nalidixic acid and ciprofloxacin resistance
$NaCiOf^R$ Nalidixic acid, ciprofloxacin and ofloxacin resistance (FQ, Fluoroquinolone group resistant)

Table 4: Presence of antimicrobial resistance genes in *S.* Typhi (n= 13) isolates

Sample ID	Antimicrobial resistance group (n)	R Profile	Plasmid-Type, size(kb)[a]	Detection of resistance genes by PCR Amplification								
				bla_{Tem}	*catA1*	*strAB*	*tetA*	*tetB*	*sul1*	*sul2*	*sul3*	Sizes of class 1 integrons (gene cassettes)[d]
KOL127	MDR (n=11)	ACQNaS	NP	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL171		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL191		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL202		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL205		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL225		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL353		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL358		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL376		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
BCH480		ACQNaS	180	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL380		ACTQNaS	IncH, 230	+	+	+	-	-	+	+	-	750bp (*dfrA7*)
KOL162	Non MDR (n=2)	TQNaCiOf	IncN, 50	-	-	-	+	-	+	-	-	1.6kb(*dfrA15,aadA1*)
KOL292		TQS	IncN, 50	-	-	+	-	+	+	+	+	1.6kb(*dfrA15,aadA1*)

Abbreviation used A, ampicillin; C, chloramphenicol; Q, co-trimoxazole; T, tetracycline; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin.



Sample ID	R Profile	Pulsotype	H58 Haplotype	MLVA Profile	MLVA Type
BCH174	Susceptible	P01	Non-H58	08-20-14-07-10-06	M13
KOL175	NaDCS	P01	Non-H58	08-13-18-07-10-06	M12
KOL265	NaCiOf Le	P02	Non-H58	10-22-06-07-11-09	M21
KOL280	NaCiOf Le	P02	Non-H58	10-22-06-07-11-09	M21
KOL377	NaDCS	P03	Non-H58	ND	ND
BCH356	NaDCS	P03	Non-H58	ND	ND
KOL152	NaDCS	P03	Non-H58	12-13-24-05-10-11	M05
KOL228	NaDCS	P03	Non-H58	ND	ND
KOL231	NaDCS	P03	Non-H58	08-17-06-07-10-09	M15
KOL246	NaDCS	P03	Non-H58	08-17-06-07-10-09	M15
KOL252	NaDCS	P03	Non-H58	08-17-06-07-10-09	M15
KOL238	NaDCS	P03	Non-H58	ND	ND
KOL251	NaDCS	P03	H58	ND	ND
KOL282	NaDCS	P03	Non-H58	08-17-06-07-10-11	M16
KOL341	NaDCS	P03	Non-H58	ND	ND
KOL356	NaDCS	P03	Non-H58	ND	ND
KOL332	NaDCS	P03	Non-H58	ND	ND
KOL337	NaDCS	P03	Non-H58	ND	ND
KOL193	Susceptible	P04	Non-H58	08-21-20-07-10-10	M14
KOL139	NaDCS	P05	Non-H58	12-04-23-07-10-11	M04
KOL328	Susceptible	P06	Non-H58	08-21-13-11-10-18	M17
KOL290	NaDCS	P07	Non-H58	ND	ND
KOL291	NaDCS	P08	Non-H58	08-12-08-07-10-18	M18
BCH217	NaDCS	P08	H58	05-09-07-07-10-18	M20
KOL322	NaDCS	P09	H58	05-32-14-12-11-17	M53
BCH5	NaDCS	P09	H58	05-38-12-11-11-27	M38
KOL236	NaDCS	P10	Non-H58	ND	ND
KOL206	NaDCS	P11	H58	05-32-10-11-11-21	M26
KOL224	NaDCS	P11	H58	05-03-10-10-11-22	M02
KOL380	ACTQSNaDCS	P12	H58	ND	ND
KOL344	NaDCS	P13	H58	05-05-11-11-11-11	M44
KOL142	NaDCS	P13	H58	05-34-14-11-11-12	M33
KOL257	NaDCS	P13	H58	ND	ND
KOL263	NaDCS	P13	H58	05-40-11-11-11-12	M40
KOL203	NaDCS	P14	H58	05-40-06-11-11-23	M46
KOL164	NaDCS	P14	H58	ND	ND
KOL162	QTSNaCiOf	P14	H58	06-34-11-11-09-19	M54
KOL195	NaCiOfLe	P14	H58	05-31-12-10-09-19	M55
KOL369	NaCiOfLe	P14	H58	ND	ND
BCH80	NaDCS	P14	H58	ND	ND
KOL239	NaDCS	P14	H58	ND	ND
KOL261	NaDCS	P14	H58	ND	ND
KOL276	NaDCS	P14	H58	05-38-11-11-11-09	M42
KOL271	NaDCS	P14	H58	05-10-11-11-10-21	M28
KOL273	NaCiOfLe	P14	H58	05-40-11-10-10-18	M19
KOL277	NaDCS	P14	H58	ND	ND
KOL278	NaCi	P14	H58	ND	ND
KOL295	NaCiOfLe	P14	H58	ND	ND
KOL131	NaDCS	P14	H58	05-30-10-11-11-23	M47
KOL138	NaDCS	P14	H58	05-30-10-11-11-23	M47
KOL310	NaCiOfLe	P14	H58	ND	ND
KOL319	NaCiOfLe	P14	H58	05-30-12-11-11-20	M37
KOL339	NaCiOf Le	P14	H58	ND	ND
KOL317	NaCi	P14	H58	05-11-10-11-11-21	M27
KOL385	NaDCS	P14	H58	ND	ND
KOL386	NaDCS	P14	H58	ND	ND
BCH436	NaDCS	P14	H58	ND	ND
KOL182	NaDCS	P14	H58	05-44-09-11-11-17	M49
KOL286	NaCi	P15	H58	05-31-11-11-11-11	M45
KOL311	NaCi	P15	H58	05-31-11-10-11-23	M57
KOL321	NaCi	P15	H58	ND	ND
KOL225	ACQSNaDCS	P16	H58	05-13-11-11-11-21	M23
KOL205	ACQSNaDCS	P16	H58	05-34-11-11-11-21	M24
KOL353	ACQSNaDCS	P16	H58	ND	ND
KOL358	ACQSNaDCS	P16	H58	ND	ND
KOL376	ACQSNaDCS	P16	H58	ND	ND
KOL191	ACQSNaDCS	P16	H58	05-32-11-11-11-21	M23
KOL127	ACQSNaDCS	P17	H58	05-16-11-11-11-21	M22
KOL207	NaCiOfLe	P18	H58	05-40-11-11-11-19	M39
KOL354	NaCiOfLe	P18	H58	ND	ND
KOL132	NaCiOfLe	P18	H58	05-30-13-11-11-19	M29
KOL230	NaCiOfLe	P18	H58	05-32-13-11-11-19	M32
KOL241	NaCiOfLe	P18	H58	ND	ND
KOL244	NaCiOfLe	P18	H58	ND	ND
KOL264	NaCiOfLe	P18	H58	05-35-16-11-11-05	M48
KOL284	NaCiOfLe	P18	H58	ND	ND
KOL148	NaCiOfLe	P18	H58	05-40-19-11-11-19	M29
KOL296	NaCiOfLe	P18	H58	05-29-12-11-11-19	M35
KOL301	NaCiOfLe	P18	H58	05-35-11-11-11-19	M41
KOL305	NaCiOfLe	P18	H58	05-32-12-12-11-19	M51
KOL366	NaCiOfLe	P18	H58	ND	ND
KOL334	NaCiOfLe	P18	H58	05-44-13-10-09-19	M56
KOL190	NaCiOfLe	P18	H58	05-34-13-11-11-19	M30
KOL174	NaCiOfLe	P18	H58	05-36-08-11-11-19	M34
KOL364	NaCiOfLe	P19	H58	ND	ND
KOL161	NaCiOfLe	P20	H58	05-09-13-12-11-19	M50
KOL381	NaCiOfLe	P20	H58	05-42-11-10-11-19	M58
KOL242	NaDCS	P21	Non-H58	12-59-10-07-10-22	M10
KOL320	NaDCS	P21	Non-H58	12-32-10-10-10-22	M03
KOL204	NaDCS	P22	Non-H58	ND	ND
KOL147	NaDCS	P22	Non-H58	12-29-09-07-10-21	M07
KOL144	NaDCS	P22	Non-H58	12-29-10-07-10-21	M06
KOL128	NaDCS	P22	Non-H58	11-31-10-07-09-22	M01
KOL160	NaDCS	P22	Non-H58	12-34-10-07-10-22	M08
KOL304	NaDCS	P22	Non-H58	12-31-10-07-10-23	M13
KOL196	NaDCS	P22	Non-H58	ND	ND
KOL180	NaDCS	P22	Non-H58	12-34-11-07-10-22	M09
KOL260	NaDCS	P23	H58	ND	ND
KOL314	NaDCS	P24	Non-H58	ND	ND
KOL292	QTS	P25	Non-H58	12-10-11-10-12-19	M59

Fig 1: PFGE Profile of 100 *S.* Typhi strains

Fig 1 : Dendrogram showing the cluster analysis of 100 *S.*Typhi isolates from Kolkata, India, 2014-16, by *XbaI*-PFGE. Band comparison was performed by using the Dice coefficient with 1.5% optimization (Opt) and 1.5% position tolerance (Tol). MLVA Profile, H58 haplotype & R Profile of the 50 strain is also shown in the figure.

Title: Seasonal dynamics of enteropathogenic bacteria in Gulf of Khambat, Gujarat: its impact on health of coastal population

Investigator: A. Palit

Work Done So Far:

Gulf of Khambat (GoK) is located in the eastern fringe of Gujarat. Anthropogenic pressure and draining of sewages in the gulf create conducive environment for the growth of many entero-pathogens. Due to presence of low lying inundated area, flood water may help to spread the pathogens from marine to inland and *vice versa*. In spite of frequent reports of enteric diseases, the route of disease transmission is not clear in Gujarat. It is assumed that favourable environmental condition triggers the proliferation of different entero-pathogens in the gulf and they may transmit to coastal population using ground/freshwater and marine organisms as transmission vehicle.

In the proposed study a multi dimensional approach will be followed to evaluate the distribution pattern of entero-pathogens in water, sediments and marine organisms of GoK, the influence of different physico-chemical indices on fluctuation of these bacteria.

FIELD EXPEDITION :

Sampling strategy

The sampling strategy involves collection of the water samples from the different points of the Gulf of Khambhat (GoK) during the different tidal regimes.

STUDY SITES :

Site 1 Koliyak :

The place is located very close to the Bhavnagar city on the GoK. The Gulf at this point is moderately wide and the tidal difference at this point is about 20 m. The point is marked by the presence of a Temple in the middle of the Gulf.

Site 2 Alang :

This site is located further south and the gulf is very wide and deep at this site. This place has the largest ship wreckage site of the world and the tidal difference is less than site 1. Oil spillage is visible in the water samples at this site.

Site 3 Ghoghola :

This site a located near Diu which is further south, the gulf is very wide at this site. This site has greater sea influence. The samples were collected from a small water channel which is very important fishing harbour.

Site 4 Porbander :

Water samples were collected from a backwater channel which has a direct link with the Arabian Sea. The water at this point flows between the Porbander city and has huge anthropogenic influence.

Site 5 Gomti estuary :

This is the mouth of the Gomti River where it opens into the Arabian sea. The site is a mixing zone of the river and sea and has a tidal range of about 5 m. The river water is used for many religious rituals.

COLLECTION OF SAMPLES:

Collection Procedure

Samples were collected from the sampling sites (about 5 meter from the shore). During the sample collection, three to four sub-samples of surface waters were collected by a metallic pot from 5m into the Gulf and pooled together in a sterile container. Subsequently, the samples were divided into aliquots of 50 mL.each and transported to the central laboratory in ice-chilled (4°C approx.), dark thermocol boxes.

Physico-chemical analysis

Immediately after collection, samples were subjected to physico-chemical analyses, viz. Temperature, pH, conductivity and salinity by a hand held multi-meter (WTW, Weilheim, Germany). The turbidity was measured consequently by a portable turbidi-meter (Eurotech, Singapore) (Table 5).

Bacteriological analysis

After transportation of the water samples to the central laboratory, microbiological parameters like Total Bacterial Count (TBC); Total Coliform Count (TCC); Total *Escherichia coli* Count (TEC); Total Fecal Coliform Count (TFCC); Cultivable *Vibrio* Count (CVC) has been determined by the spread plate method (Fig 2, 3).

Briefly, 100µl to 200µl of each of the serially diluted samples has been spread over Nutrient Agar (Becton, Dickinson) for TBC; 100µl to 1 ml of samples has been spread on Chromocult Coliform agar (Merck, Darmstadt) for TCC and TEC, on MFC agar (Hi-Media, Mumbai) for TFCC and on Thiosulphate Citrate Bile salt Sucrose Agar (TCBS; Becton, Dickinson) for CVC determination respectively followed by an overnight incubation at 37°C for all types of bacterial count and 44°C for fecal coliform count. All the samples for different parameters were repeated for at least 3 times and average values were recorded.

Table 5: Physico- chemical attributes of the GULF samples

SOURCE / PARAMETERS	TIDE	GoK				
		SITE 1`	SITE 2	SITE 3	SITE 4	SITE 5
TEMPERATURE(°C)	HIGH	27.4	26.4	32.2	29.6	31.4
	LOW	28.2	27.2	30.6	30.2	33.1
pH	HIGH	7.55	7.51	7.62	7.25	7.68
	LOW	7.56	7.71	7.73	7.06	7.82
SALINITY (PSU)	HIGH	33.7	33.3	36.3	36.9	36.1
	LOW	33.4	32.5	35.8	36.7	36
TURBIDITY(NTU)	HIGH	720	472	50.8	9.8	3.64
	LOW	556	546	40.9	10.6	0.94
CONDUCTIVITY (mS/ cm)	HIGH	53.2	52.8	56.9	55.8	57.0
	LOW	50.3	51.5	56.2	57.5	56.8

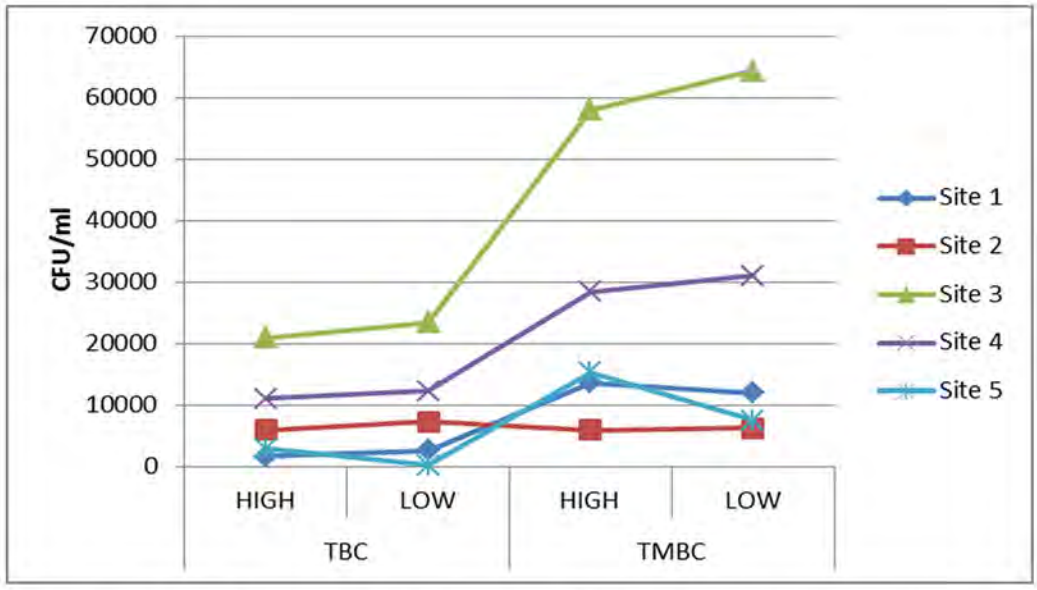


Fig 2 : Total Bacterial Count of The Gulf of Khambat, Gujarat Water Samples

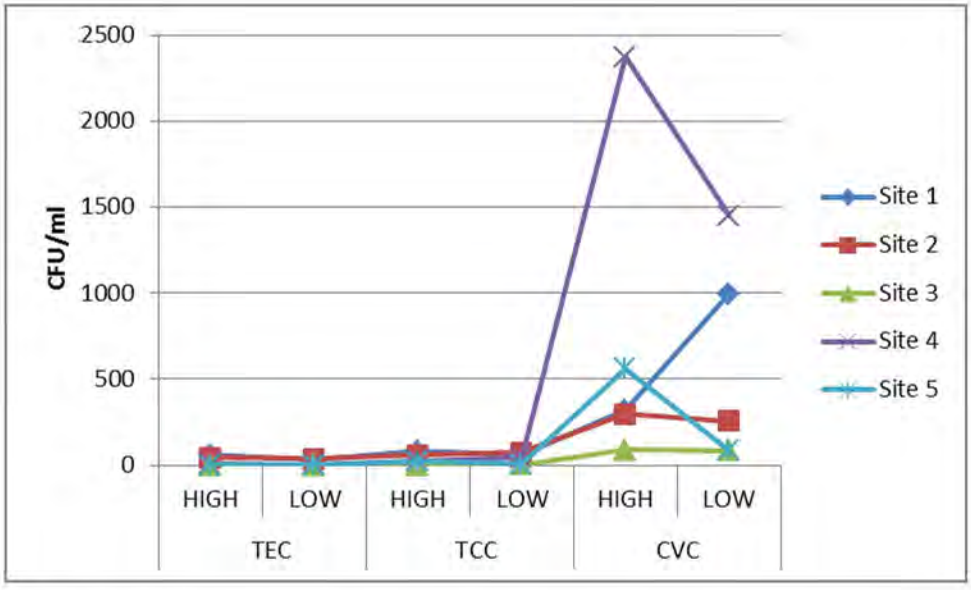


Fig 3 : *E.coli*, *Vibrio* Count of The Gulf of Khambat, Gujarat Water Samples

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

Investigator: 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:

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 (Fig-4a,b).

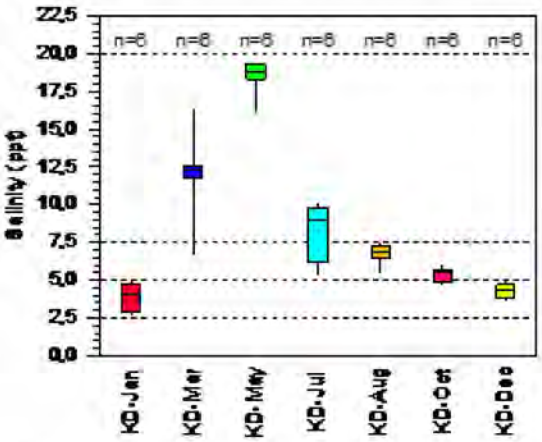


Fig 4a : Seasonal variation of Salinity at Kakdwip

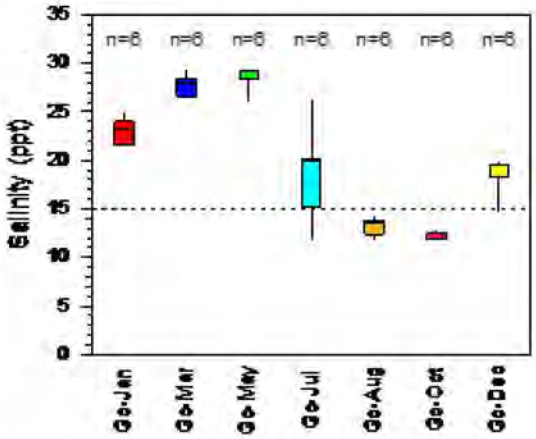


Fig 4b : Seasonal variation of Salinity at Gosaba

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 (Fig-5).

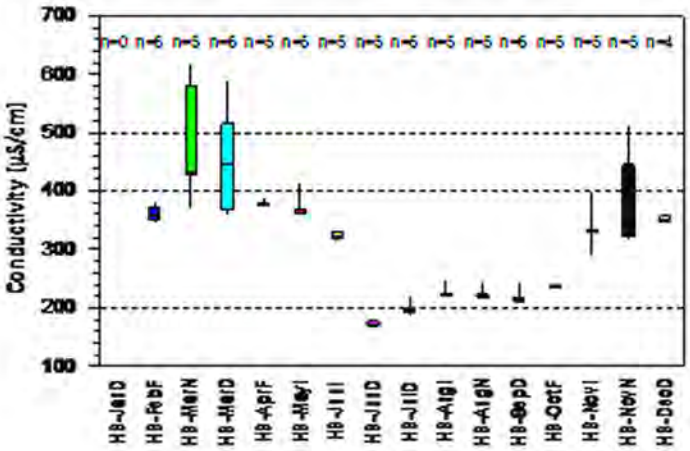


Fig 5: Seasonal variation of Salinity at Diamond Harbour

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 is in low saline inland area (Fig-6).

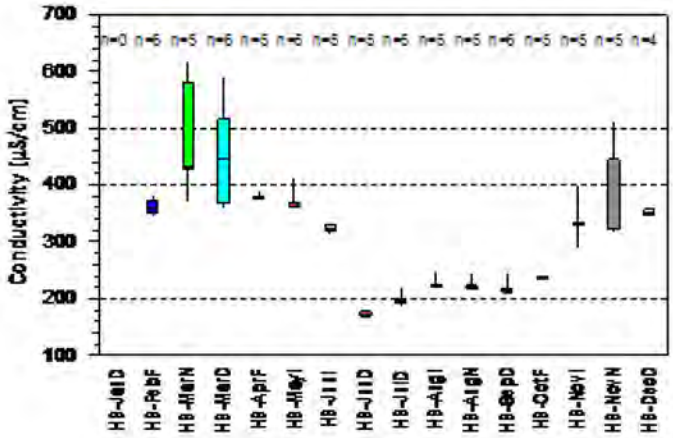


Fig 6 : Seasonal variation of conductivity at Howrah

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 rainfall/downpour could be visualized during rainy period. At two different periods, viz., June–September and February– April, the concentration of SPM at sea end stations are orders of magnitude greater, suggesting that this part of the channel represents zone of estuarine turbidity maximum (ETM).

V. parahaemolyticus could be isolated from 27/120 (22.5%) samples, being mostly prevalent in the high saline zone (Site III) (21/27), to some lesser extent at Diamond Harbour (6/21) and was completely absent at Howrah. 19 samples (15.8%) were found to harbour *V. alginolyticus* and *V. vulnificus* could be isolated from 14 (11.6%) samples. 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 from 23 water samples (19.1%) with highest preponderance at Site I (12/23), followed by Site II (8/23) and Site III (3/23) respectively. *V. cholerae* non-O1/O139 was the most prevalent enteropathogen among all the five species of non-cholera Vibrios, which was present in 42 (35%) water samples. Highest abundance of *V. cholerae* non-O1/O139 was also observed at Site I (24/42) at salinity <0.1ppt. *V. cholerae* O1 was present in 19 (15.8%) water samples and showed distinct seasonality as reported in our earlier studies. Seasonal prevalence of Vibrios was highest in the monsoon months (20–34°C), followed by summer (24–36°C) and winter (13–18°C) months respectively (Table 6). Irrespective of any organism or any site, monsoon seems to be the most favourable condition with maximum isolation rate achieved during the period.

Table 6: Physico-chemical properties of riverine-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

NTU- Nephelometric Turbidity Unit

Irrespective of the collection site, 69 *V. cholerae* isolates were obtained in the monsoon, post-monsoon and summer seasons. In winter season, we came across the least number (5) of isolates.

All of the NOVC were negative for *ctx* and O1/O139 (*rfb*) genes, but harboured a host of associated virulence genes. *hlyA* and *rtxA* genes were detected in respective highest percentages of 94% and 80% among these isolates, followed by *toxR* (28%) (Table 7). The other virulence genes (*tcp, tlcR, toxT, RJ & LJ, aldA*) could be isolated only among 3-5% (Table 7) of the NOVC. Interestingly, the isolates from Howrah depicted higher preponderance of toxin genes among them than those isolated from Diamond Harbour. The *V. cholerae* from other sites were devoid of any such toxin genes.

A total of 68 isolates were identified as non O1/ non O139 *V.cholerae* (NOVC) while 6 isolate being *V. cholerae* O1, Serovar Ogawa. No *V. cholerae* O139 could be isolated from the environmental sources.

Table 7: Toxin gene profile of *V. cholerae* isolates

Sero type	Source	Number of isolates	Toxin genes										
			ctx	*tcp*	*hlyA*	*O1/ O139*	*rtxA*	*tlc*	*zot*	*toxR*	*toxT*	*aldA*	*RJ & LJ*
NOVC	HB	46	0 (0%)	2 (4.3%)	43 (93.4%)	0 (0%)	38 (82.6%)	3 (6.5%)	3 (6.5%)	11 (23.9%)	1 (2.1%)	2 (4.3%)	2 (4.3%)
	DH	22	0 (0%)	0 (0%)	21 (95.4%)	0 (0%)	17 (77.2%)	0 (0%)	0 (0%)	4 (18.1%)	1 (4.5%)	0 (0%)	0 (0%)
VCO1	HB	5	4 (80%)	3 (60%)	5 (100%)	5 (100%)	4 (80%)	2 (40%)	3 (60%)	4 (80%)	4 (80%)	4 (80%)	4 (80%)
	DH	1	1 (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Total		74	5	5	70	2	59	5	6	24	7	7	7

Title: Comparison of boosting impact of inactivated polio vaccine (IPV) and oral polio vaccine (OPV) among infants residing in Kolkata

NICED PI: S. Kanungo, R. K. Nandy

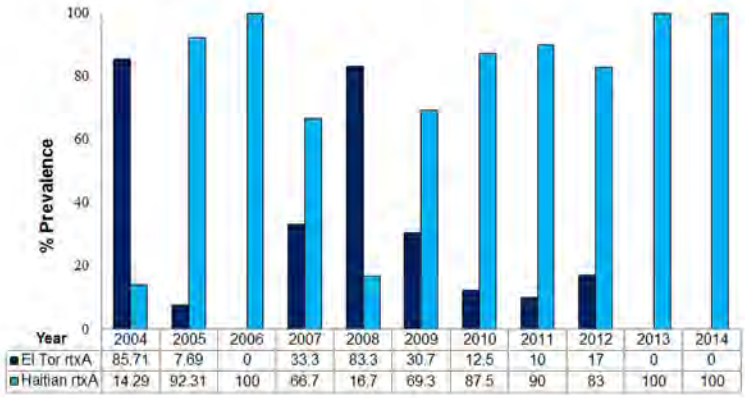
An open labelled randomized controlled trial was initiated with support from International Vaccine Institute (IVI), Korea. Through this study impact of IPV boost was estimated among 39 weeks old infants (n=172) and data was compared to boosting effect as induced by administration of OPV in age sex matched another group of subjects (n=172). As a part of the study protocol, all recruited subjects received 3 doses of OPV at 6, 10 and 14 weeks of their life in addition to doses from pulse polio immunization program and then randomized at week 39 into two groups of which subjects in one group received IPV and the other received OPV. Blood specimens were collected from the subjects at week 40 and estimation of serum neutralizing antibodies was performed at the Enterovirus Research Centre (Mumbai, India) following a standardized micro-neutralization assay to detect antibodies to poliovirus types 1, 2, and 3. Polio neutralizing serum antibody titer of 1:8 or greater at week 40 was considered seropositive while a titer of less than 1:8 was considered seronegative. Fecal shedding of poliovirus was estimated among subjects of both the groups after administration of OPV at week 52. Shedding of viruses in the stool was used as an index to represent intestinal mucosal immunity.

Direct fecal RT-qPCR was used for detection as well as quantitation of viral shedding in the stool specimens over a period of ~4 weeks since administration of OPV at week 52. Analysis showed that IPV boost resulted into significantly higher seroconversion rates for Polio type 2 ($p= 0.03$) and Polio type 3 ($p< 0.01$) as compared to OPV boost. However, intestinal mucosal immunity at week 52 infants remained comparable between two groups with a total shedding prevalence of 28%. Results of this study can be considered as a part to support the final endgame strategy of global polio eradication initiative which includes switching from trivalent oral poliovirus vaccines (tOPV) to bivalent oral polio vaccine (bOPV), and introduction of IPV. We concluded that introduction of an IPV boost into the EPI program in India would be equally beneficial to the existing OPV boost. In addition, IPV for OPV boost to be considered a step forward in the global polio eradication initiative to reduce the problem of circulating vaccine-derived poliovirus (cVDPV).

Title: Analysis of *Vibrio cholerae* O1 strains from Delhi that trace the origin of Haitian-like genetic traits

Investigator: A. K. Mukhopadhyay

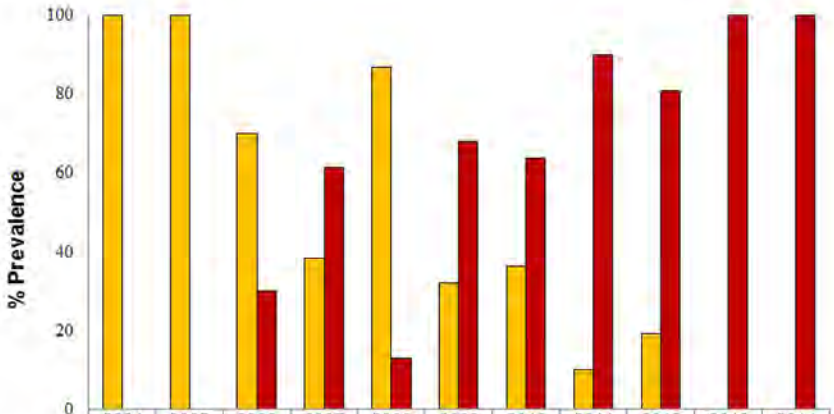
Cholera, the life threatening ancient intestinal infection still remains as a serious health burden in the developing countries where sanitation and supply of clean drinking water are limited. *Vibrio cholerae* O1 is the etiological agent of this severe dehydrating diarrheal disease. The bacterium has recently been causing outbreaks in Haiti with catastrophic effects. This episode of cholera placed the disease at the forefront of the global public health agenda. The outbreak has killed around 8000 Haitians, and infected over 600,000 to date. Recent study on the global phylogeographic patterns of the *V. cholerae* strains isolated from diverse geographic areas provide strong molecular evidence pointing to a nonindigenous source of the 2010 Haitian cholera outbreak. Numerous mutations have been reported in *V. cholerae* O1 strains associated with the Haitian outbreak. These mutations encompass among other the genes encoding virulence factors such as the pilin subunit of the toxin-co-regulated pilus (*tcpA*), cholera toxin B subunit (*ctxB*), repeat in toxins (*rtxA*), and other genes such as the quinolone resistance-determining region (*QRDR*) of gyrase A (*gyrA*), *rstB* of RS element along with the alteration in the number of repeat sequences at the promoter region of *ctxAB*. Given the numerous genetic changes in those Haitian isolates, we decided to investigate the possible origins of those variations in the Indian subcontinent. Thus, we determined the genetic traits among *V. cholerae* O1 strains in Delhi, India. A total of 195 strains isolated from cholera patients during 2004 to 2014 were analysed during this study. Our results showed that all the tested strains carried Haitian type *tcpA* (*tcpACIRS*) and variant gyrA indicating their first appearance before 2004 in Delhi. The Haitian variant *rtxA* and *ctxB7* was first detected in Delhi during 2004 and 2006, respectively (Fig 7 and Fig 8). Interestingly, not a single strain with the combination of El Tor *rtxA* and *ctxB7* was detected in this study. The Delhi strains carried four heptad repeats (TTTTGAT) in the CT promoter region whereas Haitian strains carried 5 such repeats. Delhi strains did not have any GTA deletion mutations in the *rstB* gene like Haitian strains. Overall, this study demonstrated the sequential accumulation of Haitian-like genetic traits among *V. cholerae* O1 strains in Delhi at different time frames, which may have a severe threat to the public health and the accumulation events occurred long before the Haitian cholera outbreak. Studies of such changes along with the evolution of new variant *V. cholerae* strains in different parts of the world may have implications to combat cholera.



Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
El Tor rtxA	85.71	7.69	0	33.3	83.3	30.7	12.5	10	17	0	0
Haitian rtxA	14.29	92.31	100	66.7	16.7	69.3	87.5	90	83	100	100

Fig 7: Isolation profile of variant *rtxA* in Delhi.

Occurrence of *rtxA* allele in Delhi *Vibrio cholerae* O1 strains from 2004 to 2014. *V. cholerae* O1 strains with variant *rtxA* was first time isolated in Delhi during 2004. After the appearance and gradual surge of the *rtxA*-null- mutant up to 2007, El Tor *rtxA* allele further became dominant during 2008. Then the El Tor *rtxA* was slowly replaced by the variant *rtxA* since 2009. Total replacement of El Tor *rtxA* by the Haitian *rtxA* occurred from 2013 onwards.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Classical ctxB	100	100	70	38.46	86.95	32	36.36	10	19.24	0	0
Haitian ctxB	0	0	30	61.53	13.05	68	63.64	90	80.76	100	100

Fig 8 : Retrospective analysis of *ctxB* allele in *V. cholerae* Delhi isolates.

Occurrence of *ctxB* allele type in Delhi *Vibrio cholerae* O1 strains from 2004 to 2012. *V. cholerae* O1 strains with Haitian type of *ctxB* was isolated in Delhi for the first time in the year 2006 and interestingly there was a sudden increase in the isolation profile of *V. cholerae* O1 strains with *ctxB7* allele during the year 2007 encompassing a sudden decrease in the year 2008. After this declining episode of *ctxB7* allele, the percentage of the O1 isolates with *ctxB7* allele started to increase from 2009 and around 80% Delhi strains carried ctxB7 allele in the year 2012. Total replacement of classical *ctxB* by the Haitian *ctxB* occurred from 2013 onwards.

Title: Development of a method for rapid detection of azithromycin resistant *Campylobacter* isolated from diarrhoeal patients

Investigator: A. K. Mukhopadhyay

Campylobacter species, particularly *Campylobacter jejuni*, has been documented as an important pathogen for causing acute bacterial gastroenteritis in humans. It is projected that around 400–500 million cases of diarrhea are caused by the *Campylobacter* sp. each year, worldwide. In food borne diarrheal illness, *Campylobacter* stood as second etiologic agent among the enteric pathogens. *Campylobacter* are part of normal enteric flora of a wide range of domestic animals and poultry as well as wild animals and birds. So, *Campylobacter* can be transmitted to humans mainly through the consumption of contaminated foods of animal origin, especially undercooked poultry meat, unpasteurized milk and dairy products, as well as by the ingestion of other foods that are cross-contaminated by raw poultry meat during food preparation. Though *Campylobacter* mediated gastroenteritis is self-limiting; antibiotic therapy is needed to reduce severity of disease. The most common drugs used to control *Campylobacter* mediated infections are fluoroquinolones and macrolides. High resistance towards fluoroquinolones has shifted the treatment towards macrolides. Generally, the prevalence of macrolide resistance among *Campylobacter* strains isolated from humans, broilers and cattle in the USA and Canada has been reported at around 10%. In contrast, more than 40% of *C. coli,* isolated from turkeys and swine in the USA, were resistant to this antimicrobial agent. In northern part of India, the macrolide resistance was 6.1% during 2005 and reached 22.2% in 2013 whereas, 0.7% macrolide resistance was reported in eastern India during 2008 to 2010 and increased to 4% during 2010-2012.

Macrolide resistance in *Campylobacter* is found mainly due to point mutation in V-region of 23S rRNA. We have developed a PCR based assay, which can detect the azithromycin resistant and sensitive *Campylobacter*

strains utilizing mutation responsible for the phenotype. This PCR was validated using 359 *Campylobacter* strains isolated from diarrhoeal patients at Kolkata, India (Fig 9). Antimicrobial resistance through disk diffusion method was also performed on these strains as a gold standard. PCR based detection system showed 100% sensitivity and specificity when compared with the gold standard method. Studies through sequencing analysis further confirmed the PCR result. So, our study describes a simple and rapid method for detection of mutation conferring macrolide resistance with additional feature of identification of sensitive strains.

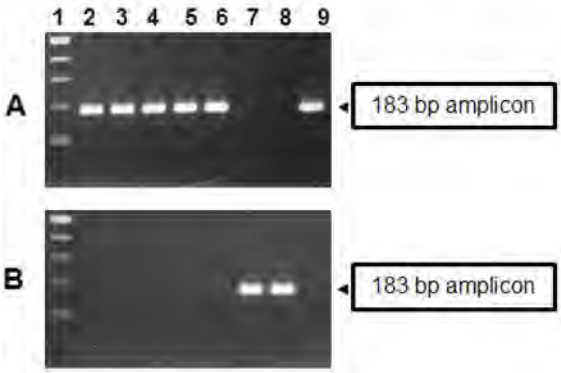


Fig 9

Fig 9 :

PCR based assay to detect the azithromycin resistant strains using primers 23srRNA-Campy-1912F / 23srRNA-Campy-2075R [A] and sensitive strains using primers 23s-rRNA-Campy-1912F / 23s-rRNA-Campy-2074N-Rev [B] in representative *Campylobacter* strains of Kolkata. Lane 1 contains a 100-bp size ladder. Lanes 2 to 6 and 9- representative resistant strains. Lanes 7 and 8- sensitive strains.

Title: Transmission of carbapenem-resistant genes

NICED PI: S. Basu

The efficiency with which an antibiotic resistance gene is transferred from one bacterium to another has huge clinical implications. How fast and how far the gene will spread depends largely on this competence to be transmitted. We studied the mode of transmission of New Delhi-metallo-β-lactamase-1 (NDM-1) both via mobile genetic elements and via outer membrane vesicles (OMVs).

OMVs are part of a secretion-delivery system used by many Gram-negative bacteria, which allows the long-distance dissemination of different bacterial proteins and DNA. The study was the first experimental evidence of intra and inter-species transfer of plasmid-harbouring bla_{NDM-1} gene in *A. baumannii* via OMVs with high transformation frequency. The *A. baumannii* strain (ST 1462) released vesicles (25-100nm) during in vitro growth at late log phase. bla_{NDM-1} and *aac(6')-Ib-cr* genes were transferred in both the *A. baumannii* ATCC 19606 and *E. coli* JM109 recipient cells. Transformation frequency of the purified OMVs was in the range of 10^{-5} to 10^{-6} and transformation frequency was gradually reduced with storage of OMVs. The size of the plasmids in the transformants and their restriction digestion pattern was identical to the plasmid in A_115. The transformants showed elevated MIC values for β-lactam group of antibiotics which confirmed the presence of bla_{NDM-1}-harbouring plasmid.

Apart from OMV-mediated transmission the other modes of transmission particularly in Enterobactericae are via mobile genetic elements such as plasmids and transposons. bla_{NDM-1} was associated with diverse scaffolds , IncF type being the prevalent one . Genetic structures surrounding bla_{NDM-1} showed its association with at least a remnant of *ISAba125* at its 5'-end and ble_{MBL} at its 3'-end in Enterobacteriacae. Many questions still remain to be answered about these elements, particularly, why certain genes such NDM can spread extensively compared to some other similar genes and efforts in this direction has potential for better health outcomes.

Title: Shigella Outer Membrane Vesicles: Novel Particles of Next Generation Vaccine

Investigators: H. Koley, U. Bhoumik

In this hour where remedy against shigellosis is much needed, our lab is already dedicated to make an efficient vaccine against the disease. With this intend, we have advanced our work to the next generation vaccine with Outer Membrane Vesicles (OMVs) secreted by Shigella. At first the protective efficacy of OMVs from *Shigella boydii* type 4 was studied. This study showed a 70% and above protective efficacy against all the six major circulating Shigella strains such as, *Shigella dysenteriae* type 1, *Shigella flexneri* 2a, *Shigella flexneri* 3a, *Shigella flexneri* 6, *Shigella boydii* type 4 and *Shigella sonnei*. These six strains can cross protect all the

50 serotypes of Shigella. So it can be concluded that if hexavalent OMVs can show protection against those strains it can be effective against all the serotypes of Shigella. Anticipating this, our next step was to study the protective efficacy of hexavalent serotype OMVs. Hexavalent OMVs immunogen demonstrated about 80% protection against all the six strains. To overcome the problem of low yield of OMVs we gradually disrupted the tolA gene of Tol-Pal system of *Shigella boydii* type 4 which then showed about 60% increased rate of OMVs production. Now we have made three tolA deleted Shigella strains such as *Shigella flexneri* 2a, *Shigella boydii* 4 and *Shigella sonnei* phase -I.: TolA mutant strain showed less virulence in new guinea pig colon loop model. There is no significant change at mRNA level of Type III secretion regulators between Wild type and TolA mutant strains .TolA protein regulates post transcriptional regulation of Type III secretion system. There is a significant change at protein level of Type-III secretion effectors proteins. We could use tolA mutant strain as a novel live attenuated Shigella vaccine (Fig-10). Ultra-structure of these OMVs was compared with WT-OMVs. Guinea pigs (specific host of Shigella) were then immunized with our trivalent mixture of OMVs (each 25µg/dose) with an interval of 0, 14th and 28th day. With the immunization dose of OMVs mixture we have done MTT assay in RAW cell line, which showed no cytotoxic effect of our trivalent immunogen. Serum agglutination with immunized sera collected from guinea pigs demonstrated homologous protection by clotting of bacteria. Our study will continue towards the protective efficacy against homologous and heterologous strains and further work of immunomodulatory signaling of innate and adaptive immunity.

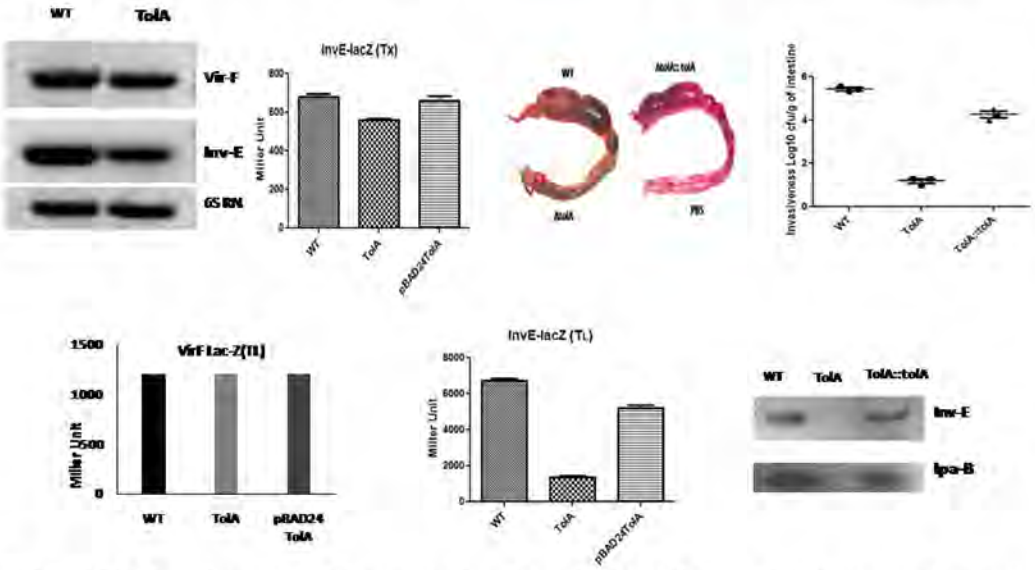


Fig: 10 TolA Regulates post Transcription Regulation of Type-III Secretion System

TolA mutant strain showed less virulence in new guinea pig colon loop model. There is no significant change at mRNA level of Type III secretion regulators between Wild type and TolA mutant strains TolA protein regulates post transscriptional regulation of Type III secretion system. There is a significant change at protein level of Type-III secretion effectors proteins. We could use tolA mutant strain as a novel live attenuated Shigella vaccine.

Title: Evaluation of host immune response of heat killed multi serotype Shigella against lethal Shigella infection mice model.

Investigators: H. Koley, D. Nag

We demonstrated after oral immunization with heat killed multi serotype Shigella (HKMS) immunogen sero-specific humoral immunity and both homologous and heterologous protective efficacy in different animal models. In our present work, we are focusing to study the host protective immune responses mainly cell mediated immune response generated by HKMS and how it affect on protective immune response in mice model. We found HKMS can significantly activate dendritic cells *in vitro* ; HKMS treated unimmunized DCs secrete Th1 and Th17 polarizing IL-12p70, IL-1β, IL-6 and IL-23 which indicate generation Th1 and Th17 immune response. Furthermore, we found generation of Th1/Th17 cell mediated immunity after oral

immunization of HKMS. Generation of cell mediated immune response is dependent on protein; we have indentified that outer membrane protein A (OmpA) of Shigella is one of the immunogenic protein of HKMS which did not alter its immunogenic function after heat treatment that can help generate cell mediated immune response (Fig 11). Then we also investigated protective immune response through intraperitoneal challenge adult mice model and we found that HKMS significantly induce protective immune response after lethal challenge of different serotype of Shigella strains. Then we have shown that protection against Shigella is dependent on both humoral and cell mediated immunity by adoptive transfer of immunized sera and splenic cells. HKMS induce cell mediated immune response without adding any adjuvant which can help to generate long term humoral immunity against Shigella.

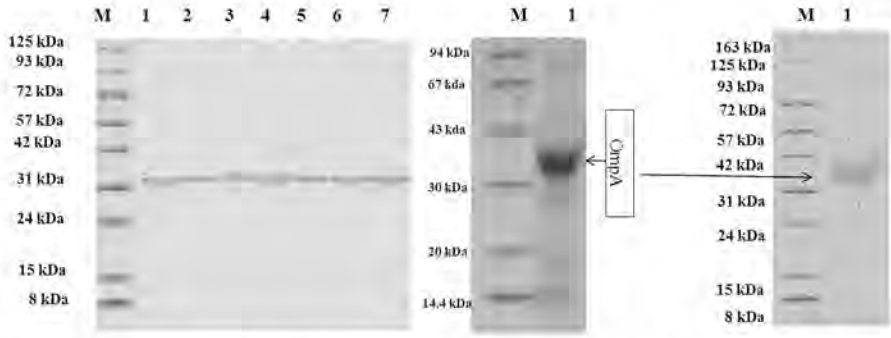


Fig-11 SDS-PAGE and Western blot analysis of HKMS Immunogen

SDS-PAGE and Western blot analysis of HKMS immunogen. (a) western blot of six strains after heat inactivation against anti-ompA sera from mice developed with BCIP-NBT shows band at 34 kDa position. (b) SDS-PAGE of purified ompA protein stained with Chomassi blue shows band of 34 kDa. (c) To confirm ompA's presence in HKMS immunogen. Western blot was carried out with purified ompA against anti-HKMS serum. It clearly shows that anti- HKMS sera contains anti-ompA antibody which gives a prominent at 34 kDa position.

Awards/ Honours Received

S.Dutta

- Awarded Prof YS Yolekar ICMR award for overall Microbiology research for the year 2014.
- Invited reviewer of the foiiowing peer reviewed international and national Journals: Indian Journal of Medical Research (IJMR), Journal of Antimocrobial Chemotherapy (JAC), Journal of Applied Microbiology (JAM), Journal of Medical Microbiology, BMC Infectious Diseases a BMC series Journal, PLOS One, PLOS Neglected Tropical Diseases, International Journal of Infectious Diseases (IJID), Clinical Infectious Diseases (CID)
- Invited as Associate Editor for BMC Infectious Diseases UK
- Invited External Project Reviewer of Swiss international Science Foundation

S. Basu

- Acted as reviewer for Lancet Infectious Disease, Antimicrobial Agents and Chemotherapy, Journal of Antimicrobial Chemotherapy, Future Microbiology, Journal of Global Microbial Resistance, Microbial Drug Resistance, BMC Infectious Disease, Infection Geneties & Evolution, PLOS One and Arab Journal of Gastroenterology .

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

S. Dutta

- Attended Scientific Advisory Group meeting of ECD held during 25-26 April, 2016 at ICMR Hqrs.
- Invited along with Shri Manoj Pant, Jt. Secretary, DHR, MoH&FW, GoI to attend a meeting of Principals and Medical Superintendents of State Medical Colleges organized by West Bengal Government on 14th May, 2016.
- Attended Food and Agriculture Organization (FAO) consultative meeting to develop a zoonotic emerging infectious disease project in partnership with Govt. of India during 20-22 May, 2016, Kolkata.
- Expert group meeting to finalize budget and proposal for the cholera vaccine implementation project in West Bengal to be funded by Gates Foundation at the Global Health Strategies, New Delhi on 27th May, 2016
- Participated in a round table discussion on "Women's Health issues: Broadening the Mandate beyond Maternal Health" on the occasion of International Women's Health Day organized by ICMR on Friday, 27th May 2016, 3.00 to 4.30 pm, at the Conference Hall, ICMR Hqrs., New Delhi.
- 2nd SAC meeting of NIPER, Kolkata at CSIR-IICB campus at 2-30 pm on 28th May, 2016.
- Attended Network meeting of WHO Collaborating Centers (CCs) in reproductive, maternal and newborn health in SEAR organized by NIRRH, Mumbai held during 7-8th June, 2016 organized by NIRRH, Mumbai.
- Delivered inaugural address at the Foundation Day symposium of the Society for Advancement of Human Development & Research held on 21st July, 2016 at NICED, Kolkata
- Attended the workshop on Impact of Climate Change on Human Health in India held during 25-26 July, 2016 at National Institute of Malaria Research, ICMR, New Delhi
- Attended meeting on the functioning of ICMR institutions as PvPI collaborating centres for research on Pharmacovigilance held on 28th July, 2016 at 2.00 pm at ICMR hq. AIIMS campus, New Delhi.
- Attended the Panel of Experts meeting to discuss strategies for determining the burden of typhoid and evaluating the role of the licensed conjugate vaccines and other interventions in India organized by CMC, Vellore held during 30-31st July, 2016 at Imperial Hotel, Janpath, New Delhi.
- Attended National Task Force meeting on Laboratory Containment of Wild Polioviruses in India on 24th August, 2016 at ICMR Hqrs., New Delhi
- Attended National Task Force meeting to finalize training modules and curriculum for the basic public health microbiology training of District Public Health Microbiologists organized by NCDC from August 29-31, 2016 at Hotel Le Meridien, New Delhi
- India Africa Health Sciences Meet (IAHSM) jointly organized by ICMR and Ministry of External Affairs, Govt. of India on September 1-3, 2016 at Vigyan Bhawan, New Delhi.
- Attending the inaugural program as special guest of honour and address the gathering at the National Seminar on "Analytical techniques for drug discovery & development from medicinal plants & natural products" organized by School of Natural Product Studies, Jadavpur University in association with the Society for Ethnopharmacology, India (SFE-India) at K.P. Basu Memorial Hall, Jadavpur University, Kolkata at 10 am on September 24, 2016
- Attended one Review Meeting taken by Hon'ble Minister of States, Smt. Anupriya Patel, held at All India Institute of Hygiene and Public Health, Kolkata at 11.45 a.m. on 24th September, 2016.
- Chief Guest of Valedictory Session of 62nd Orientation Program held at 11-30 a.m. on 27th September, 2016 at the UGC-HRDC, Jadavpur University, Salt Lake Campus.

- Attended an Informal Consultation of Experts on Typhoid Fever organized by IVD, WHO SEARO on 28-29 September, 2016 at WHO-SEARO, New Delhi
- Participated in the workshop on Strengthening Influenza Surveillance jointly organized by WHO with NCDC and IDSP during 4th to 6th October, 2016 at Hotel Lalit, New Delhi.
- Attended regional Consultation of the WHO Collaborating Centers in the South-East Asia Region to be held on 20-21 October, 2016 at New Delhi.
- Invited to attend as a key speaker to present and discuss on "NICED surveillance and plan for implementation of OCV in a district West Bengal" at the meeting for "Evidence for cholera in India" organized at THSTI on 22nd November, 2016
- Attended Typhoid Surveillance Protocol Development meeting on November 23, 2016 at THSTI, New Delhi
- 40th Annual Conference of Indian Association of Medical Microbiologists MICROCON-2016 during 25th - 27th November, 2016 at PGIMER, Chandigarh and chaired a session on enteric diseases
- National Technical Advisory Group on Immunization (NTAGI)-NTAGI-Standing Technical Sub-Committee meeting to be held from 1:00 pm to 5:30 pm on November 28th, 2016 at ICMR, New-Delhi.
- Delivered a talk on "Enteric Vaccines : Current Scenario" as the Resource Person for the 63rd Orientation Program for the teachers of universities and colleges on 30th November, 2016 at the UGC-HRDC, Jadavpur University, Salt Lake Campus.
- Attended a meeting on the role of regional VRDL at RMRIMS, Patna on 5th December, 2016
- Attended a meeting of investigators and scientific experts on Proposed Typhoid Demonstration Project-India as chairperson held on 15th December, 2016 at Navi Mumbai
- Attended FAO-ICAR/NIVEDI meeting on Laboratory based surveillance of AMR in human health and veterinary sectors at Hotel The Verda Prakyathi, Bangalore held on 18-19th January, 2017.
- Attended the 3rd meeting of the Scientific Advisory Committee meeting of National Institute of Pharmaceutical Education and Research (NIPER)-Kolkata held at 2.00 p.m. on 30th January, 2017 at IICB, Kolkata
- Attended the Enteric Fever Diagnostics: Planning for Future Success held during February 7 to 9, 2017 at the Centre de Conferences Les Pensieres in Veyrier-du-Lac, France
- Invited to chair a session of the plenary lectures at the international meeting on "Neurodegenerative disorders: current and future perspective" organized by Calcutta University in collaboration with the University of Newcastle, UK and the Institute of Neurosciences Kolkata on 12th February, 2017 at the Oberoi Grand Hotel.
- Delivering a talk on "Childhood Diarrhoea" as the Resource Person at the 64th Orientation Program for the teachers of universities and colleges on 23rd February, 2017 at the UGC-HRDC, Jadavpur University, Salt Lake Campus.
- Serum Institute of India and PATH co-hosting a Dissemination meeting in collaboration with the DBT on "MenAfriVac@:A template for Global Impact of Make in India" to celebrate the success of MenAfriVac@ to global public health; recognize partners; share results, experience and lesson learnt to be held on 3rd March, 2017 at Taj Vivanta Hotel, Sujan Singh Park, New Delhi
- Attended National Seminar on Diarrhoeal disease burden and management: Special reference to North Eastern India at Tezpur University, March 10-11, 2017 and delivered a lecture on "Enteric vaccines: current and future perspectives"
- Attended Technical Research Group meeting on Typhoid Surveillance in India - which ensured a

coordinated strategy to support, control of enteric fever in India held on 14th March, 2017 at ICMR Hqrs.

- Participated in the "Revisiting Ethical Issues in Biomedical and Health Research" by Forum for Ethics Review Committees in India (FERCI) held on 16th March, 2017 at NICED, Kolkata.
- Participated in the round table conference on "Changing concept of hygiene: Science, perception and practice" with medical and public health experts, scientists and public health engineers jointly organized by All India Institute of Hygiene and Public Health and International Scientific Forum on Home Hygiene held on 24th March, 2017 at the Sonnet, Kolkata.
- Panel discussion on the development path of Shigella vaccine for India and for the rest of the world at Hilleman Laboratories, Delhi on 29th March, 2017.
- Technical Review Group (TRG) meeting to review DeWorm3 project proposal of CMC, Vellore held under the Chairmanship of DG, ICMR. on 30 March, 2017 at ICMR HQ..

A. Palit

- Invited & participated in a PANEL DISCUSSION on "Microbiome of the Ganges at IIT, Kanpur with participant from IIT, Kanpur & Roorkee, NEERI (CSIR), Nagpur, Georgia tech Institute, USA etc.
- Organised and participated in ICMR-CSIR Collaborative Project "Seasonal dynamics of enteropathogenic bacteria in Gulf of Khambat, Gujrat: its impact on health of coastal population" and interacted with the CSMCRI Gujarat scientist for the working module in Sept 2016 at NICED, Kolkata.

R. K. Nandy

- Workshop- Participated in "Learning Engineering and Maintenance of Biomedical Research Laboratory Training" at the National Institute of Virology (NIV), Pune during August 29 to September 2, 2016.
- Meeting- Participated in "MenAfriVac: A Template for Global Impact of 'Make in India' at New Delhi, jointly organized by PATH, USA; Serum Institute, India and Bill & Melinda Gates Foundation during March 3, 2017.
- VRDL Training Workshop- Course facilitator and faculty in "Workshop on Diagnosis of Emerging Viral Diseases" at National Institute of Cholera and Enteric Diseases" during March 28- 29, 2017.

A. K. Mukhopadhyay

- Mr. Angesom Abraham Gebremeskel, Lecturer in Department of Biology, Eritrea Institute of Technology, Asmara, Eritrea awarded the NAM S&T Centre Research Training Fellowship for developing Country Scientists (RTF-DCS) to carry out research activity in India for 6 months. He worked on a project entitled "Isolation, Identification and Characterization of *Helicobacter pylori* strains in biopsy samples collected from patients with gastroduodenal problems in India" at the Bacteriology Division of this institute during February 18 – August 12, 2016.
- Dr. Keya Sen, Faculty, University of Washington Bothell, received the prestigious Fulbright -Nehru Global Flex Award (Research) for a period of two months and had worked (July 20 to Sept 15, 2016) at the bacteriology division of NICED to carry out the project entitled "Are Crows a nuisance to public health or can they have a positive contribution to society: A molecular microbial investigation of the diarrheal pathogen *Campylobacter jejuni* carried by crows" under this program.
- Invited to attend the "Gut Microbiome 2016–An International Perspective" organized by the Department of Microbiology & Enteric Diseases Division, Kasturba Medical College, Manipal University, Manipal during October 21–22, 2016 and delivered a talk on "The enigmatic gastric pathogen *Helicobacter pylori*: Balancing the double-edged sword**".**

- Delivered an invited talk on "The Saga of gastric pathogen *Helicobacter pylori*: Are All the *H. pylori* same?" in the ISG Single Theme Meeting on Peptic ulcer and *H. pylori* during January 28 and 29, 2017 at Bhubaneshwar, India.
- Dr. Mukhopadhyay was invited by US - Japan Cooperative Medical Sciences Program on Cholera and the International Vaccine Institute to attend the US -Japan Cooperative Medical Sciences Program's 19th International Conference on the Emerging Infectious Disease in the Pacific Rim and to deliver a talk on "Pathogenic modulation in El Tor vibrios: evidence from recent Indian strains harbouring Haitian traits" during February 07-10, 2017 in Seoul, South Korea
- Invited in the 7th AMRICON Medicine Update 2017 organized by the Dept. of Academics & Health Research, AMRI Hospitals at Hotel ITC Sonar, Kolkata during March 18-19, 2017 and delivered a talk on "*H. pylori* - current management strategies".

S. Basu

- Nominated to attend the FAO consultative meeting to develop a zoonotic emerging infectious disease project in partnership with Government of India , Kolkata, 20-22 May, 2016.
- International Conference on Contemporary Antimicrobial Research, Silchar, India, 14-17 November, 2016. A bug called *Acinetobacter* and drug called Carbapenem: experience from a neonatal unit. Somdatta Chatterjee, Subhasree Roy, Suchandra Mukherjee and Sulagna Basu (Invited talk)
- 104th Indian Science Congress, Tirupati, India, 3-7 January, 2017. Neonatal Sepsis due to drug-resistant *Acinetobacterspp*: a storm in the cradle. Somdatta Chatterjee, Saswati Datta, Subhasree Roy, Tapas Som and Sulagna Basu (invited talk)
- Nominated to attend the National training program on Entrepreneurship Development & Management for Women Scientists and Technologists , Entrepreneurship Development Institute, Ahmedabad, 6-10 February 2017

H. Koley

- Hemanta Koley, Dhrubajyoti Nag, Suhrid Maiti, Debaki Ranjan Howlader, Priyadarshini Mukherjee, Ritam Sinha, Sumio Shinoda. Heat-killed multi serotype Shigella immunogens induce Th1 and Th17 cell mediated adaptive immune responses in mice model 19th US-Japan Cooperative Medical Sciences in Seoul, South Korea Feb. 7-10, 2017.

BIOCHEMISTRY

The Division of Biochemistry primarily focuses on in-depth understanding of the molecular mechanisms of host pathogen interaction using biochemical approaches. The molecules of interest are *Vibrio cholerae* chitinases, chitin-binding proteins, their regulators and colonization factors of enterotoxigenic *Escherichia coli* (ETEC). Researchers of this division address characterization of these microbial proteins in relation to structure and pathogenesis of enteric diseases and host response. Knowledge generated aids in a greater understanding of the complexity of bacterial pathogenesis in the host. Further, information gathered is being applied to establish molecular tools for detection of virulence markers in pathogenic strains during infection.

Scientist:
Dr. N. S. Chatterjee, Scientist-F

Post-Doctoral Fellow:
Dr. Epshita Chatterjee

Pre-Doctoral Fellow:
Ms. Rhishita Chourashi, UGC (SRF)
Mr. Debjyoti Bhakat, ICMR (JRF)
Mr. Suman Das, ICMR (JRF)

Ph.D. Awarded:
Dr. Moumita Mondal received Ph.D. from the University of Calcutta
Title of the thesis: *Vibrio cholerae* chitinase ChiA2 and its role in environment and host intestine.

Dr. Anusuya Debnath received Ph.D. from the University of Calcutta
Title of the thesis: Studies on structure-function relationship of colonization factor antigen CS6 of enterotoxigenic *Escherichia coli*

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

Principal Investigator: N. S. Chatterjee

Vibrio cholerae O1, a cause of epidemic diarrheal diseases, normally resides in aquatic environment associated with the chitinous exoskeletons of zooplankton and utilizes chitin as the sole nutrient source by chitin utilization pathway. Presently, our studies are directed towards the understanding of this chitin utilization pathway of *V. cholerae* in environmental survival, horizontal gene transfer and pathogenesis. Chitin utilization pathway requires two factors, chitin binding protein and chitinases. The chitinases and the chitin utilization pathway are regulated by a two-component sensor histidine kinase ChiS (VCA0622) in *V. cholerae*. In recent studies these two factors are also shown to be involved in *V. cholerae* pathogenesis. However, the role played by their upstream regulator ChiS in pathogenesis is yet to be known. During this period, we investigated the activation of ChiS in presence of mucin and its functional role in pathogenesis. We found ChiS is activated in mucin supplemented media. The isogenic *chiS* mutant ($ChiS^-$) showed less growth compared to the wild type strain ($ChiS^+$) in the presence of mucin supplemented media. The $ChiS^-$ strain also showed highly retarded motility as well as mucin layer penetration invitro. Our result also showed that ChiS was important for adherence and survival in HT-29 cell. These observations indicate that ChiS is activated in presence of intestinal mucin and subsequently switch on the chitin utilization pathway. In animal models we found reduced fluid accumulation and colonization during infection with $ChiS^-$ strain. We also found $ChiS^-$ mutant with reduced expression of *ctx*A, *tox*T and *tcp*A. The cumulative effect of these events made *V. cholerae* $ChiS^-$ strain hypovirulent. Hence, we propose that ChiS plays an important role in *V. cholerae* pathogenesis.

Title: Molecular characterization of Enterotoxigenic *Escherichia coli* colonization factors

Principal 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. Colonization factors (CF) play the important role in initiating the disease and had been the major vaccine targets. However, vaccines against CFAs had poorly performed which may be due to neglecting the other non-classical virulence genes. During this period of investigation, we aim to understand the distribution of enterotoxins, colonization factor (CF) and non-classical virulence factor profiles of clinical ETEC isolates between 2008 and 2014 from two Kolkata hospitals. So far, we have screened 171 samples isolated as the sole source of infection. We used PCR for this genotyping study using gene specific primers against 2 enterotoxin genes, 15 common CF and 5 common non-classical virulence factors. All the ETEC strains were positive for enterotoxin genes. Among the 171 ETEC isolates, both enterotoxins est and elt genes were present together in 61% strains followed by 23 % est alone and 16% elt alone. Fifty-seven percent of the ETEC (97/171) expressed one or more CF. CS6 was the prevalent classical CF gene (39%) followed by CS21 (36%). Sixty-one percent ETEC (104/171) had genes for one or more non-classical virulence factors. The secreted serine protease autotransporter eatA was the common non-classical virulence gene (66%) and adhesion factor etpA (44%) was second common. Twenty-seven percent of the strains were negative for any CF or non-classical virulence factors. The results showed that est with or without elt, CS6 with or without CS5 and with or without eatA were present in 23% of clinical ETEC strains (28/123) analyzed. Presence of CS6 and CS21 along with Eat A and EtpA among the isolates in this region suggests need of multivalent ETEC vaccines.

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

N. S. Chatterjee

- National Workshop on Biosafety and Biosecurity organized by Defence Research and Development Establishment at Gwalior in collaboration with Ministry of External Affairs (MEA), Govt. of India, during May 19-20, 2016.

CLINICAL MEDICINE

The Division of Clinical Medicine carries out both hospital-based surveillance studies and translational research. Two major surveillance studies are going on for over 20 years. Under one surveillance project, every 5th hospitalized patient of all age groups from the ID & BG Hospital, Beliaghata, Kolkata is surveyed on randomly-selected two consecutive days in a week. Another project is conducted at the DR. B. C. Roy Post Graduate Institute of Pediatric Sciences, Kolkata where children upto the age of 12 years suffering from diarrhoea and dysentery and attending the hospital OPD are enrolled. A second study in the same hospital surveys for enteric fever through blood culture and Widal tests. Laboratory research at the division focuses on two major areas: host-pathogen interaction in human *Salmonella* Typhi and Paratyphi infection and mucosal immune responses in the intestine. The major emphasis is on the identification of novel virulence factors of *Salmonellae* and the host immune responses, with an aim to develop novel antimicrobial agents and vaccines. A protein subunit vaccine against *S.* Typhi and *S.* Paratyphi developed by the division is currently in the process of entering clinical trials and national and international patents are pending for a synthetic peptide designed to treat Gram negative bacteraemia and sepsis. Studies have been published on the role and regulation of cationic antimicrobial peptides, the so-called endogenous antibiotics in intestinal immune responses and modulation of immune response by indigenous probiotic bacteria in the animal models of inflammatory bowel diseases.

Scientists

Dr. S. Das, Scientist-E
Dr. P. Indwar, Scientist-B

Staff :

Mr. A. Pal, Technical Officer-A
Mr. K. G. Saha, Technician-B
Mr. S. Turi, MTS (General)

Postdoctoral fellows:

Dr. Ayan Lahiri – Research Associate (CSIR project)
Dr. Shreya Dasgupta – ICMR Centenary Postdoctoral Fellow
Dr. Debdut Naskar – Research Associate (DBT)
Dr. Jayadev Joshi – Scientist II (ICMR project)

Pre-doctoral Students:

Mr. Bhupesh Kumar Thakur - SRF (ICMR) (Thesis submitted)
Ms. Pujarini Datta - SRF (DST-INSPIRE)
Mr. Asim Biswas - SRF (CSIR)
Ms. Atri Ta – Technical Assistant (OUP3/4) (Thesis submitted)
Mr. Sayan Das - SRF (CSIR)
Ms. Rimi Chowdhuri - Technical Assistant (OUP3/4)
Mr. Rahul Shubhra Mondal – Scientist I (ICMR project)
Mr. Krishnendu Das – JRF (ICMR)

PhD Awarded:

Dr. Nirmalya Dasgupta received Ph.D. from Department of Biochemistry, University of Calcutta.
Title of Thesis: Differentiation-induced regulation of gene expression in the intestinal epithelial cells and its role in homeostasis.

Title: Regulation of innate immune responses at the intestinal mucosa

Investigator: S. Das

We have demonstrated that viral double-stranded RNA (dsRNA) transcriptionally regulates murine cathelicidin (mCRAMP) expression through phosphorylation of Sp1 by PI-3 kinase / PKCζ pathways activation and Sp1 recruitment to the mCRAMP promoter. This results in reduced intestinal bacterial load and mucosal inflammation in *Shigella flexneri* 2a-infected mice after intra-rectal administration of dsRNA (Fig 12). In addition, we reported that persistent intestinal expression of Ccl20, a chemokine for dendritic cells with antimicrobial functions, is regulated transcriptionally by AP1 (cjun/cfos) and non-canonical NF-κB (RelB/ p52) downstream of MEK-ERK and NIK-IKK-α-NF-κB2 (p100) phosphorylation, respectively.

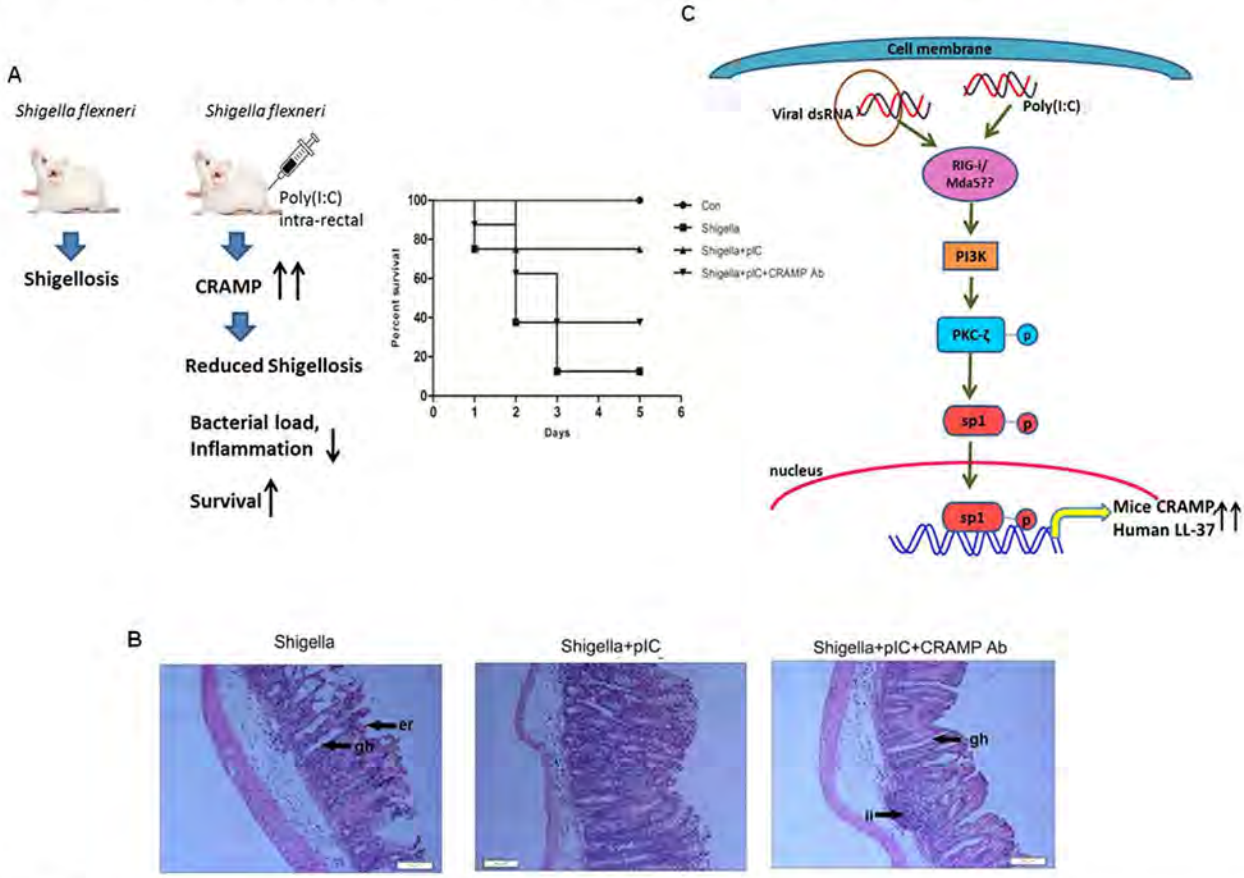


Fig 12 : Regulation of cathelicidin expression by viral dsRNA in the colonic epithelial cells. A. (Left) Cartoon showing *Shigella flexneri* infection of mice. (Right) Kaplan- Meyer Plot of mice survival. B. Histopathology of colonic tissues of mice treated as above. C. Cartoon showing the signaling pathways regulating cathelicidin expression.

Title: Host-pathogen interactions in human salmonella infection

Investigator: S. Das

We had earlier reported that T2942, an AIL family protein of *Salmonella* Typhi is essential for invasion of intestinal epithelium and functions autonomously of type three secretion system. Subsequently, we found that T2942 binds to the cell surface receptor tyrosine kinase Met with high affinity, leading to its phosphorylation and activation of the downstream signaling pathways involving Src, PI3-K and Rac-1. This in turn leads to Rac-1 dependent and localized actin polymerization at the invasion sites (Fig 13).

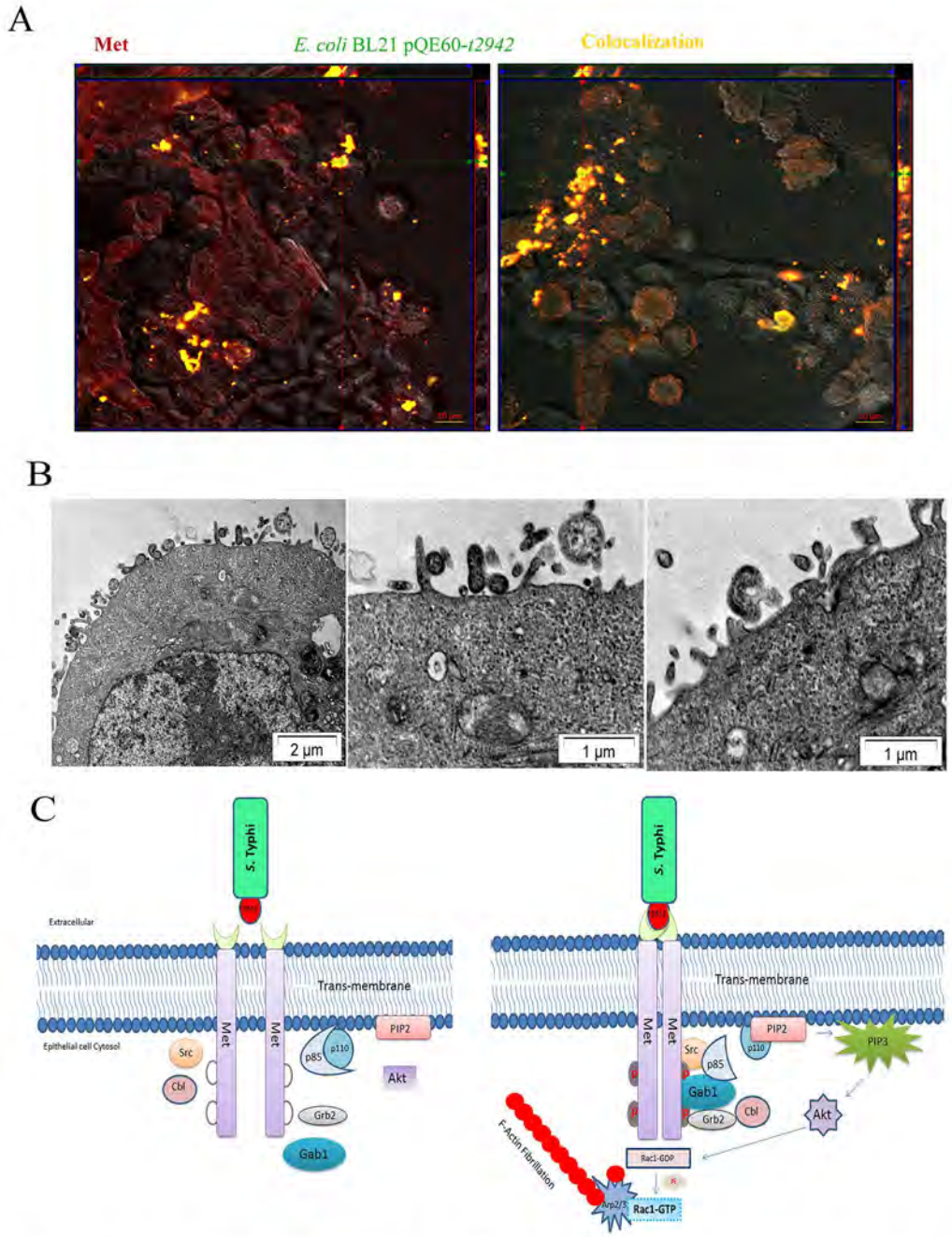


Fig 13. Activation of the Met signaling pathways in the colonic epithelial cells by *Salmonella* Typhi adhesion/invasion protein T2942. A. Confocal micrographs of HT-29 cells infected with *E. coli* BL21 expressing T2942, showing co-localization of cell-surface receptor Met and T2942. B. Transmission electron micrographs of infected HT-29 cells showing localized membrane ruffles (actin polymerization), the so-called 'zipper entry' mechanism of the bacteria. C. Cartoon showing the scheme of intracellular signaling following T2942-Met interaction.

Title: Butyrate-induced regulation of intestinal homeostasis

Principal Investigator: S. Das

Despite being a known HDAC inhibitor, butyrate suppresses colonocyte Syk tyrosine kinase expression by deacetylating histones 3 and 4 around the transcription start site (TSS), precluding RNA Polymerase II binding to the promoter and inhibition of transcription. In contrast, butyrate augmented C/EBPα levels, which competed out the transactivator protein Oct-1 from binding to Polo-like kinase 1 (PLK1) promoter and was associated with increased deacetylation near the TSS. The above functions of butyrate mediate differentiation and apoptosis of colonic epithelial cells.

Title: Computational predictions of host-pathogen interactions and their validation

Principal Investigator: S. Das

Bacterial lysR-type transcriptional regulators (LTTRs) are attractive targets for anti-virulence drug development strategy. To design novel LTTR inhibitors or repurpose the existing drugs for LTTR inhibition, we screened the fragments generated from FDA-approved drugs, followed by fragment-joining and molecular substructure search in the PubChem database. This helped us to identify an existing antiviral drug, ribavirin as a potent inhibitor of *Vibrio chelorae* LTTR, AphB. Experimental validation *in vitro* and *in vivo* proved that ribavirin is capable to prevent *V. cholerae*, *Salmonella* Typhi and enteropathogenic *E. coli* infection through LTTR inhibition.

Clinical Research:

Hospital-based surveillance of diarrhoeal diseases and enteric fever in children – Stool/rectal swab and/or blood is collected from the patients and sent to NICED laboratories for the diagnosis of 26 enteric pathogens including bacteria, viruses and protozoa

Title: Clinical study to evaluate the association of neurological manifestations in rotavirus diarrhoea among hospitalized children under three years of age

Principal Investigator: P. Indwar

Co-Investigators: M. K. Bhattacharya, M. C. Sarkar, A. Mukhopadhyay, S. Ganguly, NICED; S. Das; Dr. B. C. Roy Post Graduate Institute of Paediatric Sciences Kolkata

Fifty seven patients admitted with diarrhea and neurological symptoms were enrolled, thirteen were positive for rotavirus diarrhea and rotavirus RNA was detected in cerebrospinal fluid (CSF) of four patients. Adenovirus was seen in 4 stool samples but CSF sample did not show presence of adenovirus. All the patients presented with convulsion of 2-3 episodes and were discharged within 5-6 days.

Awards/ Honours Received

S. Das

- Elected as the Fellow of the West Bengal Academy of Science and Technology (WAST), 2016.

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

S. Das

- Organized 3-day workshop on "Bioinformatics Applications for Biologists" at NICED on May 30th to June 1st, 2016 (attended by 20 participants).
- Sayan Das, Ph.D. student presented a poster at "The EMBO Meeting" held at Mannheim, Germany from 10 - 13 September 2016. (Received travel support from Department of Biotechnology, India).

- Rimi Chowdhury, Ph.D. student presented a poster at "The EMBO Meeting" held at Mannheim, Germany from 10 - 13 September 2016 (Received travel support from Department of Biotechnology, India).
- Rimi Chowdhury, Ph.D. student presented a poster at "The Keystone Symposium: S1, Translational Vaccinology for Global Health" held at London, England, UK from October 25 – 29, 2016. (Received Keystone Symposia Global Health Travel Award)
- Sayan Das, Ph.D. student presented a poster at "The Keystone Symposium: S1, Translational Vaccinology for Global Health" held at London, England, UK from October 25 – 29, 2016. (Received Keystone Symposia Global Health Travel Award).
- Dr. S. Das delivered a lecture at the 3rd International Meeting on Advanced Studies in Cell Signaling Network (CeSiN2016) held at CSIR-Indian Institute of Chemical biology, Kolkata on December 18-20, 2016.
- Dr. S. Das delivered a lecture at the National Level Seminar organized by the Indian Immunology Society - Odisha Chapter at National Institute of Science Education and Research (NISER), Bhubaneswar on 6th January, 2017.
- Dr. S. Das taught at the Refresher Course for College and University Teachers on Recent Trends in Life Sciences held at Jadavpur University, Kolkata on 7th January, 2017.
- Organized 2-day workshop on "Clinical Bioinformatics" at NICED on February 7th and 8th, 2017 (attended by 8 participants).
- Dr. S. Das delivered a lecture at the CAS-Phase II sponsored symposium on 'Emerging Trends in Biology' organized by the Department of Biochemistry, held at Ballygunge Science College, Univercity of Calcutta, Kolkata on 17th March, 2017.
- Taught Medical Microbiology to the MSc Microbiology (4th Semester) students of the University of Calcutta.

P. Indwar

- Attended Health Research Fundamentals- an online training course conducted by NIE Chennai from 23rd January 2017-17th March 2017.
- Attended workshop on "Ethics in Human Health Research and Good Clinical Practice" held at NICED, Kolkata on 16th March 2017.

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.

This division primarily focuses on good data management practices and is also compliant with Good Clinical Practices (GCP) to produce the reliable, complete and accurate data from the various health research projects of this institute, appropriate statistical analysis of the research data and finally publication in peer reviewed Journal.

This division also has a crucial role for data management and creation of diarrhoea database from ongoing hospital based diarrhoeal diseases surveillance at Infectious Disease Hospital (IDH), and DR. B. C. Roy Post Graduate Institute of Pediatric Sciences, in Kolkata to identify the pattern of diarroeagenic enteric pathogens. The causative organism of diarrhoea and antimicrobial resistant pattern of cholera and Shigella are communicated on weekly basis to IDH and different department of State Government to help the physicians for proper patient management of diarrhoeal diseases.

This division provides data management support including data entry/verification to various studies undertaken in this institute and also other collaborative projects viz. the project on Multisite human respiratory infections surveillance network in India and Integrated Diseases Surveillance Programme (IDSP) and rotavirus vaccine trial with PATH vaccine solutions.

The process has been initiated to conduct research methodology and biostatistics workshop in Government medical colleges in West Bengal to enhance the research skill of undergraduate and postgraduate medical students to undertake some research on important public health problem.

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

Final goal is to extract important findings from research data using modern and appropriate statistical techniques and to publish in peer reviewed Journals.

Scientist:
Dr. B. Manna, Scientist -F

Staff:
Mr. S Shil, Technical Officer-A

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

B.Manna

- Attended Influenza surveillance workshop organized by WHO at New Delhi during 4-6 October, 2016
- Attended 61st Annual National Conference of Indian Public Health Association (IPHACON-2017) and 1st State Conference of IPHA Rajasthan at Jodhpur, Rajasthan during 24-28 February, 2017

ELECTRON MICROSCOPY

The electron microscope is used mainly for research and diagnosis. The techniques in routine use are negative-staining analysis, Kleinschmidt's protein monolayer technique of DNA, partial denaturation mapping and heteroduplex analysis of DNA, protein-free spreading methods of DNA and RNA, immunoelectron microscopy, ferritin labeling, ultramicrotomy, darkfield electron microscopy and electron diffraction, cryoelectron microscopy, three-dimensional image reconstruction technique including tomography, environmental scanning electron microscopy and atomic force microscopy.

Staff:

Ms. A. Sarbajna, Technical Officer -A
Mr. S. Kumar, Technician-B
Mr. B. R. Mallick, MTS (Technical)

Predoctoral Fellow:

Ms. S. Das CSIR (SRF)

EPIDEMIOLOGY

Considering ICMR-National Institute of Cholera & Enteric Diseases as a premier national public health institute, Division of Epidemiology is deeply associated with carrying out community-based research on both communicable & non-communicable diseases of public health importance as well as malnutrition through a team of committed public health workers. Based on felt-need of the region as well as country, scientists of this division worked on exploring the problems of diarrhoeal diseases, HIV/AIDS, hepatitis, STIs, influenza, dengue, diabetes, hypertension, arsenicosis, under-nutrition and many other health related events in recent past. Other important roles played by this division were responding to public health emergency situations such as conducting epidemic investigations and suggested necessary measures to the local health authority for its control, supporting state public health machinery to combat various epidemics caused by emerging & re-emerging infectious pathogens from time to time, conducting vaccine trials with the aim of finding out better enteric vaccine/s for prevention & control of diarrhoeal diseases, building up of regional as well as national public health capacity through various teaching & training programmes. It also participated in major national health surveys/programmes such as District Level Household Survey, National HIV Sentinel Surveillance Programme etc.

During the year under report, Division of Epidemiology was associated with randomized controlled trial on oral vaccines through involvement of its two extramural projects, one on efficacy of penta-valent rotavirus vaccine trial & another on exploratory study to understand the biological basis of under performance of oral vaccines in India with a nested open trial to see IPV boost.

Another study was conducted among health care providers of slum dwellers within Kolkata city revealed that the perceived responsiveness of the healthcare providers appeared to be much better for the non-qualified practitioners compared to qualified practitioners in that study area.

The Division was involved with carrying out two hospital-based surveillances – one on diarrheal diseases (at I.D. & B.G. Hospital, Kolkata) and the other on viral respiratory infections (at College of Medicine & Sagore Dutta Hospital, Kamarhati). Another project was also initiated on relationship between climate change and waterborne diseases in two village panchayats in the Sundarbans area of South 24-Parganas.

Additionally, epidemiology division in collaboration with another researcher from a Chennai-based institute was entrusted with work of data analysis of National Integrated Biological and Behavioral Surveillance (IBBS) for High Risk Groups (HRGs) by National AIDS Control Organization (NACO). It was highlighted that increased pharmaceutical injecting and its contribution to HIV-epidemic in India, necessitates an immediate and appropriate response particularly focusing on 'core essential combination prevention and treatment approaches.

The Division had also prepared itself to undertake a study to investigate the disease burden of influenza among elderly population in Kolkata through a community-based surveillance mechanism, which is a part of the multi-city project (other sites are Delhi, Chennai & Pune), funded by CDC, Atlanta, through All India Institute of Medical Sciences as a coordinating institute. Similarly National AIDS Control organization has also entrusted this Division as lead institute to undertake an operational research titled a situation analysis of health facility preparedness for screening and treatment of syphilis among antenatal clinic attendees in India'. This would be a multi-regional operational research project (West Bengal, Hariyana, Maharastra, Tamil Nadu & Assam) with the objective of strengthening of elimination activities of congenital syphilis burden in India, which is a national mandate.

Scientists:

K. Sarkar, Scientist-F
S. Panda, Scientist-F
A. K. Deb, Scientist-E
S. Kanungo Scientist- C (till August 2016) & Scientist-D from Sep-2016 onward
F. Debnath, Scientist- B (till August 2016) & Scientist-C from Sep-2016 onward
P. Chatterjee, Scientist-B (joined in January 2017)

Staff:

Ms. S. Manna, Technical Officer- A (Retired in February 2017)
Mr. R. Saha, Technical Officer A
Mr. C. Mandal, Technical Assistant
Mr. A. Chakraborty, Technician-C
Mr. S. Routh, Technician-B

Post Doctoral Fellow:
Dr. T. Mahapatra

PhD Awarded:

Dr. Mitali Sen received Ph.D. from Visva-Bharati University.
Title of the thesis: Socio-cultural influences on preventive and management practices related to child survival: A community-based study in rural West Bengal.

Title: Establishing a network of population based influenza surveillance platform for elderly persons in India

Investigator: K. Sarkar

There is paucity of data on burden of influenza among the elderly population in India. In our country, surveillance for influenza is done under the aegis of the integrated disease surveillance program (IDSP), which is mostly based at participating health facilities. Due to their general frailty and access related issues, the elderly population does not frequent such facilities. Besides, it has been reported that hospital-based surveillance which only consider hospitalizations that include a diagnosis of pneumonia may underestimate the total burden of influenza hospitalizations. Hence to get the true picture of burden of infections with influenza and other respiratory viruses in this vulnerable age group and to have a better understanding of the contribution of circulating virus strains, population-based active surveillance is desirable. Because of the diverse socio-demographic and climatic conditions in India, it is imperative that the surveillance for influenza and other respiratory viruses among elderly population is broad-based, involving multiple sites in different regions of the country. Hence, this study was planned with following objectives.

Objectives :

1. To establish a network of population-based surveillance platforms for ongoing surveillance of influenza and other respiratory viruses among elderly (aged 60 years or more) in four regions of India to determine circulating influenza and other respiratory viruses strains of epidemic concern from the community

2. To demonstrate feasibility of providing real-time population-based data on circulating influenza and other respiratory viruses strain through use of portable/mobile electronic devices for data collection and sharing

3. To map the healthcare facilities and their utilization pattern in these surveillance platforms

Methodology :

This is a multi-city project with four stations - Delhi, Pune, Chennai & Kolkata. Community-based surveillance of influenza among elderly population will be done in Kolkata field. Trained field workers will

visit study households on weekly interval and enrolled subject will be screened for the presence of acute respiratory infections (ARI) through qualified & trained nurses. They will use standard data entry formats in handheld tablets. If screened positive, the field workers will inform the trained nurse who will perform detailed assessment and classify it as either acute upper (AURI) or acute lower (ALRI) respiratory infections. Validation of clinical assessment by nurse will be done against physician's diagnosis and diagnostic testing before using them in the surveillance. Any outpatient visit or hospitalization will also be inquired upon by the workers. In case a subject is known to have been admitted in a hospital from ARI, attempt will be made to visit the subject in hospital for collecting nasal and oro-pharyngeal swab.

Clinical information of illness will be collected using standardized questionnaire in electronic format. Trained nurses will perform assessment using the above defined criteria. Nurse will collect information on vital signs, date of onset of symptoms, and will do respiratory examination for rapid breathing or other indications of severe disease. In the case of a severe illness with lower respiratory tract involvement or low oxygen saturation that is identified during a weekly visit, nurse will contact the physician to conduct an assessment and arrange transportation to the appropriate health facility, including the nearest health post or local hospital. If a participant is not present at the time of a weekly visit, a 7-day symptom history will be obtained, when possible, from a caregiver within the same household, and a follow-up visit scheduled to complete the assessment and collect specimens, if the participant meets the ARI case definition.

Work done so far:

The study was supposed to be initiated in January-17 but due to some unavoidable problem with funding agency, transfer of fund was delayed and was only received in June-2017.

Approval of Scientific Advisory Committee & Institute Ethics Committee has been obtained. Study community was visited on several occasions to sensitize the local population & community leaders about this study. Investigators of CDC & AIIMS also visited the community and suggested several issues to be incorporated considering a uniform & standard procedure to be followed for this multi-city project (Delhi, Pune, Chennai & Kolkata). Visit to the laboratory was also made by scientists of AIIMS & CDC and detailed lab testing protocol including trouble shooting was finalized.

Title: Understanding HIV discordant/concordant relationship in familial, societal & program context in India - a multi-site qualitative study to inform intervention development

Investigator: S. Panda

The extramural project titled 'Understanding HIV discordant/concordant relationship in familial, societal & program context in India - a multi-site qualitative study to inform intervention development' has the potential of exploring various issues around HIV transmission in marital heterosexual union where an individual is presently married and living with a single spouse. The focus of this qualitative research study is to explore how people manage to stay HIV discordant in heterosexual marriage. This will entail understanding 'beliefs', 'barriers' and 'facilitators' pertaining to HIV-transmission within marriage. In order to achieve this, married couples concordant and married couples discordant for HIV infection will be enrolled. Presently a memorandum of understanding is being signed between Investigators, Co-Investigators, Institution Heads and the National AIDS Control Organization.

A Report titled 'National Integrated Biological and Behavioral Surveillance for High Risk Groups' published by the National AIDS Control Organization (NACO) was anlysed by Dr S Panda. The findings drew attention towards the immediate need for policy and program change pertaining to HIV transmission among people who inject drugs (PWID) in India.

Title: The interplay of climate and non-climate factors in determining the risks and predicting outbreaks of waterborne diseases

Investigators: A. K. Deb (PI); S. Dutta (Project Coordinator); A. Palit (Co-PI); F. Debnath, A. De

The objectives of the project titled "The interplay of climate and non-climate factors in determining the risks and predicting outbreaks of waterborne diseases" include: (a) to improve our understanding of the role of climate variability on occurrence of diarrheal diseases, adjusted for the effects of relevant non-climate factors; (b) to determine the linkage between temporal changes in water quality and occurrence of diarrheal diseases, including diarrheal outbreaks; and (c) to develop a composite index for generating early warning for diarrheal outbreaks. The work encompasses collection of both retrospective and prospective data from various sources. While retrospective disease data will be collected from the Integrated Disease Surveillance Program (IDSP), other relevant data (such as various developmental indicators over the years) are being collected from different government/non-government sources. A prospective community-based diarrhea surveillance has also been planned to detect pathogen-specific diarrhea cases and diarrhea outbreaks during the study period. Relevant climate data will be obtained from the Regional Meterological Department. The data collection tools for prospective data collection have already been developed and approvals from local panchayat bodies have also been obtained for the start of the community-based diarrhea surveillance.

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

Principal Investigator : S. Kanungo

Co-Investigators : M.K.Bhattacharya, B.Manna, A. K. Mukhopadhyay, R. K. Nandy, M. Chawla Sarkar, and Dr. Dilip Paul, Professor, and MSVP, Dr. B. C. Roy Post Graduate Institute of Paediatric Sciences, Kolkata

The Serum Institute of India, developed an oral live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine. The vaccine was tested for efficacy and safety in healthy infants in several regions of India representing different demographic, climatic and sociocultural factors. Total sample size was 7500 and vaccine and placebo allocation ratio was 1:1. NICED was a part of this multi centric phase III trial and first subject was dosed on 15th Aug 2014. 700 children aged 0-6 weeks were recruited and were dosed for three times concurrently with UIP vaccine at one month interval (Pic 1). The dosing was completed on 27th May 2014 (Table 8). Field clinics were set up since initiation of the project, manned by physicians and community health workers. Active surveillance was established in order to detect the gastroenteritis cases and other adverse events at the earliest (Pic 2). The adverse events were managed and documented as per protocol. Total of 1812 episodes of Acute Gastroenteritis cases were detected and stool samples were collected. The diseased subjects were followed up till the resolution of the episode. Total of 117 Serious Adverse Events had been managed and reported to the regulatory authorities within stipulated timeline. None of the serious adverse events were related to the Investigational product. The surveillance was conducted up to the age of two years of each subject and ended on 06 March 2017. Primary analysis revealed that the vaccine is safe and efficacious against Severe Rotavirus Acute Gastroenteritis.

Pic1: Subject receiving intervention in study clinic

Pic 2 : Subject followed up in clinics

Table 8 : Overall Study Status

Events	Numbers
Subject consented	**736**
Screen failure	36
Received Dose-1	700
Received Dose-2	697
Received Dose-3	693
Completed 9th months visit	689
Completed 12th months visit	687
Completed 15th months visit	684
Completed 24th months visit	683
Immunogenicity cohort	35
Early termination(including death cases)	17

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

Principal Investigator : S. Kanungo

Co-Investigator : B. Manna

The intervention and follow up phase of the study was completed in September 2015 where the impact of a multi-component intervention in improving non-qualified practitioners' (140 at the baseline and 124 at the end-line as 16 were lost to follow up between baseline and 2 months post-intervention) diarrhea related knowledge and practice to provide appropriate clinical management of diarrhea in urban slums of Kolkata was assessed. Then as per the recommendation of the Scientific Advisory Committee, exit interviews of patients (3-5 per provider) seeking care from the participating providers for diarrhea were conducted during April-May 2016 and another round of assessment (6 months after the post-intervention assessment) was conducted by administering the same questionnaire (used in baseline and post intervention) was administered to the participating practitioners (110 as 14 were lost to follow up between post intervention and one year post-intervention) between April and June 2016, to see whether the retention of the post-intervention change was sustained over time. 8 months after the intervention the domain-specific mean score (in a scale of 10) was 6.11 (5.78-6.44) for symptoms, 7.74 (7.32-8.17) for occurrence and spread, 6.39 (6.11-6.67) for cholera, 7.64 (7.42-7.86) for prevention, 7.10 (6.85-7.35) for management and 6.89 (6.67-7.11) for ORS. Overall knowledge score (in an overall scale of 100) was 70.08 (68.40-71.75) (Fig 14). It was found that overall and cholera related knowledge improved further during the one year follow up assessment and in most of the domains the improvement was sustained. Biggest reduction was for the causation and spread (Table 9). Also, our exit interviews informed us that practitioners whose knowledge improved significantly could satisfy their patients more regarding treatment. Patients treated by non-qualified physicians whose knowledge improved significantly through the intervention were twice more likely [OR=2.19(1.12-2.91)] to be satisfied by the quality of received services. Subjects attending practitioners with significantly improved practices were more likely [OR=1.76(1.24-1.99)] to be satisfied with their cost of treatment.

Results: Changes in domain-specific knowledge scores

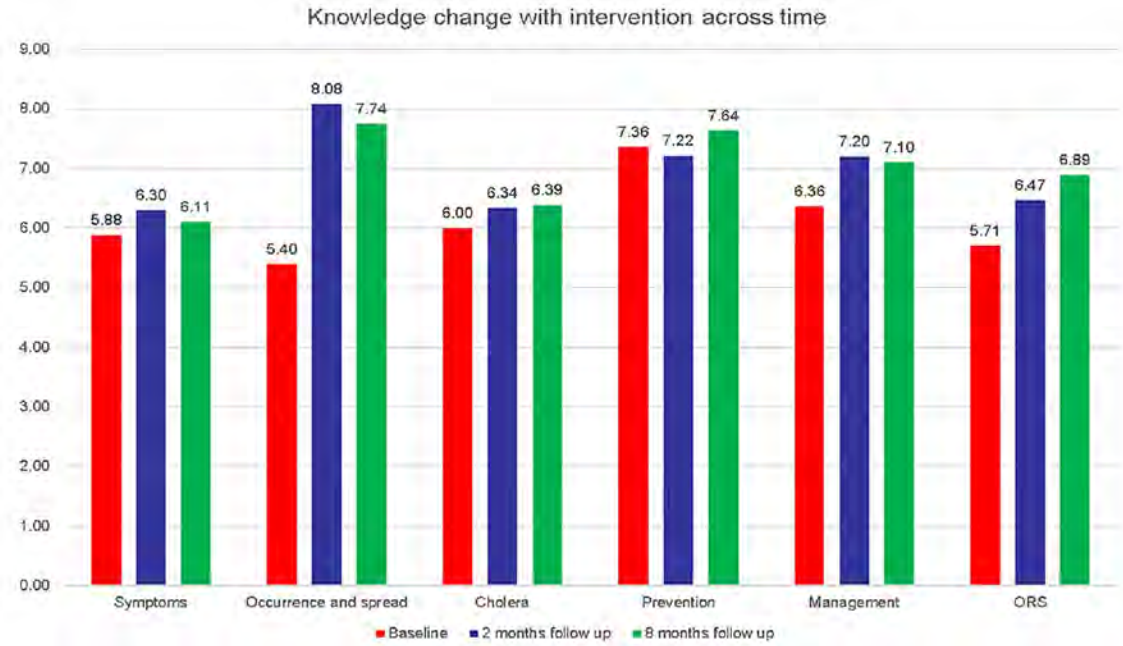


Fig 14: Impact of a multicomponent intervention on sustained improvement in knowledge about diarrhea among practitioners of urban slum of Kolkata across time

Table 9: Between 2 post-intervention assessments 6 months apart, Change and in overall and domain-specific mean knowledge scores regarding diarrhea among practitioners of urban slum of Kolkata

Domains	Mean Knowledge Scores (95%CI)		P value
	2 months Post-intervention	8 months Post-intervention	
Symptoms of diarrheal diseases	6.30(5.97-6.63)	6.11(5.78-6.44)	0.0611
Etiology and spread of diarrheal diseases	8.08(7.66-8.50)	7.74(7.32-8.17)	<0.0001
Cholera	6.34(6.05-6.63)	6.39(6.11-6.67)	0.0811
Prevention and control of diarrheal diseases	7.22(6.94-7.49)	7.64(7.42-7.86)	0.4944
Management of diarrheal diseases	7.20(6.93-7.46)	7.10(6.85-7.35)	<0.0001
Oral rehydration solution	6.47(6.23-6.70)	6.89(6.67-7.11)	<0.0001
Overall Knowledge score (in a scale of 100)	69.25(67.66-70.84)	70.08(68.40-71.75)	<0.0001

Title : 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

Principal Investigator : S. Kanungo

This intramural prospective study was completed during July 2016, when three round of quantitative data collection regarding the morbidity among under five children (in three separate age-groups: 0-11, 12-23, 24-59 months), consequent health-seeking by their care-givers and perceived responsiveness of the healthcare service providers was completed in urban slums of Kolkata. To meet the objectives of understanding the patterns of diseases suffered by the under-five population in urban slums, evaluation of caregivers' perception about common illnesses, estimation of the related healthcare responsiveness as perceived by the caregivers and assessment of the treatment seeking behavior of caregivers regarding ailments and exploration of possible interrelationship among these estimates and their variations across the strata of socio-demographic aspects among under-5 children detailed data analyses have been conducted. Overall Fever was the most common ailment [15.17% (12.83-17.52)], followed respiratory disorders [6.53% (4.92-8.15)] and gastro-intestinal problems (diarrhea) [5.09% (3.66-6.53)]. ENT [2.66% (1.61-3.71)] and skin diseases [0.55% (0.07-1.04)] were other disorders observed. 30% (274) of the participating subjects sought care from non-qualified practitioners, during ailment of their children (Fig: 15). Availability of non-qualified practitioners was highest. They were also most easy to get an appointment. Care-seekers found both private providers (qualified and non-qualified) to be more respectful than their counterparts in Government hospitals. The behavior of the non-treating staffs were similar across providers in terms of showing respects. Privacy was least maintained in Governmental sector. Non-qualified ones listened to the care-seekers most carefully, and their communication were best intuitive for the care-seekers. In Governmental hospital autonomy was least regarding involvement of the care-seekers in decision making. Confidentially were most assured, availability of choice regarding providers were most profound and treatment fees were most affordable for these providers (Table 10).

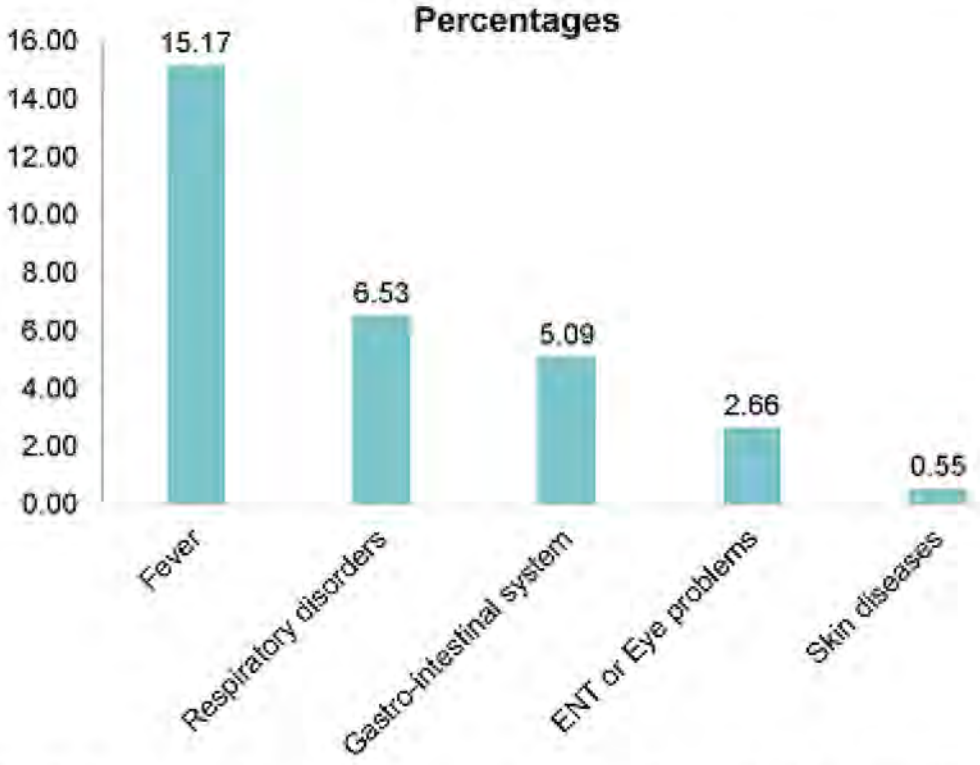


Fig 15 : Distribution of common ailments among under-5 children in urban slum areas of Kolkata

Table 10. Perceived HSR and Health-seeking among caregivers of under-5 children in urban slum areas of Kolkata

Health system responsiveness components	Qualified private practitioners	Government hospitals	Non-qualified practitioners
	Mean score	Mean score	Mean scores
Availability	2.52(2.45-2.60)	1.11(1.09-1.13)	3.69(3.61-3.76)
Ease of getting appointment	1.38(1.32-1.43)	0.69(0.64-0.73)	1.84(1.74-1.95)
Respectful treatment by provider	1.57(1.51-1.63)	0.76(0.72-0.81)	1.87(1.77-1.97)
Respectful treatment by staff	1.55(1.49-1.61)	1.32(1.28-1.37)	1.63(1.54-1.72)
Privacy was maintained	1.55(1.49-1.61)	0.73(0.68-0.78)	1.72(1.63-1.82)
Listened carefully	1.48(1.43-1.53)	0.68(0.63-0.72)	1.76(1.67-1.86)

Title: Rates of early initiation of breast feeding (EIBF) and exclusive breast feeding (EBF) up to 42 days of post-partum period and factors associated with failure to early initiation of breast feeding in rural West Bengal

Investigator : F. Debnath (PI); Co -PI: N. Mondal, BMOH, Dhaniakhali Block, WBHS, Govt. of West Bengal

Co- Investigator : S. Chakraborty, CMOH, Hooghli, Govt. of West Bengal

This is an ongoing intramural research project. The primary objectives of this study are to estimate proportion of newborns put on early breast feeding after birth, to estimate proportion of infants exclusively breast fed up to 42 days' post-partum period. The secondary objectives are, to determine the factors associated with failure of early initiation of breast feeding, to determine the factors associated with early interruption of exclusive breast feeding at 42 days' postpartum period, to estimate proportion of exclusively breast fed infant up to six months of age among those who will be exclusively breast fed up to 42 days' post-partum period. We opted a cohort study design for this purpose. We are conducting this study in Dhaniakhali Block of Hooghli District, where we need to recruit 319 newborns, assuming 38.3% of them are put on early initiation of breast feeding, odds ratio of two, 80% of power, 95% confidence interval and accounting for 10% of non-response. After obtaining all necessary approvals, we have started recruiting study participants who delivered at Dhaniakhali Rural Hospital. So far, we have recruited 37 mothers & their newborns in our study. The work is under progress. We are collecting information on sociodemographic, knowledge and attitude regarding breast feeding, delivery related issues, newborns' information, information related to EIBF & EBF using semi structured questionnaire. Post recruitment every infant is visited every week up to 42 days' post-partum period to collect information on EBF. Another visit will be paid one weak prior completing six months to collect information on EBF till that period, but this visit will only be paid to those who remained to be exclusively breast fed up to 42 days' post-partum period.

Awards/ Honours Received

F. Debnath

• Awarded MPH (Epidemiology & Health system) Degree by SCTIMST, Trivandrum

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

K. Sarkar

- Attended a national workshop on Operational Research, organised by National AIDS Control Organisation in collaboration with WHO & other partner organisation, held in New Delhi, during 30 Jan - 2 February 2017.
- Organised Two workshops on `Improvement of nutritional status among school children' in Auditorium of Kanchanjungha Stadium, Siliguri, on 8 & 9 December 2016. A large number of school teachers, BDOs, SDO & Mid Day Meal supervisors attended the workshops

Workshop on malnutrition in progress at Siliguri Kanchanjungha Stadium Auditorium

Workshop on malnutrition at Kanchanjungha Stadium Auditorium on 9-Dec. 2016

S. Panda

- In relation to 'Stop Diarrhea' project conducted in four States of India, Dr S Panda attended 'Technical Advisory Group Meetings' organized by Save the Children in mid-August, 2016 and end-January, 2017 in New Delhi.
- Dr S Panda attended the 'Expert Group Meeting' organized by ICMR-Headquarter during 18th – 19th December, 2016 in New Delhi to reflect on research activities on viral hepatitis in India with a special focus on hepatitis C.
- In tune with the requirement of University Grant Commission (UGC) for faculties of different colleges in West Bengal, Dr S Panda delivered lecture on 5th January, 2017 at the 'Department of Life Science and Biotechnology' of Jadavpur University as part of the refresher orientation/training course.
- In the 'Capacity Building Workshop on Operational Research, Ethics and Data Analysis' organized by the National AIDS Control Organization (NACO), Dr S Panda participated as a faculty member during 30th January – 2nd February, 2017 and played his role as a plenary speaker as well as facilitator of group work.
- National Seminar on 'Diarrheal disease burden and management: Special reference to North Eastern India' was organized by Tezpur University, Assam where Dr S Panda upon invitation delivered a key note speech on 'Diarrheal Disease Studies from north-east India: summary observations and research considerations' during 10th – 11th March. 2017.
- In the meeting on 'R&D blueprint development for action to prevent epidemics in response to WHO guidance document' organized by ICMR Headquarter during 14th – 16th March, 2017, Dr S Panda presented his views on 'how to proceed with and decide the outline for Indian response to promote strategic research for emerging/re-emerging infectious diseases of epidemic potentials' and participated in the ensuing discussion.
- Towards expanding the multi-dimensional and dynamic South-South cooperation for developing excellence in clinical and bio-medical research on HIV/AIDS, ICMR invited Dr S Panda to participate

in a meeting of eminent scientists from the South Africa Medical Research Council (SAMRC) during 27th – 29th March, 2017.

A. Deb

- Attended the workshop on Management of Viral Hemorrhagic Fever and Respiratory Infections Network Laboratories held at ICMR-National Institute of Virology, Pune on April 4, 2016.
- Attended the "Unnat Bharat Abhiyan (a flagship program of Ministry of HRD, Govt. of India) Workshop on Health care" organized by IIT-Kharagpur in Kolkata on May 9, 2016.
- Attended a workshop on "Strengthening Public Health Education and Research in West Bengal" organized by the Society for Health and Demographic Surveillance, Dept. of Health & Family Welfare, Govt. of West Bengal and held in Kolkata on June 21, 2016.
- Attended a workshop on "ICMR-WHO Regional Consultation for Draft National Ethics Guidelines for Biomedical and Health Research Involving Human Participants" held at ICMR-National Centre for Disease Informatics and Research, Bengaluru on October 4, 2016.
- Attended DST Sponsored training course on" Science & Technology for Rural Societies", held during November 7 – 11, 2016 at Indian Institute of Public Administration, New Delhi.
- Attended a training on Good Clinical Practice held at ICMR-NICED, Kolkata on March 16, 2017.

S. Kanungo

- Attended African Advanced Course of Vaccinology Course (Afro-ADVAC) held on 18-29 Sep 2016 in Muldersdrift, Johannesburg, South Africa.
- Guest Speaker at the preconference CME at 44th Annual National Conference IAPSMCON 2017 on topic Enteric Vaccines : Current updates at Kolkata on 9th Feb 2017
- Oral presentation at the 61st Annual National Conference of Indian Public Health Association (IPHA) and 1st State Conference of IPHA Rajasthan titled "Health system responsiveness as a modifier between perception and treatment seeking behavior among caregivers of under-5 children in urban slums: an idea to ponder upon" held from 24th Feb 2017 to 26th Feb 2017 organized by Dept. of Community Medicine and Family Medicine, AIIMS Jodhpur, Rajasthan
- Chairperson of free paper session at the 61st Annual National Conference of Indian Public Health Association (IPHA) and 1st State Conference of IPHA Rajasthan held from 24th Feb 2017 to 26th Feb 2017 organized by Dept. of Community Medicine and Family Medicine, AIIMS Jodhpur, Rajasthan
- Attended 10th International Conference on Typhoid and Other Invasive Salmonelloses, held on April 4-6, 2017 in Kampala, Uganda

F. Debnath

- Attended 6th training programme on "Science, Technology and Emerging Trends in Governance" Sponsored by DST, Govt. of India and conducted by Indian Institute of Public Administration, New Delhi during February 13-17, 2017
- Attended Workshop on" Ethics in Human Health Research and Good Clinical Practice" Organized by ICMR -National Institute of Cholera and Enteric Diseases, Kolkata & Forum for Ethics Review Committees in India on March 16, 2017
- Part of organizing committee for two days training workshop on the diagnosis of emerging viral diseases Sponsored by DHR-VRDL and organized at ICMR – NICED, Kolkata during March 28-29, 2017

IMMUNOLOGY

The traditional view is that the innate immunity is nonspecific and lacks memory, whereas adaptive immunity is characterized by specific antigen recognition and memory. However, this is no longer an accurate or useful categorization of the immune system as the receptors used by innate immune cells are specific. Thus, rather than innate and adaptive immunity being viewed as mutually exclusive, it is now apparent that the truth lies in shades of grey with blurring boundaries.

Compared with conventional B and T cells, innate B and T cells are enriched in mucosal tissues, where pathogens are first encountered. Memory T cells are usually considered to be a feature of a successful immune response against a foreign antigen, and such cells can mediate potent immunity. However, in mice, alternative pathways have been described, through which naïve T cells can acquire the characteristics and functions of memory T cells without encountering specific foreign antigen or the typical signals required for conventional T cell differentiation. Such cells reflect a response to the internal rather than external environment, and hence such cells are called innate memory T cells.

Development and functional properties of 'innate-like' T and B cells were studied for their potential role in regulation of protective immunology. The pathogen-associated molecule porin promotes preferential differentiation of the innate-like CD8 single-positive T cell subset in association with promyelocytic leukemia zinc-finger up-regulation and interleukin (IL)-4 production. On priming with anti-CD3 antibody, porin augmented TLR2 and IFN-gamma indicating a role of the TLR ligand in acquisition of innate memory response. Our study also reveal that culture system can be manipulated with LPS (PAMP) and HSP70 (DAMP) as well as conditions for expansion of 'innate-like' PD1+CD8+ T cells or PD1-CD8+ T cells *in vitro* for conducting immune therapy. In presence of LPS these cells also express suppressor of cytokine signaling (SOCS) 1 and thus suppress IL-10 expression. In contrast, heat shock protein (HSP) 70 down-regulated TLR4 augmenting the anti-inflammatory cytokine IL-10. In association with IL-10 release of IFN-gamma was abrogated.

To identify the molecules that allow manipulation of innate cells and orchestration of optimal immune response, the shift in cytokine milieu was also verified on innate B-1a cells. IL-10 is responsible for the long term survival of B-1a cells in culture, which is initially promoted by IL-15 followed by the appearance of SOCS1 which resulted in the cells switching to IL-12 expression. Interleukin (IL)-15, a key manipulator of innate T-cell function also modulates B-1a cell activity by augmenting activation markers, turning them towards type 1 polarization and immunoglobulin (Ig) expression which is significant in the context of gut immunity.

B cells with 'innate-like' functions also include marginal zone B cells. MZ B cells express high levels of the complement receptors CD21/CD35 and the nonclassical MHC molecule CD1d. The population is heterogeneous, multireactive and interacts with a wide variety of bacterial antigens and self-antigens. Our results show unlike FSL-1 and Pam3CSK4, porin does not stimulate the TLRs on these cells. Besides the TLRs, the activation molecules CD25 and CD69 remains unresponsive to porin showing MZ B cells are differentially driven by the molecule. Porin-induced expression of TOLLIP, known for down-regulating TLR2 and TLR4 and NLRC5 the central player for turning MZ B cells to "regulatory B10" cells via IL-10 production. Thus our work advances the knowledge towards the mechanisms that drive the unique development and function of both innate T and B lymphocytes.

The above is a compilation from data obtained from the two projects: Cytokine Regulation of Porin Stimulated B-1a Cell and Generation of Culture-differentiated Innate Memory CD8 Cells with Toll-like Receptor Expression and Responsiveness to Pathogen/Danger-associated Molecules.

Scientists:

T. Biswas, Scientist G
R. Biswas, Research Scientist

Staff:

S. Kumar Shaw, Technician-B
N. Chandra Mondal, MTS

Ph.D. Awarded:

Dr. Debolina Sinha received Ph.D. from Jadavpur University
Title of the Thesis: Porin Differentially Regulates Cytokine Expression of Follicular Zone and Marginal Zone B Cells.

Dr. Amlan Kanti Ghosh received Ph.D. from Jadavpur University
Title of the Thesis: Regulation of B-1a Cells by Interleukin-15 for Mucosal Immune Response.

Awards/ Honours Received

T. Biswas

- Adjudicated Ph.D. thesis & viva voce at PGIMER, Chandigarh (2016).
- Invited reviewer for Mucosal Immunology (Nature Publishing Group).

PARASITOLOGY

Parasites cause serious health problem across the globe with special emphasis in developing countries due to lack of proper resources and sanitation. The situation is further complicated by the emergence of drug resistant newer clones. Progress in controlling, eliminating or eradicating parasitic infections is a key part of the International health agenda. Keeping this in mind this Division of National Institute of Cholera and Enteric Diseases (NICED) is conducting various research programmes on *Entamoeba histolytica*, the causative agent for ameobiasis, and major enteropathogens like *Giardia lamblia*, *Cryptosporidium*, other coccidians and protists and different Soil Transmitted Helminths (STH) which are major health concerns in India.

The main research objective is the study of host-parasite relationships at large. A special emphasis is given to ectoparasites of cattle, vector borne diseases of large and small animals and increasing understanding of human parasitic diseases like Giardiasis, Amoebiasis, Cryptosporidiasis etc. can serve as the basic foundation for further development in screening, diagnosis and therapeutics research. The division has a long tradition of research on the biology of major diarrhoea causing parasites at its transcriptomics, proteomics and metabolomics level along with the molecular epidemiological studies. More recently, the lab has been implicated with the monitoring of Soil transmitted Helminthes (STH) of the school children between 5-12 years and STH prevalence mapping in North Eastern states of India.

As per the directives from Ministry of health and family welfare this division has initiated State-wide prevalence surveys of STH in technical cooperation and partnership with WHO, DtWi, State Governments for school aged children. An expert core committee has been formed under Ministry of Health and Family Welfare, Government of India, for nationwide strategy planning, development of guidelines, prevalence survey, mapping and deworming procedure where again the division of Parasitology of NICED, ICMR is one of the member. Depending upon the burden children can be included in regular deworming program. It helps in decreasing DALY rates, morbidity, decreased malnutrition and growth.

Modified Kato Katz technique for rapid identification of soil transmitted helminths (STH) has been introduced by our lab. It is low cost and highly effective. Identification of STH (soil transmitted helminthes) made easier and very cost effective. No need to make local labs for rapid identification within 1 hour of sample collection. The division has developed new diagnostic approach for better microscopy and molecular detection. Identification of parasites made easier and highly specific with Triple Feces Test (TFT technique).

The division has also been involved in the quality control assay program in partnership with AIIMS, New Delhi, KMC, Manipal, PGIMER, Chandigarh, SGPGI, Lucknow and CMC Vellore for parasite identification.

With the use of transcriptomics, proteomics and metabolomics the Parasitology division at NICED has already undertaken intervention strategies for controlling the diseases caused by these human pathogens. The strategies include: 1) prevention of host-parasite interaction, 2) identifying new genes and proteins, specific for the parasite that can be potential target sites for drug development, 3) targeting sub-cellular organelle, mitosome for anti-giardial drug design, 4) developing metabolite-based therapeutic agents derived from indigenous plants, 5) structure-function relationship of important enzymes like arginine deiminase/phospholipase B 6) protein kinases in relation to cell signaling, 7) programmed cell death in *Giardia*, 8) development of diagnostics, 9) stress survival and regulation of virulence gene expression in *Giardia lamblia*, 10) role of diverse intra and extracellular small signalling molecules in expression of genes essential for growth, survival in *Giardia*,11) genetic mapping of STR loci of *E. histolytica* genome to understand rapid emergence of new pathogenic clones 12) comparative genomics of *Entamoeba histolytica*.

The division has standardized PCR and ELISA based detection procedure of major parasites and also for simultaneous detection of three parasites namely *Giardia, Cryptosporidium and Entamoeba*. The Division of Parasitology has developed high resolution genotyping based identification of strains with probable hidden pathogenicity. The division has developed a new high resolution genotyping of *Entamoeba histolytica* to assess its virulence and pathogenecity. They have also been able to identify the enormous genetic diversity and different pathogenic markers and association of *Entamoeba histolytica* genetic patterns with disease outcome by high resolution Multilocus sequence typing system (MLST) in Kolkata. Besides that they have assessed helminth burden among different age groups in West Bengal with special emphasis on children of low socio economic class and evaluated a new TriCombo ELISA kit (Techlab) for the simultaneous identification of E *histolytica, G lamblia* and *Cryptosporidium* directly from stool samples. The division has arranged several seminar and workshop for the training on parasite identification and biosafety issues of the local laboratories and teaches how to handle stool samples and all the details about medical parasitology. This division serves as a resource in training on the identification of parasites by microscopy, PCR and ELISA. The division has contributed the knowledge about enteric parasites and STH including the epidemiology, trends of STH and school health issues, biology, clinical features, diagnosis, treatment, prevention and control.

Scientists :

Dr. S. Ganguly, Scientist-E
Dr. N. Mandal, Scientist-B

Staff :

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

Pre Doctoral Fellows :

Sumallya Karmakar ICMR (SRF)
Rituparna Sarkar CSIR (JRF)
Sanjib Kumar Sardar ICMR (JRF)

Ph D Awarded :

Dr. Dibyendu Raj received Ph D. from University of Calcutta

Title of the thesis : Studies on the oxidative stress management in *Giardia lamblia.*

Title : Studies on the Redox Homeostasis and antioxidant signaling in *Giardia lamblia*

Principal Investigator : S. Ganguly

Protozoan programmed cell death or apoptosis is an important factor in the survival of the parasite and its pathogenicity. The most amazing aspect of protozoan cell death is in its molecular architecture. To date, protozoa lack most of the components of the highly complex cell death machinery studied in multicellular organisms. Hence the unique apoptotic machinery in protozoa can be exploited for the development of therapeutic drugs and diagnostic markers.

This chapter focuses on *Giardia*, an intestinal protozoa undergoing stressed induced calcium dependent arachidonic acid mediated apoptotic like cell death.

To understand the mechanism of the mode of cell death we have examined the effect of arachidonic acid on trophozites under oxidative stress. As arachidonic is the result of phopholipid degradation by different intracellular phospholipase A2, we have detected arachidonic acid by spectrofluorometric analysis. It has been observed that arachidonic acid induced lipid peroxidation in *Giardia* and the confocal study shows that arachidonic acid triggers the apoptotic like cell death in *Giardia* in the presence of calcium. The calcium regulation revealed that intracellular free calcium is required to activate a pathway which is prostaglandin pathway (Fig.16). To confirm the prostaglandin production we have measured concentration of prostaglandin in both normal and stressed *Giardia*. This is the first report that we have identified the presence of prostaglandin pathway in *Giardia lamblia.*

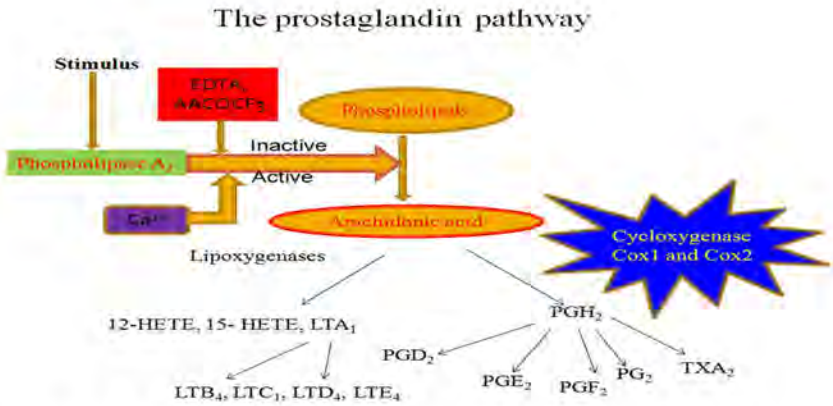


Fig 16 : Model representation of the conventional prostaglandin pathway.

Neither phospholipase A1 (PLA A1) nor phospholipase A2 (PLA A2), nor their respective genes, have been identified in *Giardia lamblia*, even though they are essential for lipid metabolism in this parasite (Javier Vargas-Villarreal et al, 2007). To assess the phospholipase activity we have inhibited the enzyme by different blockers. These results suggest that *Giardia lamblia* possesses several PLA2 isoforms that may be soluble or associated with membranes. Moreover, to involve in *Giardia lamblia* phospholipid metabolism, PLA2 could play important roles in the cytopathogenicity of this parasite.

Title : Genetic variations of *Giardia lamblia*

Principal Investigator : S. Ganguly

Co-PI : Prof. Tomoyoshi Nozaki, Director, Division of Parasitology, NIID, Japan

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 microarray for hybridization procedure for fishing out these particulate candidate regulators.

To elucidate this problem, *Giardia lamblia* DNA library (microarray) has been used containing more than 10000 clones to identify the differentially regulated genes in the oxidative stressed cells than the control set. The differentially regulated genes fall into five groups of functionally related proteins. These functional categories are: (i) metabolic enzymes; (ii) structural proteins; (iii) kinases and phosphatases; (iv) cell cycle and proliferation controller; and (v) cell death regulators.

The study has depicted a view on the genes that are differentially expressed during oxidative stress and taking important roles in *Giardia* stress management. NADH oxidase, NADH ferredoxin oxidoreductase, pyruvate ferredoxin oxidoreductase, thioredoxin reductase, nitroreductase, arginine deiminase, alcohol dehydrogenase etc. have been found to make a good network for oxidative stress management. This also indicates that several pathways are involved in this situation, as the enzymes are the representatives of some important biochemical pathways in other eukaryotes. In addition with this, a good number of hypothetical proteins are there that indicates their significant role in stress regulation. Some other proteins like Hsp90, β-giardin, cysteine rich VSPs, CAM kinase, serine threonine protein phosphatase etc. also play some important roles in *Giardia* survival.

NADH oxidase can directly convert oxygen to H_2O. Pyruvate-ferredoxin oxidoreductase can produce reduced ferredoxin from oxidative one and generates Acetyl CoA and CO_2 from pyruvate (Brown et al., 1998; Han et al., 2012). In case of metronidazole, toxic nitro-radicals are detoxified by nitroreductase generating NH_2OH and NH3. Peroxiredoxin converts H_2O_2 to H_2O. In this way the enzymes form a beautiful network to regulate the oxidative stress.

Cell death regulation has also been studied along with cell survival strategy by oxidative stress management. Cell cycle analysis revealed that increased oxygen tension affects the cell cycle progression and during death we found a sub G_1 peak in flow cytometer. Further studies confirmed that the parasite cells are not dying through necrosis but via a programmed cell death mechanism. In *Giardia*, cellular membrane distension and exposure of annexin V-associated phosphatidyl serine residues on the outer leaflet of the plasma membrane were interpreted as common denominators with canonical programmed cell death (PCD) i.e. the conventional cell death mechanism. Apart from this, in eukaryotes, DNA is known to undergo a recursive shearing process that in turn generate fragments of defined length, as seen from the typical electrophoretic ladder profile during PCD. When the same analysis was performed on *Giardia lamblia* dead cells, a low molecular weight persistent smear devoid of any banding pattern was observed recurrently. This feature unambiguously differentiates *Giardia lamblia* PCD from other described cell death forms and suggests the existence of a different underlying DNA fragmentation mechanism in organisms devoid of mitochondria. Similar type of report has been obtained in case of *Trichomonas vaginalis* (Chose et al., 2003). PI has shown typical DNA degradation in the dead cells. But caspase or other proteases not have been found to be responsible for this type of death. Cells after incubating with protease inhibitors when underwent stress, still they committed death in programmed pattern which is confirmed by Annexin V test and DNA fragmentation, same as before. Two cases in *Leishmania* and *Blastocystis* have been reported where also the PCD is independent of caspase pathway or other proteases (Nasirudeen et al., 2005), (Dutta et al., 2007). May be these early branching eukaryotes (EBE) including *Giardia lamblia*, undergo PCD using a novel pathway (Ghosh et al., 2009, Bagchi et al., 2012). In the later section of this study, some genes have been proved to be regulated during the cell death using quantitative real time PCR. Programmed cell death protein like protein, cytochrome, cathepsin etc are playing significant role in cell death determination where as GRP98 and protein for cell viability (PCV) are anti PCD and their expressions are inversely proportional with commencement of cell death.

Oxygen concentration always plays a vital role in the survival strategy and commitment of death from lower groups of organisms to higher eukaryotes. With the changes in global oxygen concentration from one era to another along thousands of years, lives on earth have evolved themselves from obligatory anaerobes to obligatory aerobic system via several facultative and mutual adaptations (Dowling *et al.*, 2008; Ginger *et al.*, 2010). Currently, the detail mechanism is not clear on how these severe changes occurred gradually with time. Studies on oxidative stress regulation in *Giardia* and similar early branching eukaryotes (EBE) may shed some new ideas about these changes.The information generated from the experiments can be used in chemotherapeutic interventions and new drug designing. As it has been observed, oxidative stress is regulating different cell biological activities in this primitive parasite, evolution of higher eukaryotes with aerobic metabolism, sexual reproduction, and programmed cell death under higher oxygen tension can be studied further in detail.

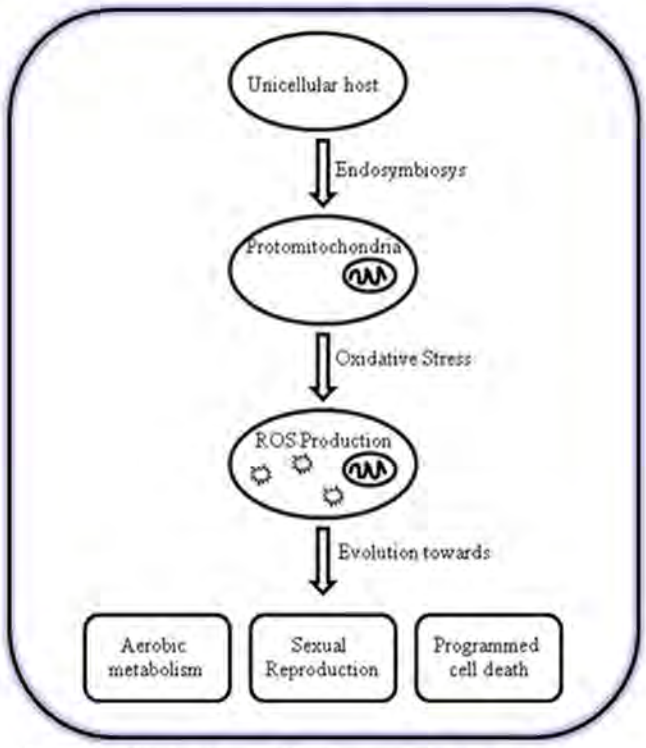


Fig- 17: Evolution of programmed cell death

Much information about the molecular cell biology and infection biology of *Giardia* spp. has accumulated during the past years. However, this is only the start of an intensive research period that will see *Giardia* spp. being used as model systems to understand genome function and evolution, gene expression, cell biology and intestinal immune responses. The genomes of several *G. intestinalis* isolates from different assemblages are being sequenced currently, and this will make it possible to develop methods for transfection, gene knockouts, functional genomics and gene expression studies. This will rapidly turn the diplomonads into a model system representing a branch of eukaryotic diversity that is currently very poorly studied. This is needed to complement the more established model systems such as yeast and *Caenorhabditis elegans*, so that we can gain an understanding of the basic principles of the eukaryotic cell and the mechanisms of reductive evolution and adaptation that have resulted from the parasitic lifestyle of these diplomonads. *Giardia* spp. will also be important model systems for other pathogenic protozoa and for studies of mucosal immunity (Ankarklev *et al*., 2010).

Awards/ Honours received

S. Ganguly

- Selected as advisory committee member of Department of Microbiology at Saint Xaviers University, Kolkata (Autonomous).
- Govt. of India expert committee member under Ministry of Health and Family Welfare, Government of India for conducting STH Mapping and deworming program in India.

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

S. Ganguly

- Dr Ganguly has been invited to "Annual Program review meeting for Soil Transmitted Helminthes in Delhi on 6- 7 Sept, 2016. Dr. Ganguly was invited to present progress of his work on STH mapping. He presented (oral) data of his project.

- Organizer and Resource person for a training program organized by NICED for training on "Identification of Soil Transmitted Helminths using Kato Katz technique", at National Institute of Cholera and Enteric Diseases During July 2-11, 2016.

- Organized and presented a paper in NICED-NIID, Japan-India joint bilateral meeting held in Kolkata from 12-13 Dec, 2016

- Invited participant in the "Confocal Microscopy Workshop – Revolutionize your confocal imaging" held in Bengaluru from 30 Jan – 1 Feb 2017.

- Invited participant as Judge in Smart India Hackathon 2017 from 31 Mar-2 Apr, 2017.

- Participated in a four day Quality management system and internal audit training course and workshop as per ISO 15189:2012 and NABL 112 from 24-27 Apr, 2017 at B M Birla Heart Research Institute, Kolkata

PATHOPHYSIOLOGY

This division is involved in several projects related to microbial proteases and their role in pathogenesis and tumor regression. Several proteases from *Vibrio cholerae* and *Escherichia coli* have been purified, cloned, expressed and characterized. The major protease secreted by *V. cholerae* is hemagglutinin protease (HAP). We purified HAP from a non-O1, non-O139 *V. cholerae* strain and showed for the first time its role in pathogenesis. HAP induced hemorrhagic fluid accumulation in rabbit ileal loop (Infection and Immunity, 2006). HAP is secreted by type II secretion system. We were the first to show that HAP along with other proteases like VesC (serine protease) are also secreted through outer membrane vesicles (Infection and Immunity, 2016).

We have also reported the association of SPATE autotransporter proteases with neonatal septicemic *E. coli* (NSEC) isolates and our study suggests that SPATEs may play a role in the pathogenesis of these isolates (EJCMID, 2014). Recently we have identified a novel metalloprotease, YghJ (SslE) from NSEC isolate. The 167 kDa protein was cloned, expressed, purified and induced pro-inflammatory response in mouse macrophages and intestinal epithelial cells (IJMM, 2016). We have also shown that YghJ could induce hemorrhagic fluid accumulation in mouse ileal loop (Microb Pathogenesis, 2017).

HAP from *V. cholerae* showed apoptotic response on several cancer cells like EAC breast cancer cells. We showed that HAP could regress tumor growth in EAC induced mice model (Apoptosis, 2016). We also identified the receptor (PAR1) on which HAP can activate and induce apoptosis (Apoptosis, 2016). HAP mediated PAR1 activation generated a novel pro-apoptotic peptide which was responsible for the activation of PAR1 and its downstream signaling pathways that induced apoptosis of malignant cells. The pro-apoptotic peptide showed PAR1 mediated apoptosis of cancer cells via MAP kinase and $NF_{K}B$ pathways. These signaling pathways also enhanced the cellular ROS level. *In vivo* studies in EAC induced Swiss albino mice model showed peptide treatment could significantly increase the survival rate of cancerous mice.

Our laboratory is also working on transport proteins involved in diarrhoea. We have showed the role of Anoctamin 6 in chloride secretion in Accessory Cholera Enterotoxin (ACE) stimulated diarrhoea (JBC, 2016). The role of tight junction proteins and its underlying pathways in inflammatory diarrhoea and the protective effect of Zn on this intestinal dysbiosis has been explored (IJID, 2016). We are also working on alteration of transport proteins and barrier functions during Zn deficiency. Further work is in progress to identify a purified compound from *Cucumis sativus* and study its role for treatment of cystic fibrosis and gastrointestinal disorder (FASEB, 2016).

Scientists

Dr. A. Pal, Scientist-F

DBT Ramalingaswami Fellow
Dr K.M. Hoque (till 28th November, 2016)

Staff
Mr. B. Roy, Technician-B

Post-doctoral students

Dr T. Ray (DST-SERB Research Associate)
Dr R. Tapader (ICMR Postdoctoral fellow)
Dr Sk. Irshad Ali (Arizona State University)

Predoctoral Fellows:

A. Mondal (SRF-ICMR)
Paramita Sarkar (SRF-DST INSPIRE)
Joydeep Aoun (SRF- ICMR)
Tultul Saha (SRF-UGC)
D. Kar (JRF-CSIR)

Ph D Awarded:

Dr. Rima Tapader received Ph. D (Microbiology) from University of Calcutta.

Title of the thesis: Studies on the role of serine protease autotransporters of *Enterobacteriaceae* (SPATEs) as a potential virulence determinant in clinical isolates causing neonatal septicemia.

Dr. Sk Irshad Ali received Ph.D from University of Calcutta.

Title of the thesis: Studies on the Epac1 Mediated Signaling in Epithelial Ion Transport and Barrier Function in Diarrheal Diseases.

Title: Molecular targeting therapy of breast cancer by PAR1 mediated apoptosis through a novel pro-apoptotic peptide

Investigator : A. Pal

Conventional cancer therapies are relatively non-specific and show side effects. For being a good therapeutic agent it should kill malignant cells without altering the survival of healthy cells by targeted strategies that specifically disrupt oncogenically active cell surface receptors. Several proteases and peptides have been reported to have anti-cancer activity. Due to the advancement in large scale synthesis of peptides it will be possible to make peptide based anti-cancer drugs more affordable to patients. In an earlier study we showed PAR1 mediated apoptosis of breast cancer cells by *Vibrio cholerae* hemagglutinin protease (HAP) and designed a novel pro-apoptotic peptide "PFISED". HAP and the pro-apoptotic peptide "PFISED" showed PAR1 mediated apoptosis of breast cancer cells via MAP kinase and NF_KB pathways. These signaling pathways enhanced the cellular ROS level. PAR1 is over expressed in malignant cells and the level of ROS in malignant cells is reported to be higher than the normal healthy cells. Malignant cells cross the threshold level of ROS faster than the normal healthy cells and induced intrinsic pathway of apoptosis. HAP and "PFISED" peptide mediated PAR1 activation induced apoptosis of breast cancer cells without altering the survival of healthy cells and may be used as a novel targeted therapy for cancer treatment. (Fig 18)

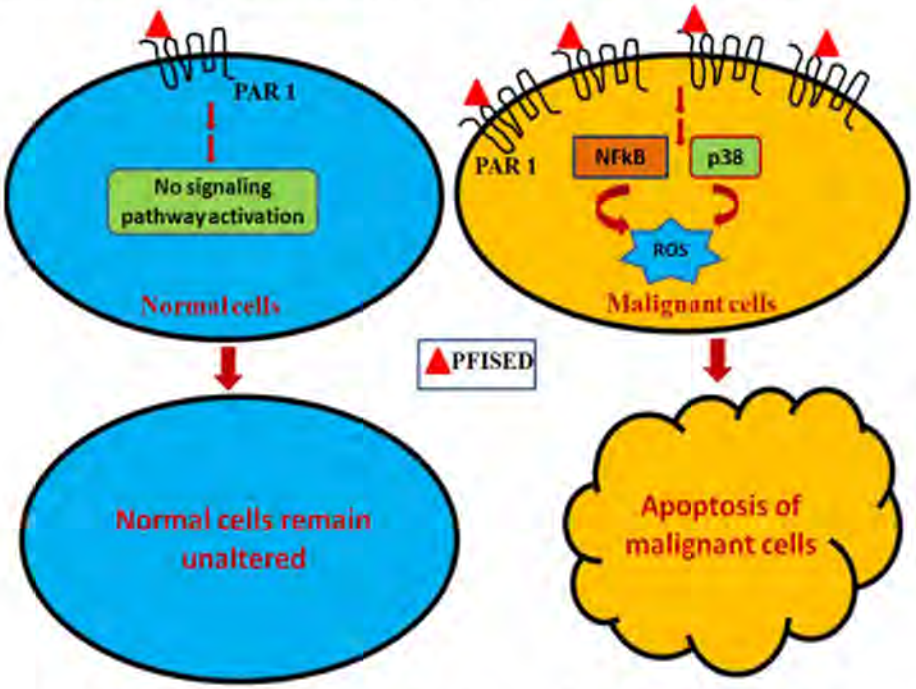


Fig 18: Novel pro-apoptotic peptide "PFISED" mediated targeted therapy for cancer The pro-apoptotic peptide "PFISED" causes PAR1 activation and trigger downstream signaling pathways (NFkB and p38) that induced apoptosis by increasing cellular ROS level. PAR-1 is over expressed in malignant cells compared to normal cells. Thus the novel pro-apoptotic peptide "PFISED" causes PAR-1 mediated apoptosis of malignant cells where as normal healthy cells remain unaltered in the same environment.

Title: YghJ (SslE), a secreted lipoprotein of neonatal septicemic *E. coli* induces TLR2 mediated inflammation in macrophages with the involvement of NFκB and MAP kinase signaling

Investigator : A. Pal

YghJ, also known as SslE of diverse *E. coli* pathotypes is a cell surface associated and secreted lipoprotein harbouring M60 metalloprotease domain. Importantly, YghJ was identified as a potential vaccine candidate for extraintestinal pathogenic *E. coli* which provided nearly complete protection from sepsis in a mouse model. Sepsis is characterized by the overproduction of diverse mediators such as proinflammatory cytokines and so far no factor other than LPS is known to stimulate the secretion of these cytokines. In our earlier study we first showed that YghJ from neonatal septicemic *E. coli* (NSEC) can trigger the production of a wide array of cytokines in murine macrophages which are essentially implicated in the pathogenesis of sepsis. Furthermore we found that YghJ can cause extensive tissue damage in mouse ileum. However, the signaling pathway leading to YghJ mediated proinflammation is still unexplored. In this study, we first report that YghJ induces over expression and activation of both TLR2 and TLR1 on mouse macrophage RAW264.7 cells. Immunoprecipitation shows that YghJ specifically binds to TLR2/TLR1 heterodimer and also activates MyD88 and TRAF6. Moreover, YghJ induces phosphorylation of ERK1/2, JNK1/2 and p38 and nuclear translocation of p50-p65 to trigger the MAP Kinase and NFκB signaling pathways. Pretreatment of macrophages with specific inhibitors against each signal molecule showed the involvement of ERK1/2, JNK1/2 and NFκB in the secretion of IL-1 (IL-1α and IL-1β) cytokines and involvement of p38 and ERK1/2 in the secretion of TNF-α. To further confirm the prerequisite of TLR2 in YghJ mediated proinflammation, RAW264.7 cells were transfected with TLR2 siRNA. Interestingly, YghJ induced activation of both NFκB and MAP Kinase signaling pathways was completely abrogated in TLR2 siRNA-transfected cells. Moreover, significant reduction of YghJ stimulated cytokine secretion was found in TLR2 knockdown cells. In addition, our study also demonstrates that YghJ enhances cellular ROS level, and stimulates NO production both of which are indicative of sustained inflammation (Fig 19). The secreted cytokines and chemokines from activated macrophages play important role in discrimination of M1 and M2 polarization of macrophages as well as attract and activate T lymphocytes. The cytokines (IL-1α, IL-1β, TNF-α) induced by YghJ are representative of type I polarization (M1). In our present study we further validate the M1 polarization of macrophages by investigating the chemokine profile induced by YghJ. Interestingly, our results demonstrate that YghJ stimulate the production of chemokines representative of M1 polarization such as MIP-1α, MIP-1β, RANTES, CXCL9 and CXCL10.

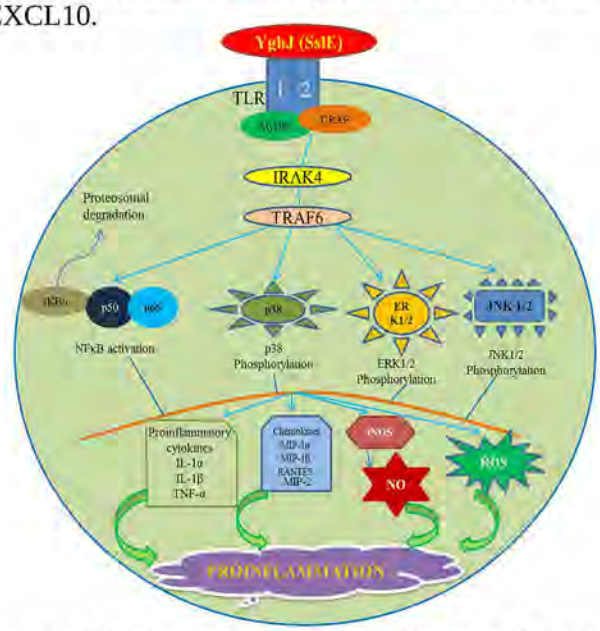


Fig 19 . YghJ (SslE) induced proinflammation in mouse macrophages is TLR1/2 mediated through the activation of NFκB and MAP kinase signaling pathways.

YghJ triggered activation of TLR1/2 heterodimer and subsequent recruitment of adaptors MyD88, activation of TIRAP which in turn recruits and activates TRAF6 and other downstream molecules. This leads to the IκB proteosomal degradation followed by nuclear translocation of p50 and p65 and also phosphorylation of p38,ERK1/2 and JNK1/2. The activation of NFκB and MAP kinase signaling pathways leads to the secretion of various proinflammatory cytokines such as TNF-α, IL-1 α, IL-1β, different M1 chemokines such as MIP-1α, MIP-1β, MIP-2, RANTES and also other inflammatory hallmarks such as cellular ROS and NO. These together leads to the proinflammatory response of macrophages by YghJ.

Title: Zinc ameliorates altered intestinal barrier functions and proinflammatory responses induced by *S. flexneri* through Mitogen-Activated Protein Kinase- Dependent modulation

Investigator : K. M. Hoque

Zinc (Zn) has emerged as a major anti-diarrheal therapeutic and preventive strategy in the current management of diarrhea; however the mechanisms by which Zn regulates barrier functions in the setting of intestinal infection when pathogens like *Shigella* influence epithelial permeability by modulating TJ proteins remains unknown. Here we investigated the potential benefits of Zn in restoring impaired tight junction network preceding inflammatory responses following *Shigella* infection, using polarized T84 monolayers and in vivo mouse model.(Fig.20) Basolateral infection triggered time dependent decrease in transepithelial electrical resistance followed by an increase in paracellular permeability of FITC-labelled dextran and altered ion selectivity. Immunofluorescence study revealed a redistribution of the tight junctional transmembrane protein Claudin-4 and Claudin-2 from an intercellular to an intracellular location, while subcellular fractionation demonstrated accumulation of tight junction proteins increased to the cytoplasm following infection. Zn ameliorates this perturbed barrier function and comparable redistribution of tight junction components by modulating phosphorylation levels through ERK1/2 activation. Treatment with Zn prevents elevated secretion of intestinal specific inflammatory cytokines IL-6 and IL-8 limiting inflammatory responses. C57BL/6(B6) mice challenged with virulent *S.flexneri* showed significant increase in paracellular transport of FITC dextran and severe bacillary pathogenesis. Oral Zn supplementation diminished bacterial shedding, restored barrier function and helped in clinical setting of diverse pathophysiological symptoms provoked upon *Shigella* infection. When taken together, these findings suggested novel mechanisms of Zn action involving ERK1/2 activation in restitution of altered barrier function along with inflammatory responses during shigellosis, thus having a therapeutic potential in acute inflammatory diarrheal diseases.

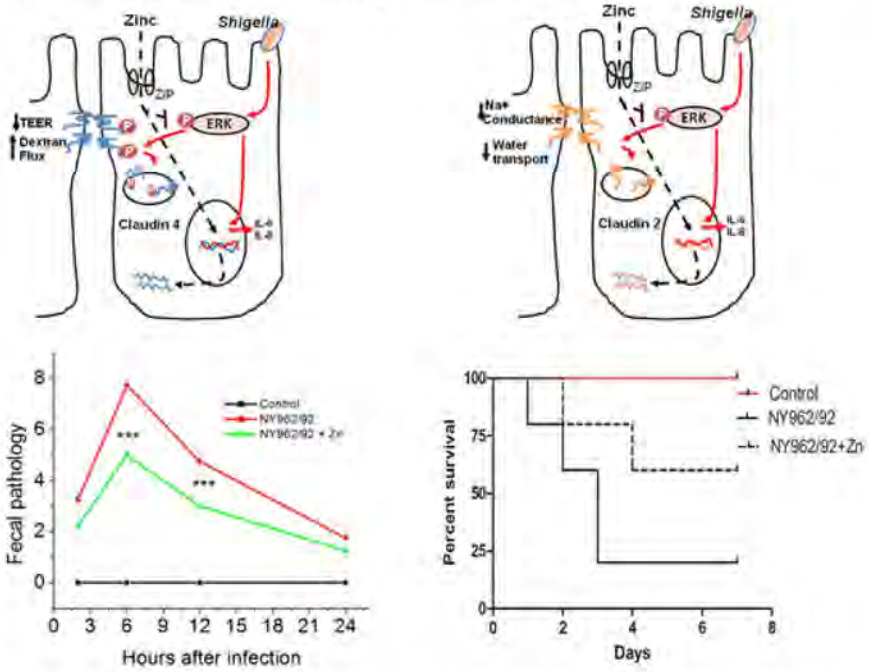


Fig 20 : Zinc mediated enhancement of barrier function during *Shigella* infection. Schematic illustrations showing the mechanism of Zinc modulated regulation of (A) Claudin-4 and (B) Claudin-2 in *Shigella* infection. *Shigella* pathogenesis in adult mice after intraperitoneal (i.p.) infection with *S. flexneri* 2a strain (NY962/92; 5×10^8 CFU/mL) in presence and absence of oral Zn supplementation. (C) Fecal pathology scored with feces on the basis of color and consistency and (D) survival rates at different post infection time points.

Awards/ Honours received

A. Pal

- Examiner for M Sc 2nd Semester Practical examination at the Department of Microbiology, Bidhannagar College on 24th June, 2016
- Elected as Recorder of the Section of Medical Sciences (including Physiology for 2016-17 and 2017-18) in the 104th and 105th Session of Indian Science Congress
- Nominated as a government representative for the Renewal of registration and reconstitution of the Institutional Animal Ethical Committee for CPCSED, New Delhi for Dey's Medical Stores 12th Jan, 2017, National Research Institute of Ayurvedic Drug Development 17th Feb, 2017, BCDA College of Pharmacy and Technology, Barasat 11th April, 2017

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

A. Pal

- Delivered a talk at the 104th Indian Science Congress held at Tirupati from 3-7th January, 2017
- Co-opted as an external expert member of the Board of Post Graduate studies in Physiology, Burdwan University on 20-02-2017
- Delivered a talk at the National Symposium on Oxidative stress in Health and Diseases at the University of Kalyani 30-31st March, 2017

K. M. Hoque

- Mirajul H Kazi, Saha T, Woodward W, Guggino WB. Identification of Ano1 (Tmem16A) activator purified from Cucumis sativus: Its role for treatment of cystic fibrosis and gastrointestinal disorders. At the Experimental Biology Meeting, April 2-6, 2016 held in San Diego Convention Centre, San Diego, CA, USA.
- P. Sarkar, T. Saha, P. Ghosh, J. Aoun, M. K. Chakraborty, M.H. Kazi.Dietary Zinc deficiency induces epithelial barrier dysfunction and altered intestinal ion transport in T84 cells. International Conference on perspectives of cell signaling and molecular Medicine, Jan 8-10, 2017 held in Bose Institute, Kolkata.

VIROLOGY

Diagnosis of diarrhoeagenic viruses and their molecular characterization to understand the genetic diversity

The team in Division of Virology of ICMR-NICED is actively associated with surveillance studies being conducted by the National Institute of Cholera and Enteric Diseases to understand the role of different viral etiological agents in circulation, including new variants and emerging diarrhoeagenic viruses, in and around Kolkata that are associated with diarrhoea cases. Molecular characterization studies enable us to gather knowledge on the genetic diversity and. phylogenetic nature of the circulating enteric viruses with focus on Groups A, B, and C Rotaviruses, Caliciviruses viz. Norovirus and Sapovirus, Astroviruses, Picobirnaviruses, emerging three segmented etiological agent and Adenoviruses.

Understanding HIV epidemic and HIV drug resistance mutation in East and N-Eastern India.

ICMR-NICED being a leading government public health institute has been providing technical and research inputs for prevention and control of HIV in the country with particular focus on East and North-Eastern states of the country. Virology Division with state of art laboratory infrastructure and dedicated skilled human resources is actively involved with the national HIV program and having the distinction of detecting the 2nd HIV positive case in the country in the year 1986. As NACO-Regional Institute (East) for HIV Surveillance, ICMR-NICED has been conducting sentinel surveillance for the states of Andaman & Nicobar, Chhattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal for ANC attendees, FSW, MSM, IDU, Trans Genders, Long Distance Truckers and Migrants.

Antiretroviral therapy (ART) has led to the emergence of HIV drug resistance (HIVDR) mutations. HIVDR limits the success of ART and therefore needs to be monitored. Our study has revealed non-appearance of major or minor HIVDR among ART naive individuals indicating absence of transmitted HIVDR in Kolkata, a metropolitan city in eastern India. It seems that people living with HIV (PLHIV) in this area might be benifitted with national ART program. However presence of major and minor HIVDR mutations among PLHIV exposed to ART indicates requirement of in-depth studies to unveil the current scenario of HIVDR among ART exposed PLHIV at different stages of ART exposure.

Studies towards understanding dynamics of Host-Rotavirus interaction

The NICED Virology lab is one among very few laboratories in India which studies basic aspects of virus replication, and the functional aspects of host-virus interaction. The focus is towards identification of cellular proteins which are modulated during Rotavirus infection, understanding the underlying mechanisms by which rotavirus exploits cellular proteins for their propagation in the host cells.

Surveillance of Influenza and other respiratory Viruses

Hospital based surveillance for respiratory virus infected cases in outpatient department and hospitalised cases is ongoing to monitor seasonality, emergence of new subtypes and monitoring antiviral resistance among circulating influenza strains. The study is done in collaboration with NIV, Pune and Center for Disease Control and Prevention, Atlanta USA. The laboratory also provides service for influenza diagnosis during outbreaks of acute respiratory infection.

Influenza virus diagnostics:

For adequate measures to be taken to detect and fight against infectious diseases, their surveillance is essential. Referral samples from different hospitals from acute respiratory illness are being tested for detection of influenza A H1N1. Apart from West Bengal samples from other states are also included for detection and further studies.

Study related to establishing a network of population based influenza surveillance platforms for elderly persons in India has been formulated in collaboration with CDC-AIIMS. This multi centric study will be useful to understand the incidence of influenza in elderly population in India and also to understand the seasonal pattern

of influenza among the elderly.

Isolation of influenza virus from the referral samples is ongoing in tissue culture with an objective to characterize and study on host-virus interactions

Scientists:
Dr. T. Krishnan, Scientist-F
Dr. M. K. Saha, Scientist-F
Dr. M. Chawla-Sarkar, Scientist-E
Dr. A. K. Chakrabarti, Scientist-D

Staff:
Dr S. Bhunia, Technical Officer-A
Dr S. K. Sadhukhan, Technical Officer-A
Mr. S. Omesh, Technical Officer-A
Ms. M. Mullick, Technical Officer-A
Ms. P. Bhaumick, Technical Officer-A
Mr. K. Sen, Technical Assistant
Ms. P. De, Technician-B
Md. M. Hossain, Technician-B
Ms. C. Das, MTS (General)

Pre-Doctoral Fellows:
Edwin Anthony, UGC-MANF (JRF)
Arpita Mukherjee, UGC-(SRF)
Upayan Patra, ICMR-(SRF)
Anindita Benerjee, DST Women Scientist
Urbi Mukherjee, UGC-(JRF)
Rakesh Sarkar, UGC-(JRF)
Ms.Piyali Ghosh, Project Assistant

Ph.D. Awarded:
Dr. Shampa Chanda received Ph.D. from the University of Calcutta
Title of the Thesis : Analysis of Role of "RNA Interference" and Mechanism by Which Rotavirus Counteract RNAi during Rotavirus Infection

Title: Detection of emerging viruses among acute gastroenteritis cases in Kolkata, India

Principal Investigator : T. Krishnan

Faecal specimens of approx. 3498 cases were screened for detection of non Group A rotaviruses by agarose gel electrophoresis, followed by ethidium bromide staining, from IDH Kolkata (Fig.21) and BCRIPS, Kolkata (Fig.22) The characteristic electropherotype of trisegmented genome was detected by agarose gel electrophoresis showing the emergence of a different etiological agent associated with viral gastroenteritis 254 cases with trisegmented genomic RNA profile were detected among diarrhoea cases. The emerging etiological agent showing three segmented genomic RNA profile was detected in diarrhoea cases of different age groups viz. 59 cases (23.22%) aged below 5 years; 13 cases (5.10%) aged above 5 years but below 12 years; 18 cases (7.08%) aged above 12 years but below 18 years; 164 cases (64.56%) in the adult age group above 18 years.

The dehydration status was different in the 151 diarrhoea cases that were sole-positive for the emerging etiological agent viz. some dehydration was observed in 121 cases (78.9%); severe dehydration in 23 cases (15.12%); no dehydration was seen in 7 cases (5.88%)

The nature of the faecal specimen also varied in these 151 diarrhea cases; the stool was watery in 115 cases (79.83%) loose stool in 33 cases (17.64%) and bloody mucoid stool in 3 cases (2.52%).

The positive cases also presented with other symptoms viz. only vomiting in 32 cases (15.12%); only abdominal pain in 11 cases (8.40%); only fever in 3 cases (1.68%); both abdominal pain and vomiting in 45 cases (26.89%); both vomiting and fever in 13 cases (7.56%); both abdominal pain and fever in 5 cases (4.20%); the three clinical symptoms viz. vomiting, fever and abdominal pain was observed in 23 cases (17.64%) and only diarrhoea without any of the other three clinical symptoms was recorded in 19 cases (18.48%).

The emerging etiological agent with trisegmented genomic RNA was the only detected pathogen in 151 cases; but in the remaining 103 cases, it was detected with co-infection of other enteric pathogens; one or more virus in 28 cases (21%); one or more bacteria in 55 cases (31%); only one parasite in 7 cases (2.52%). mixed co-infection was observed in some instances viz. bacteria and virus in 5 cases (4.20%); bacteria and parasite in 5 cases (2.52%); virus and parasite in 3 cases (2.52%).

The next generation sequencing results indicate that the three segmented etiological agent is a hitherto unreported new agent associated with acute watery diarrhoea in Kolkata, India. The emerging etiological agent (n=31) was also detected in faecal specimens of OPD cases (approximately 400) screened from Dr. B. C. Roy Institute of Pediatric Sciences (BCRIPS) in Kolkata.

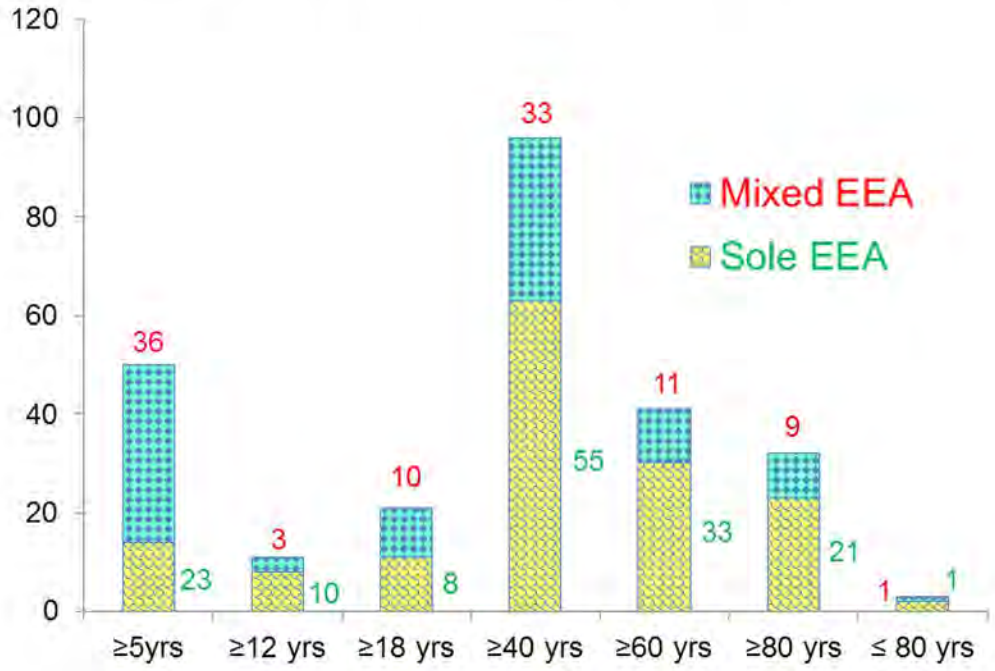


Fig 21. Detection of emerging etiological agent (EEA -n=254 -IDH) among acute watery diarrhoea cases in Kolkata, India

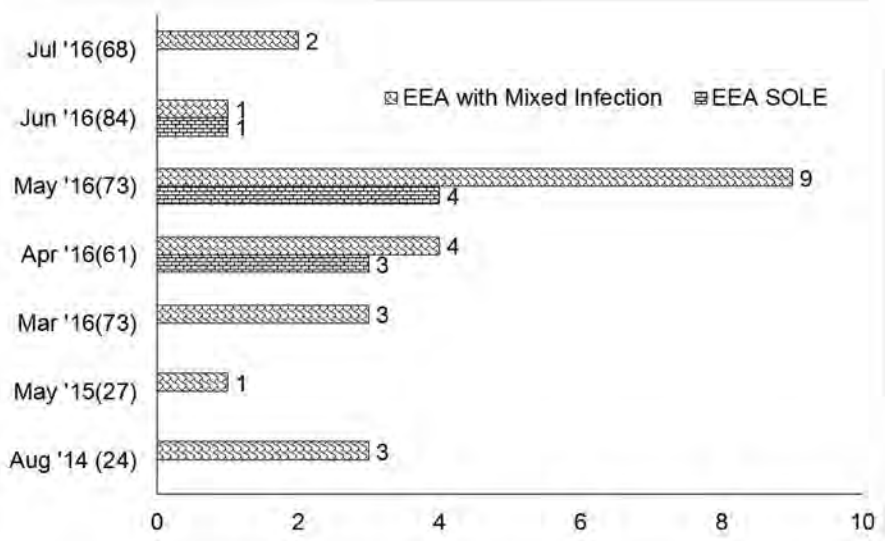


Fig 22: Detection of emerging etiological agent (EEA) as sole and mixed infections (August 2014 to July 2016) from OPD cases at BCRIPS, Kolkata

Title: HIV risk among injecting drug users in Nagaland: Analysis of HIV surveillance data

Principal Investigator: M. K. Saha.

India has one of the largest and most robust HIV Sentinel Surveillance (HSS) activities in the world. Six leading Government Public Health Institutes (AIIMS, ICMR-NARI, ICMR-NICED, ICMR-NIE, PGIMER, and RIMS) have been involved, as Regional Institutes for HSS, in implementing the Indian national HSS. HSS has been the core activity in tracking HIV epidemic with the aim to provide accurate and consistent information about the level and trend of epidemic. It helps in fine tuning the planning for strengthening, prevention and control of the epidemic. ICMR-NICED has been implementing the project for the states of A&N Islands, Chhattisgarh, Meghalaya, Nagaland, Sikkim and West Bengal for ANC attendees, FSW, MSM, IDU, Trans Genders, Long Distance Truckers and Migrants.

HSS data indicated that the HIV epidemic still remained concentrated among injecting drug users (IDUs) in Nagaland, N-E India. Among 1856 IDUs tested, HIV sero-positivity was 2.21%. Mean age of study participants was 28 years, 98.09% were males, 67.92% were never married/divorced/separated/widowed, 61.09% were educated up to secondary level, 55.66% were urban residents, 54.96% attended for needles/ syringes collection, 46.15% were unemployed/students, 34.99% injected drug twice a week, 33.66% used drugs for 1-3 years. Older subjects and unmarried/divorced/widowed/separated were less likely to inject drugs for long. Educated youths, truck drivers and rural residents were more likely to inject drugs for longer duration. Higher frequency of injecting drug was associated with increasing age, unskilled work, business and rural residence. Risk of HIV among unmarried/divorced/widowed/separated and among those who tried for opioid substitutes was higher. Interventions programs targeting older, educated, employed, unmarried/ divorced/separated/widowed, male IDUs of rural Nagaland might help to control HIV.

Title: HIV Drug Resistant Mutations among ART naïve People Living with HIV in Kolkata, India.

Principal Investigator : M. K. Saha.

Emergence of HIV Drug resistance (HIVDR) compromises the effect of antiretroviral therapy (ART), resulting in treatment failure. Even HIVDR was reported from individuals not exposed to antiretroviral therapy (ART) indicating occurrence of transmitted HIVDR among People Living with HIV (PLHIV). Genotyping of HIV among a cohort of 47 consecutive ART-naïve PLHIV in Kolkata, India, was conducted during 2013-15. Protease (PR) and reverse transcriptase (RT) genes were sequenced for HIV-1 subtyping and phylogenetic analysis and to detect HIV Drug Resistance Mutations (DRM) comparing sequence data with Stanford HIVDR database. All samples were of Clade C subtype. No major or minor HIV DRM was detected. Only accessary mutations were detected among 9 samples (Table 11). Absence of major or minor HIV DRM and marginal presence of accessary polymorphic mutations, among a cohort of 47 consecutive ART-naïve PLHIV revealing non-existence of transmitted HIVDR and most likely transmitted HIV might not be present here. Thus the first line ART seems be an effective choice.

Table 11: HIV-DR mutation analysis employing Stanford HIV-Drug Resistance Database

HIV-DR mutation analysis employing Stanford HIV-Drug Resistance Database	
Mutation	**Implications of the Accessory Mutation detected**
K20R	Highly polymorphic, PI-selected accessory mutation
L 89M	Improves HIV-1 replication fitness with other PI-resistance mutations. Common polymorphism not associated with reduced PI susceptibility. A consensus amino acid in most non-B subtypes.
V82I	Highly polymorphic substrate-cleft mutation, not selected by PIs. It is consensus amino acid in subtype G viruses.
A71V/T	A71V/T are polymorphic, PI-selected accessory mutations. Increases replication of viruses with other PI-resistance mutations.
A71I/L	A71I/L are non-polymorphic, PI-selected accessory mutations. Increases replication of viruses with other PI-resistance mutations.
T74S	T74S is PI-selected accessory mutation, polymorphic in most non-B subtypes
G48V, G48M	G48V and G48M are non-polymorphic mutation selected in viruses with multiple PI-resistance mutations.
V75I	V75I is a relatively non-polymorphic accessory mutation that often occurs in combination with the multi-NRTI resistance mutation Q151M. When V75I occurs alone its clinical significance is uncertain.

However, what happens to ART exposed PLHIVs in eastern India has rarely been studied. It is pertinent to examine the outcome of first line ART in resource-limited settings, particularly in Indian context because ART has been scaled up and now being provided to all PLHIV. An exploration to find out HIV DRM among 5 first line ART exposed (one year) PLHIVs showed emergence of DRM. Initial data suggests that the major DRMs were associated with nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI). This observation warrants further in-depth studies on virologic failure and of HIV DRM among first line ART exposed PLHIV for better understanding of HIVDR dynamics as well as planning evidence based clinical management.

Title: Immobilization of α amylases onto graphene oxide nano-support for activity enhancement and thermo-stability

Principal Investigator : M. K. Saha

Amylases, as biocatalysts, having wide usage in industrial applications (processed food industries, additives in detergents, waste-water treatment, bio-pulping, and bioremediations). Efficiency of enzymes are increased manifold by immobilization on suitable matrices that offers reusability and operational stability by altering its thermodynamic characteristics. Nanomaterial supports are being widely used as provider of larger surface area for the substrate to interact with the enzyme coupled with increased loading capability. It is the higher surface to volume ratio of the nanomaterials which increase immobilization efficiency, storage and reusability of the enzyme. Bio-engineering involving graphene has gained headway in recent past with promising results for its stereo selectivity and bio-solubility. Graphene oxide (GO), owing to its varied flexibility in biological applications as nano-support has found its use in production of biocatalysts, biosensors, and drug delivery vehicles. The use of glutaraldehyde as a cross linker for enzyme immobilization on graphene confers increased structural stability to GO-enzyme complex.

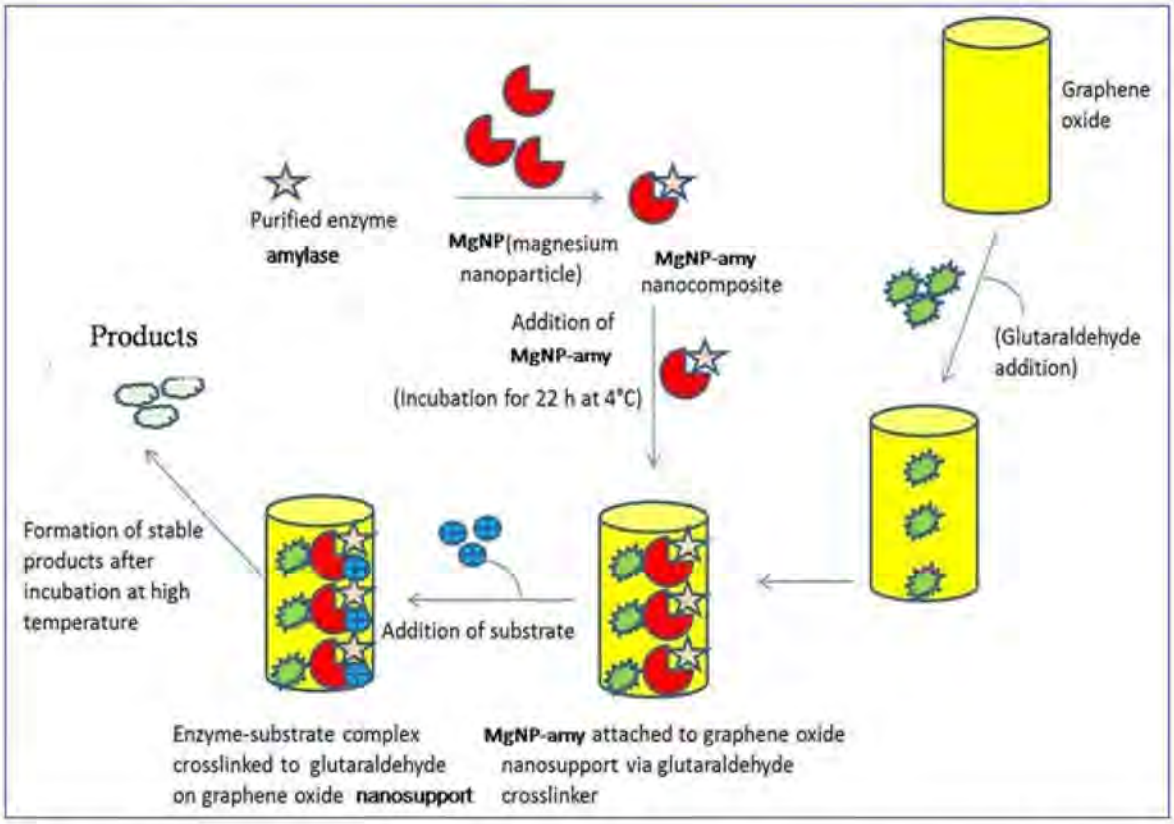


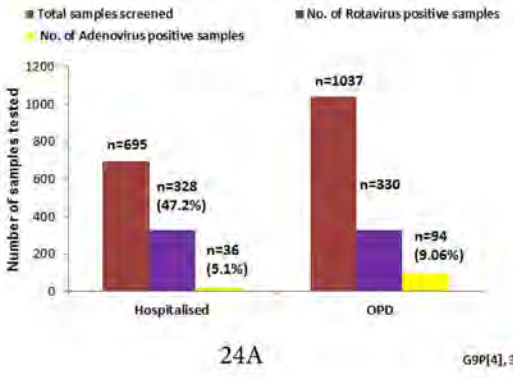
Fig 23: Immobilization of alpha amylase on graphene oxide nanosupport employing glutaraldehyde crosslinker

An immobilization technique was devised for conferring psychro-stability to a cold active α -amylase (amy) enzyme by the use of magnesium nanoparticle (MgNP) and graphene oxide (GO). The GOMgNP-amy nanocomposite showed enhanced enzymatic activity and thermos-stability at both upper (90°C) and lower (8°C) temperature extremes. The GO-MgNP-amy showed increased affinity towards substrate, reflected in the decrease in its K_m by 2.35 and 14.9-fold at 8°C and 90°C, respectively, than the untreated enzyme. GO-MgNP-amy showed 2.34- fold and 4.29-fold increase in V_{max} at 8°C and 90°C, respectively, than the untreated enzyme. When compared to native enzyme at 90°C, GO-MgNP-amy had t1/2 (half-life) increased by 44-fold. Again at 8°C, GO-MgNP-amy had t1/2 increased by 6.48-fold compared to the native enzyme. The enzymatic activity of GO-MgNP-amy was retained even after 12 repeated uses and showed storage stability at 4°C for more than 120 days. The ability of GO-MgNP to sustain and aggravate enzyme activity and stability at temperatures beyond the optimal range could be beneficial in bioprocessing industries which requires functioning at these extreme ranges of temperature.

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

Principal Investigator : M. Chawla Sarkar

As part of the Institutional diarrhoeal disease surveillance and National Rotavirus Surveillance Network, rotavirus (RV) surveillance is conducted by NICED to assess prevalence of Rotavirus infection among hospitalized children and to monitor circulating strains in the region. This surveillance is a part of the national network to provide baseline information as RV vaccine has been introduced in national immunization program in four states in India. Vast diversity in the RV genotypes and rapid emergence of novel types due to recombination in developing countries raise concern, thus comparison of pre-vaccination data with post vaccine scenario will be important for determining vaccine efficacy. A total of 1732 samples were tested during 2016-17, of which 658 (38%) were positive for rotavirus (Fig 24). Genetic characterization of positive strains revealed predominance of G1P[8] and G3P[8] genotypes during this period, while other genotypes like G1P[4]/P[8]/P[6]; G2P[4]/P[8]; G3P[4]; G9P[8]/P[4]; G12[P6]/P[8] were observed at low frequency. Emergence and circulation of G3P[8] strains in Eastern India has been observed in Eastern India after more than a decade. In addition to RV, 130 (7.5%) were Adenovirus (HADV) positive. HAdV-40 was found to be the predominant genotype while HAdV-41 co-circulated, at very low frequency.



24A

24B

Fig24 . A. Rotavirus and Adenovirus positivity among children (< 5yr) seeking healthcare facility for treatment of gastroenteritis. During diarrhoeal disease surveillance (April 2016-March 2017), hospitalized patients were enrolled from Pediatric wards of ID & BG Hospital and Institute of child health, patients from Outpatient department (OPD) were enrolled in BC Roy Post Graduate Institute of Pediatric Sciences. Stool samples were tested by Rota-Adeno rapid diagnostic kit. **B.** Pie diagram representing distribution of circulating genotypes of Rotavirus strains in Kolkata.

Title: Identification of Host Determinants which modulate Rotavirus Infection and to assess their small molecule inhibitors as anti-viral candidates

Principal Investigator : M. Chawla Sarkar

Phospho-proteomics platform has been widely used to identify post-translational dynamics of cellular proteins in response to viral infection. The present study was undertaken to assess differential tyrosine phosphorylation during early hours of Rotavirus (RV) SA11 infection. Seventy-one differentially tyrosine phosphorylated proteins were identified in response to RV-SA11 infection at 2hpi. Chaperone proteins showed highest enrichment followed by nucleic acid binding proteins, cytoskeletal proteins and hydrolases. Hsp60 was further found to be phosphorylated by an activated form of Src kinase on 227th tyrosine residue. Interestingly, tyrosine phosphorylation of mitochondrial chaperonin Hsp60 was correlated with its proteasomal degradation at 2-2.5hpi. The level of mitochondrial NSP4 was found to be positively regulated by mHSP60. Phosphorylation and subsequent transient degradation of mitochondrial Hsp60 during early hours of RV-SA11 infection resulted in inhibition of premature import of NSP4 into the mitochondria, thereby inhibiting early apoptosis. Overall, the study highlighted one of the many strategies rotavirus adopts to evade premature apoptosis for efficient viral propagation (Fig 25).

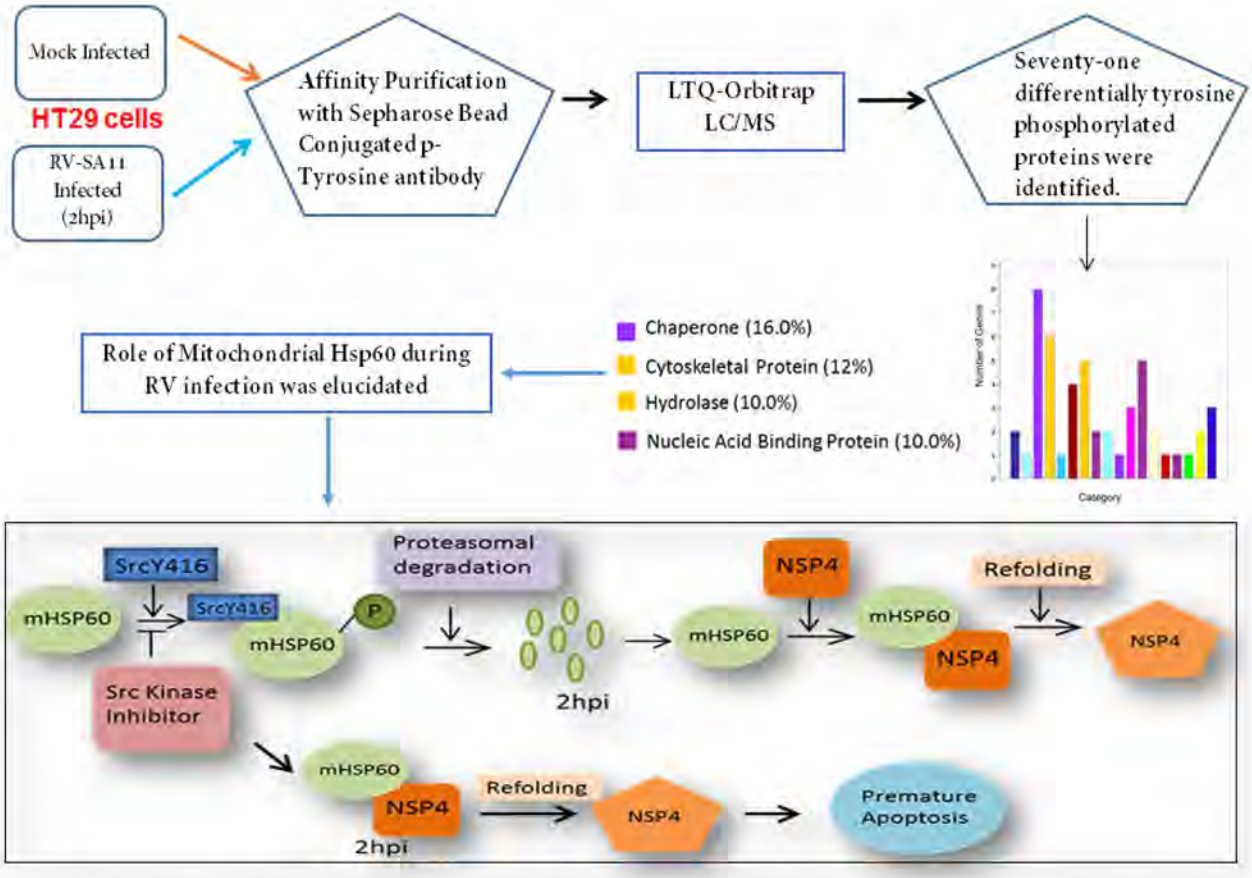


Fig 25. Schematic representation of modulation of mitochondrial chaperonin Hsp60 by tyrosine phosphorylation during Rotavirus infection. Mitochondrial Hsp60 is degraded following Tyr-Phosphorylation resulting in delay in translocation of NSP4 to mitochondria and consequent Rotavirus induced apoptosis.

Title: Hospital based Surveillance for Respiratory Viruses in Outpatient and Inpatients seeking treatment for acute respiratory illness.

Principal Investigator: M. Chawla Sarkar

Hospital based surveillance for panel of respiratory viruses was initiated in Sagar Dutta Medical College and Hospital in Kolkata as part of a Multi-centric study co-ordinated by NIV Pune in 2016. A total of 517 clinical samples have been collected from suspected Acute Respiratory Infection (ARI) cases and tested for 12 Respiratory viruses by real time PCR. During the study period (Oct 2016-March 2017), Human Metapneumovirus (19.7%), Adenovirus (19.1%), Rhinovirus (18.3%) were predominant among ARI cases. Other viruses including Influenza, RSV, Parainfluenza I-IV were observed at a very low frequency (1-2%). The study is ongoing.

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

Investigator: A. K. Chakrabarti

Vibrio phage Reference Laboratory is involved in study of phage typing. *V. cholerae* isolated from 6 different states including West Bengal were sent to the phage Reference Laboratory for phage typing study. Strains received were confirmed as *V. cholerae* O1 biotype EI Tor. Most of the strains found as serotype Ogawa and few were found as serotype Inaba. Using a panel of typing phages the strains were discriminated into different phage types. Our result indicated the presence of two different types. Type 2 and Type 4 when typed using the conventional scheme of Basu and Mukherjee. However the strains can be discriminated in different types using the new phage typing scheme. Among the several types phage type 27 was the major type.

Awards/ Honours received

T. Krishnan

- Member of Editorial Board of the World Journal of Clinical Infectious Diseases for a term period from 2016 to 2019.
- Invited to join the Editorial Board of the Asian Journal of Medicine and Health as Academic Editor from 24th August 2016.
- Sentinels of Science Award in recognition of being one of the 'Top overall contributors to peer review in science and research and number of verified pre-publication reviews completed between 1 October 2015 and 17 September 2016' in the section Immunology and Microbiology.

Dr. M. K. Saha

- Member, Expert Committee, Strengthening of Quality Control Testing Procedure of Immuno Diagnostic Kit Laboratory (IKDL), National Institute of Biologicals, New Delhi, Govt of India.
- Member, Technical Resource Group, Laboratory Services, NACO, New Delhi, Govt of India
- Member, Technical Expert Committee for strengthening of scientific activities of Molecular Diagnostic Laboratory, National Institute of Biologicals, New Delhi, Govt of India.
- Vice-Chancellor's nominee, Selection Committee for Promotion under Career Advancement Scheme for Assistant & Associate Professor, Jadavpur University.
- Resource Person for evaluation of performance of participants (faculty members of different universities) of Orientation Program, UGC-Human Resource Development Centre, Jadavpur University.
- Expansion of scope of NABL accreditation,ICMR-NICED lab to include Bacteriology lab beside Virology.
- Baseline assessment of Molecular HIV Lab (HIV Viral load assessment) at Banaras Hindu University as expert.
- Chairman & Judge, Technical Session, National Symposium on Quality Assurance in Higher Education, UGC-Human Resource Development Centre, Jadavpur University.

Conferences/ Seminars/ Workshops/ Trainings Attended/ Organised

M. K. Saha

- Organized workshop on EQAS & NABL Accreditation for State Referral Laboratories organized during 20-21 September 2016 at ICMR-NICED.
- Expert Group consultation on HIV surveillance and estimation, NACO, CDC, UNAIDS and WHO, at New Delhi, 27-29th Sept 2016.
- Attended as focal person of Regional Institute (E) in the National Pre-Surveillance meeting for HSS at NIHFW on 28 & 29th Nov 2016.
- Participated in Training of Trainers on Quality Management System (QMS) in CD4 Laboratories during 28-29th Dec 2016 at Pune.
- Regional Training of Trainers for HIV Sentinel Surveillance organized at ICMR-NICED during 4-5 January 2017.
- Participated as resource person in the Training in 'QMS in CD4 Labs' at Guwahati – 14 & 15 Feb 2017
- Organized training as resource person in coordination with CDC on"Implementation of Quality Management system (QMS) at CD4 Laboratories" at ICMR-NICED, Kolkata on 21st& 22ndFeb 2017.
- EQAS workshop & Panel sera distribution for SRLs was organized at ICMR-NICED on February 23rd 2017.
- HSS laboratory personnel training organized by NACO-Regional Institute (East) on 24th February 2017 at ICMR-NICED.

M. Chawla Sarkar

- The World of Microbes: Pathogenesis, Environment and Evolution., 17th October 2016, Bose Institute, Kolkata. Strategies adopted by Rotaviruses for a successful infection: "It's all about hijacking the cellular machinery to invade the cell". M. Chawla Sarkar : Oral Presentation.
- 5th Molecular Virology Meeting, 11-12 February 2017, THSTI, NCR Biotech Science Cluster, Faridabad. Strategies adopted by Rotavirus for a successful infection: Its all about hijacking the cellular Proteome. M Chawla Sarkar: Invited Lecture.
- National Seminar on Diarrhoeal Disease Burden and Management: With reference to North East India. March 10th-11th 2017 at Tezpur University, Assam. Surveillance and molecular characterization of Rotavirus and Adenovirus strains among children seeking health care facilities with acute gastroenteritis in Kolkata, Eastern India during 2011-2016. A Banerjee, S Mullick Bagchi, B Manna and M Chawla Sarkar : Invited Speaker.
- National Seminar on Diarrhoeal Disease Burden and Management: With reference to North East India. March 10th-11th 2017 at Tezpur University, Assam. Molecular characterization of rotavirus strains in children hospitalized with gastroenteritis in West Bengal. M. K Nayak, N Ganguly, C Ghosh, P Niyogi, S. Panda and M Chawla-Sarkar: Poster presentation.

A.K Chakrabarti

- Delivered oral presentation entitled "Avian influenza viruses: a potential threat to public health" on October 28, 2016 organised by NICED Scientific forum.
- Participated in a Workshop on Responsible Conduct of Research Involving Humans- Principles and Methods of Good Clinical Practice" on 16th March, 2017.
- Participated and provided training on viral detection by Real Time RT-PCR on a hands on Workshop on Laboratory Diagnosis of Emerging Viral Diseases during March 28th to 29th 2017 at ICMR-NICED. Delivered oral presentation and provided hands on training on "Cell Culture Techniques"

SERVICES

SERVICES PROVIDED BY THE INSTITUTE:

1. **Quality Assurance of Enteric Bacteria** through Gastrointestinal Tract Pathogens Repository (GTPR):

The ICMR-National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India, WHO collaborating center for research and training, maintains repository of enteric pathogens isolated from diarrheal diseases surveillance including patients admitted to Infectious Diseases and Beliaghata General Hospital (ID & BG Hospital, Kolkata). Apart from those isolates, the institute also receives large number of *Vibrio cholerae* strains every year from hospitals across India for confirmation, and phage typing. Support is provided to the microbiological/clinical laboratories of Government/non-Government hospitals in different states by confirming the identification of *Shigella sp.*, *Salmonella sp.*, *V. parahaemolyticus* and diarrheagenic *Escherichia coli.*

The 'Gastrointestinal Tract Pathogen Repository' (GTPR) is an other facility that was initiated with funding support from Indian Council of Medical Research (ICMR), under the aegis Department of Health Research (DHR), Government of India, in 2011 for archiving of bacterial enteropathogens. Main tenet was to provide ample scope for the researcher to carry out retrospective studies with analysis and to understand the changing characters of the pathogens over period of time. After completion of the support, the facility started functioning since 2016 with intramural support and remained as valued source for archived enteropathogens for researchers inside/outside of the Institute. The repository includes well characterized 786 clinical *V. cholerae* strains Kolkata isolated between 1996 and 2016 in kolkata and from different parts of India. Details of archived strains are available at www.gtpr.org.in which also includes a number of pathogenic bacteria like diarrheagenic *E. coli* (n=15), *Salmonella enterica* serovar Typhi (n=12), *V. parahaemolyticus* (n=1) and *Shigella boydii* (n=1). During 2016-17 period, GTPR characterized 207 strains received from 17 institutes/ hospitals from 9 states (Chhattisgarh, Gujrat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Uttrakhand, and West Bengal) which belonged to *Shigella spp.*, *Salmonella spp.*, diarrheagenic *E. coli, V. cholerae* and *V. parahaemolyticus.*

Antimicrobial resistance among pathogens, causing nosocomial and community-acquired infections are alarming aspects of clinical microbiology. As pathogens are becoming resistant to conventional therapy, attentions are focused on the development of vaccines for long and protective immune responses. Chicken is the most common bio-reservoir of *Salmonellae* that are affecting humans. Non-conventional antibiotics enhance the chance of MDR pathogens. We are working on the formulation of novel OMVs based immunogen against poultry salmonellosis and campylobacteriosis. Oral immunization with combination OMVs of circulating *Salmonella* and *Campylobacter* strains could help the poultry be freed from the infection. This will benefit society in maintaining the health of the poultry bird (industrial aspect) and infection free poultry products and will reduce the chance of *Salmonella* and *Campylobacter* infections to the human (clinical aspect).

2. A) **Diarrhoea Treatment Unit** functions as an outpost at the OPD of Dr B. C. Roy Post Graduate Institute of Pediatric Sciences and offers diagnostic service to suspected diarrhoeal diseases and typhoid fever patients (stool/ blood culture and sensitivity, molecular diagnostics, Widal test) and provides treatment while carrying out hospital-based surveillance.

B) **Training and support on parasite detection and isolation:** Field studies have been performed during last fiscal year in Chakdah, Nadia, West Bengal for investigation of presence of different enteric parasites by improper hand wash.

The study was also conducted in Arunachal Pradesh, Assam, Nagaland, Manipur, Mizoram,West Bengal for identification of different soil transmitted helminthes among school children. besides Quality Control and Quality Assessment support facility in eastern India for parasitic detection

3. **Quality Assurance for HIV Testing :**

External Quality Assurance Scheme is one of the important tools to assess the performance of the laboratory and its ability to generate accurate results. National Reference Laboratory of ICMR-NICED is the proficiency testing provider for HIV antibody testing for the States Reference Labs (SRLs) of A&N, Assam, Jharkhand, Meghalaya, Mizoram and Orissa.

- Conducting Proficiency Testing for 12 SRLs and approx. 432 ICTCs under these states.
- Confirmation of all indeterminate or discordant samples identified by attached 12 SRLs.
- Quality Assurance for HSS Lab result (Retesting of all positive and 5% negative).
- Testing laboratory for HIV Sentinel Surveillance 2017.
- Referral service for confirmation of HIV testing results of the samples received from different SRLs and other organizations.
- Testing of HIV Dried Blood Spot samples for HIV Sentinel Surveillance for High Risk Group and Bridge Population (2017). (Table 1 & Table 2)

Table 1: External Quality Assurance/ Confirmation of samples from SRLs/ NRLs

Name of States	Name of SRLs/ other organizations	Samples received	Concordant Result at NRL	Discordant Result at NRL
West Bengal	School of Tropical	04	04	00
Manipur	RIMS, Imphal	02	02	00
Meghalaya	NEIGRIHMS, Shillong	01	01	00

Table 2: HIV Sentinel Surveillance 2017 (ANC): Quality Assurance from SRLs under NACO NRL, NICED, Kolkata and other Testing Centres (samples received from April 2016 to March 2017)

Sl. No	Name of SRLs/ Testing Centre	Samples sent by SRL		Samples rejected by NRL	Confirmed Result at NRL		Discordant
		[illegible]	[illegible]		[illegible]	[illegible]	
1.	Rajendra Institute of Medical Sciences, Ranchi, Jharkhand	35	01	00	35	01	00
2.	Patliputra Medical College, Dhanbad, Jharkhand	38	02	00	38	02	00
3.	MGM Medical College, Jamshedpur, Jharkhand	16	02	00	16	02	00

HSS sample testing

Referral Services

National Reference Lab, NICED has been entrusted with the responsibility of verifying results for samples sent by Hospitals. Samples tested, result communicated within the turnaround time, analyzed the root cause of discordance and trained the referring lab personnel for improvement and technical capacity building. Most of the samples are positive for HIV antibody indicating improvement of quality of the referring labs. (Table 3, Table 4, Table 5)

Table 3: Referral Service done for the Institutions at NACO NRL, ICMR-NICED, Kolkata

Sl. No.	Source of Sample	No. of sample Tested	No. of sample Positive
1	Command Hospital, Kolkata	51	45
2	Cord Life Science India Pvt. Ltd.	01	00

Table 4: HIV Sentinel Surveillance 2017 (ANC samples): HIV Testing
Testing Centre data, NACO-NRL, ICMR-NICED, Kolkata

District	Site Name	Sample received	Sample rejected	Sample tested
Kolkata	Abhinash Dutta Maternity Home	401	01	400
Kolkata	Vidyasagar State General Hospital	402	02	400
Nadia	Nabadwip State General Hospital	401	01	400
Nadia	Aranghata BPHC	113	25	88
Nadia	Ranaghat Sub-Divisional Hospital	200	00	200
24 Parganas (N)	Madhyamgram Rural Hospital	203	03	200
24 Parganas (N)	Barasat District Hospital	200	00	200
TOTAL SAMPLE TESTED= 1888				

Table 5: HIV Sentinel Surveillance 2017 (ANC samples): RPR Testing
Testing Centre data, NACO-NRL, ICMR-NICED, Kolkata

District	Site Name	Sample received	Sample rejected	Sample tested
Kolkata	Abhinash Dutta Maternity Home	401	01	400
Kolkata	Vidyasagar State General Hospital	402	02	400
Nadia	Nabadwip State General Hospital	401	01	400
Nadia	Aranghata BPHC	113	25	88
Nadia	Ranaghat Sub-Divisional Hospital	200	00	200
24 Parganas (N)	Madhyamgram Rural Hospital	203	03	200
24 Parganas (N)	Barasat District Hospital	200	00	200
TOTAL SAMPLE TESTED= 1888				

Training

NACO-NRL of ICMR-NICED conducted Training programs for Medical Officers, Lab/Program Supervisors and Medical Lab Technologists for HIV testing as and when requested by different organizations. Every year two workshops are organized for 12 State Reference Laboratories under NRL.

Participants- EQAS Workshop

NABL Accreditation:

ICMR-NICED expands the scope of NABL accreditation in accordance with ISO 15189:2012 in the field of Medical Testing which undergoes the discipline of Microbiology and serology.
The scope of accreditation includes HIV immunoassay, HIV molecular Assay (HIV PCR) and different Microbiological Culture & Widal test.

Diagnostic Kit Evaluation by Consortium of NRLs at ICMR-NICED

Quality assurance of HIV, HBV & HCV diagnostic kit is essential to combat against spread of these viruses. To ensure supply of quality kits for all public health facilities including blood banks, a robust mechanism has been developed by a Consortium of National Reference Labs following the uniform procedure countrywide to evaluate performance of commercial kits. Request for evaluation is routed through the consortium secretariat, NARI, Pune, and all the labs are assigned the task for evaluation in a predefined rotational basis to avoid any bias (Table 6).

Work in progress at Laboratory

Table 6: Kit Evaluation by consortium of NRLs, ICMR-NICED, Kolkata

Type of Kit Evaluated	No. of Kit/ Batch Received	No. of Kit/ Batch Rejected	Reason for Rejection	No. of Kit/ Batch accepted and Evaluated	No. of Kit/ Batches meet the required Sensitivity	No. of Kit/ Batches meet the required Specificity	Total no.of batches complying with specification
HIV ELISA	01	00	NA	01	00	01	00
HIV RAPID	23	02	Temp on arrival at Lab beyond acceptable limit of 2-8°C	23	22	23	22
HBsAg ELISA	19	00	NA	19	19	19	19
HBsAg RAPID	00	00	NA	NA	NA	NA	NA
HCV ELISA	01	00	NA	01	01	01	01
HCV RAPID	02	00	NA	02	02	02	02
TOTAL	46	02	---------	46	44	46	44

Integrated Counseling & Testing Centre (ICTC)

The main functions of the ICTC are:

- Conducting HIV diagnostic tests.
- Conducting VDRL test for High Risk Group (HRG).
- Providing basic information on the modes of HIV transmission and promoting behavioural change to reduce risk.
- Providing psychosocial support to HIV positive people.
- Link HIV positive people to treatment and care services.
- Free condom distribution.
(Table 7, Fig 1)

Table 7: HIV testing details at ICTC (April 2016-March 2017)

Total Tested	Positive	Positivity	HRG Tested	Referred from RNTCP	HIV-TB Co- infection
1135	17	1.49%	718	143	0

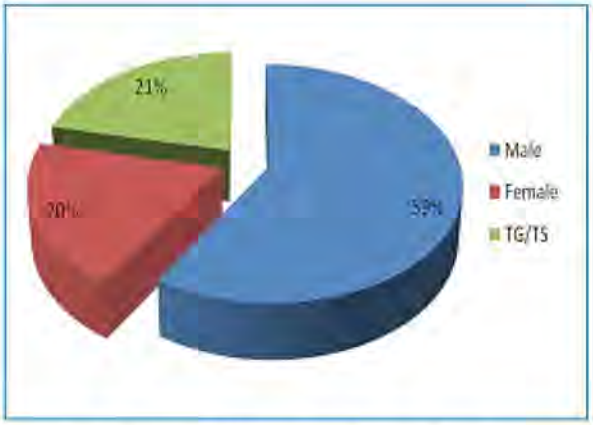


Fig 1: ICTC client details gender wise

High standards of testing are maintained at ICTC by using 3 test principles for diagnosing HIV. There are established external quality assurance scheme (EQAS) for ICTC through State Reference Laboratory. NICED ICTC secured 100% concordance result in EQAS.

From April 2016 to March 2017, total 1135 persons were tested for HIV in ICTC. Among them, 17 were found HIV positive and linked to ART centre for pre ART registration and CD4 testing. 718 HRGs were tested for HIV during this period. Almost 80% of them were tested for syphilis.

HIV Rapid test at ICTC

Early Infant Diagnosis

Molecular Diagnosis of HIV among babies (up to 18 months) born to HIV infected mothers using Dried Blood Spot (DBS) employing state of art molecular assay is being done at ICMR-NICED Regional Reference Lab (RRL), for the first time in the country in such a massive scale 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). Evidence generated rationalized change of national ART regimen for preventing mother to child HIV transmission as well as use of only DBS sample (removing whole blood sample test for confirmation) for molecular diagnosis of HIV.

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

National Institute of Cholera and Enteric Diseases (NICED) is one of the 6 Regional Reference Laboratories (RRL) among AIIMS, NICED, NITR, MUniv, NIMHANS & NARI, under NACO, performing RealTime HIV-1 Qualitative *in vitro* amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from Dried Blood Spot (DBS) samples. In NICED, EID program has been started from August, 2010 initially with three states, West Bengal, Orissa and Chhattisgarh. With gradual success of the program, the North Eastern states (Jharkhand, Bihar, Assam, Manipur, Mizoram, Nagaland, Meghalaya, Arunachal Pradesh, Sikkim, Tripura, and Andaman & Nicobar Islands) were also included under NICED-RRL (Molecular HIV Laboratory).

Work in progress at EID Lab

Presently, 1200 ICTCs are involved in collection of DBS samples in 14 states under NICED-RRL for DBS HIV-1 TNA PCR. A National Testing Algorithm comprising of two sections according to the age group of the child (Algorithm A: for infants < 6 months and Algorithm B: for child 6-18 months) have been followed for HIV exposed infants in this EID program for detection of HIV-1 DNA. All DBS HIV-1 PCR reactive/detected specimens are further confirmed by a 2nd Confirmatory HIV-1 PCR of the same sample.

A total of 2370 DBS samples were received at NICED-Regional Reference Laboratory (Molecular HIV Lab) and accepted for testing, according to sample acceptance criteria and among them a total of 2318 DBS samples were tested for the period of 01.04.2016 to 31.03.2017 and their status is depicted in Table and Figure depicted below. (Table 8 & Fig 2)

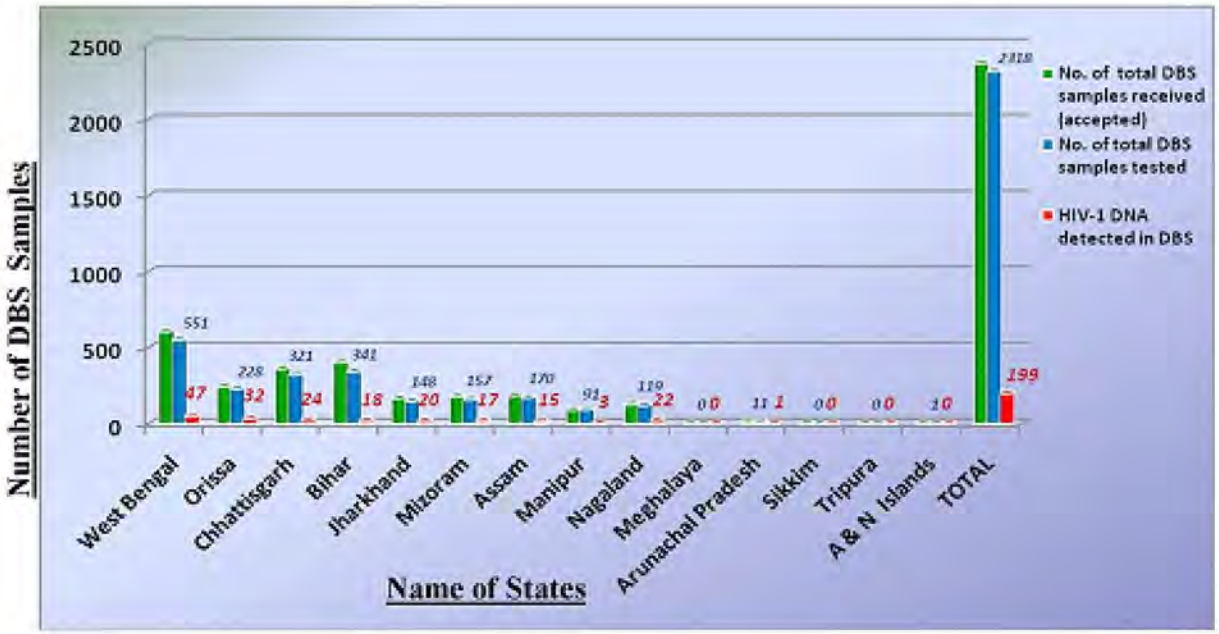


Fig 2: Status of EID DBS samples received and tested (with HIV-1 positivity) at ICMR-NICED from April 2016 to March 2017

Table 8: Status of EID DBS samples received and tested (with HIV-1 positivity) at ICMR-NICED from April 2016 to March 2017

Name of States	No.of total DBS samples received (accepted)	No.of total DBS samples tested	HIV-I DNA detected in DBS
West Bengal	605	551	47
Orissa	245	228	32
Chhattisgarh	362	321	24
Bihar	403	341	18
Jharkhand	166	148	20
Mizoram	176	157	17
Assam	185	170	15
Manipur	90	91	3
Nagaland	125	119	22
Meghalaya	0	0	0
Arunachal Pradesh	12	11	1
Sikkim	0	0	0
Tripura	0	0	0
A & N Islands	1	1	0
TOTAL	2370	2318	199

Regional Institute (East) for HIV Surveillance

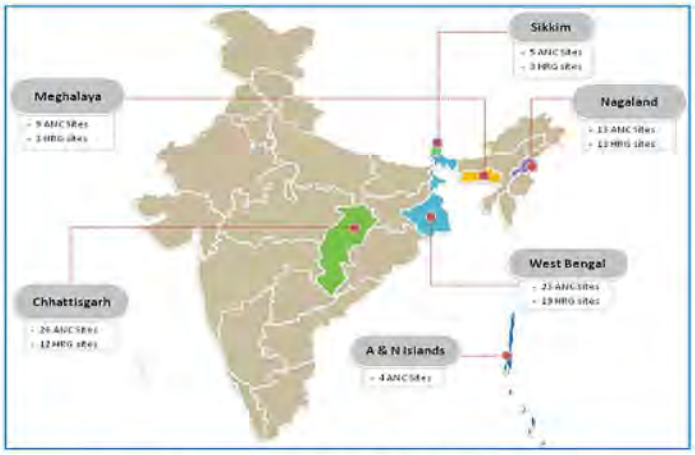
The activity of Regional Institute (East), ICMR-, involves implementation of HIV Sentinel Surveillance (HSS) among Antenatal Clinic (ANC) attendees and High Risk Group (HRG) for the East and North Eastern states with the aims to monitor the (i) trends and prevalence of HIV infection, (ii) distribution 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. RI (E) also has an important role in data entry and data management of HSS.

Spectrum of Activity

- Technical support & guidance to State AIDS Control Society (SACS) in overall planning & implemen tation of HSS activities in eastern Indian states, facilitating smooth implementation of surveillance ac tivities by liaising with concerned state authorities addressing specific problems at sentinel sites/ testing labs.
- Technical validation & approval of new site through review of relevant data & site visits.
- Conduct Regional Pre & Post-surveillance co-ordination & planning meetings, Regional Trainings and Workshops for HSS – ANC and HRG round.
- Technical & Supervisory support for state level training of site & lab personnel.
- Monitoring & Supervision during HSS (ANC and HRG round) 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.
- 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.



State wise site details: ICMR-NICED region

Monitoring visit at North Sikkim

Training Reports & Activity Reports of RI (E)

Plasma Viral Load Assay for HIV

HIV Viral load assay, under NACO, is being conducted at ICMR-NICED – Molecular HIV Laboratory for East & N-Eastern region, for ensuring efficacy of ART and taking evidence based decision for initiation of further treatment. Quantitative measurement of HIV level in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection and has been shown to be an essential parameter in prognosis and management of HIV infected individuals. Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma HIV RNA levels (viral load). The goal of antiretroviral therapy is to reduce the HIV virus in plasma to below detectable levels of available viral load tests. ICMR-NICED is one of the Laboratories under NACO that uses Abbott RealTime HIV-1RNA assay which is an *in vitro* reverse transcription polymerase chain reaction (RT-PCR) assay for the quantization of HIV-1 in human plasma. ICMR-NICED Molecular HIV laboratory restarted HIV viral load assay for the patients under ART for monitoring effectiveness of on-going treatment as per national guidelines and also to assist in HIV drug resistance mutation assay.

Presently, there are two linked centres, one in West Bengal and the other in Chhattisgarh, sending specimens to ICMR-NICED for HIV Viral Load test.

For the period of 01.04.2016 to 31.03.2017, 911 Viral Load samples were received at ICMR-NICED; a total of 968 samples (including some previous pending samples (n=57)which were received before April 2016) were tested for HIV viral load from the particular period.

The status of the samples is depicted in the Table and Figure below. (Table 9 & Fig 3)

Table 9: Status of HIV Viral Load Assay for patients under ART for the period 01.04.2016 to 31.03.2017

968 NO. OF SAMPLES TESTED		HIV-1 Viral Load <400 copies	HIV-1 Viral Load Copy No. 400-10000	HIV-1 Viral Load Copy No. >10000	HIV-1 Viral Load NOT DETECTED
Received (1.4.16 - 31.3.17)	**Received earlier**				
911	57	175	113	297	383

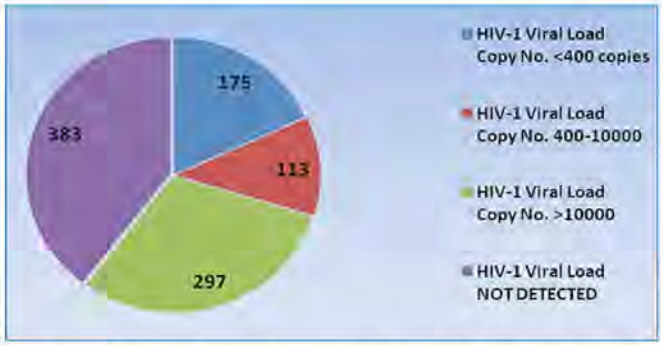


Fig 3: Status of HIV Viral Load Assay for patients under ART for the period 01.04.2016 to 31.03.2017

Exposure visit of HIV experts from Bangladesh:

A Joint Exposure Visit of delegates from Govt of Bangladesh and NGO officials of Bangladesh took place as per directives of NACO, Govt of India, during 26-31st October 2016. The objectives of the visit were to learn the best practices on Care Support and Treatment Program for the people living with HIV through Govt-NGO Collaboration.

The dignitaries who visited ICMR-NICED were:

1. Dr. Anisur Rahaman Director & Line Director, National AIDS/STD Program (NASP), Bangladesh
2. Dr. Tarit Kr Saha, Asst Director & Program Manager (In-charge) NASP.
3. Dr. Mansurul Alam, Professor & Head, Deptt of Dermatology, Chittagong Medical College & Hospital
4. Ms. Habiba Akter, Execitive Director, AAS.
5. Mr. Md Sariful Alam, Project Director, CST-1, AAS
6. Dr. Jebun Nessa, Project Director, CST-3, CAAP.

Explaining ICMR-NICED activities for Indian National AIDS Control Programme

The team visited National HIV Reference Laboratory, NICED, on 29th Oct, 2016, NICED Scientists explained role of National Reference Lab activities to ensure quality at different tier labs in the HIV program in India. The interactive exercise included explaining activities of the state-of-art Molecular Assay for HIV for Viral Load Estimation, Early Infant Diagnosis using Dry Blood Spot (DBS) sample and HIV Testing Quality Assurance and HIV Sentinel Surveillance for Indian national AIDS Control Program.

4. **NICED virology lab** is a referral lab for H1N1/2009 testing in West Bengal since 2009. Service to the state is provided by testing clinical samples of referred Severe Acute Respiratory Illness cases from hospitals in Kolkata and surrounding districts. During 2016-17, 258 referred cases were tested, of which 3 were influenza A/H1N1 positive, 8 Influezna A/H3N2 and 7 Influenza B positives. Reports are sent to State Heath authorities and NIV Pune. Referred samples from different hospitals of acute respiratory illness cases are being tested for detection of influenza A H1N1. Samples from other states were also included for testing.

5. **Phage Typing Laboratory**: As a WHO collaborating center, ICMR-NICED use to receive strains from different medical colleges and research institutes across the country for biotyping, serotyping and phage typing study. Total of 267 *V. Cholerae* O1 biotype El Tor strains received for this study were confirmed in Vibrio Phage Reference Laboratory and used for phage typing study. Using the set of the typing phages *V. cholerae* strains can be discriminated into several different phage types. Reports of the result were communicated to the concerned authority in time.

6. **The Biomedical Informatics Center** under the Division of Clinical Medicine (PI: Dr. Santasabuj Das) 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.

7. **NICED-Virus Research and Diagnostic Laboratory (VRDL)** is a designated Regional Level (Grade – 1) laboratory under the VRDL network established by Department of Health Research as a part of implementation of the Scheme "Establishment of a Network of Laboratories for Managing Epidemics and Natural Calamities" to strengthen infrastructure of viral diagnostics in India. After release of grant-in-aid in September 2014, testing for viral pathogens was initiated in July 2015. Following visit of ICMR consultants in July 2016, all personnel (scientific and technical) were recruited in September 2016. The entire setup has staff strength of 18 which includes scientists, technical and other support staff

Role in diagnosis and outbreak investigation

Since its inception NICED-VRDL has been involved in activities related to routine viral diagnostics and viral outbreak investigation. On request of and in partnership with State Health Authorities NICED-VRDL has provided extensive laboratory support towards viral outbreak management. Referred samples from Medical Colleges and other Public Health setups have been screened for respective viruses during Influenza A H1N1 outbreak of 2015-16 and Dengue outbreak of 2016-17. With state-of-the- art facilities, NICED-VRDL is equipped to perform molecular tests (PCR/ real time PCR) and immunological tests (antigen and antibody detection) for viral diseases. Apart from involvement in outbreak investigation, NICED-VRDL routinely on request provides diagnostic services (immunologic and/or molecular) for Dengue (including serotyping), Chikungunya, Influenza A H1N1 and Zika virus to Medical Colleges and other Public Health setups. A record of all investigations performed at NICED-VRDL during the period 2016-17 is summarized below.

Table 10: Investigations performed at NICED-VRDL during the period 2016-17

Investigations performed	Total tested	Total Positive
Dengue NSI Antigen ELISA	11536	4547
Dengue IgM antibody ELISA	3272	1299
Chikungunya IgM antibody ELISA	1446	524
Dengue PCR (serotyping)	335*(All NS1 positive)	318
Chikungunya PCR	301	32
Zika PCR	309	0
H1N1 PCR	566	135

*Details of Dengue serotyping

Total tested	DENV-1	DENV-2	DENV-3	DENV-4	RNA negative
335 (all NS1 positive)	155	89	29	45	17

Reporting of test results is done directly to patients, clinicians and concerned health authorities. Furthermore all VRDL testing data is uploaded to the VRDL online/offline data capture system to enable collation of nationwide data.

Collaboration and Research

Though the direct catchment area of NICED-VRDL is the state of West Bengal yet it has collaboration with other Viral Diagnostic Laboratories (VDLs) such as Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar and Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. Through this collaboration NICED-VRDL aims to promote joint research and provide diagnostic and training support to the VDLs.

Training of Health care Personnel

Being a Regional Laboratory NICED-VRDL is committed to the responsibility of organizing training workshops for health care personnel of the state and other VDLs. In this regard a training workshop on Laboratory Diagnosis of Emerging Viral Diseases was conducted on March 28-29, 2017 at ICMR-NICED with participants from Medical Colleges and VDLs of West Bengal, Bihar, Jharkhand. The primary focus of the workshop was to provide hands on training on immunological and molecular diagnostic techniques of emerging diseases.

Training of Health care Personnel

The training workshop received overwhelming appreciation from the participants and requests were made for more of such workshops in future.

Further development and into the future

Further development of laboratory infrastructure and civil works is under process. Once completed it will be a state-of-the-art facility equipped with immunological, molecular and tissue culture modalities for diagnostic and research activities in virology.

8. **Short term training** was accorded to 40 post graduate students from various universities all over the country to prepare a dissertation based on the research work, as part of their curriculum by the various scientists of the Institute (Table 11).

Table 11: Short term trainees for the period April 2016 to March 2017

Institution/University	No. of trainees
Bidhannagar College (affiliated West Bengal State University)	4
National Institute of Technology, Durgapur	3
St. Xavier's College	4
University of Calcutta	11
Vellore Institute of Technology	1
Vidyasagar University	17
Total	40

9. **Awareness activities** were undertaken by the members of the 'Hygiene Committee of NICED' in numerous schools (Table 12) and community settings in the city and focusing on various health issues **under SWACHH BHARAT campaign.**

Table 12: Swachh Bharat activities held by ICMR-NICED

Place of activities			Campaigns conducted	Participants	Number of participants attending the events
Schools	**Type of school**	**Medium of teaching**			
	Primary Nutritive value of different foods, steps of hand washing and home remedies in diarrhea were discussed	Bengali	1	Students	75
		Hindi	1	Students	
	Secondary/ Higher Secondary Dengue, environmental pollution, safe water, food safety, steps of hand washing were some of the topics covered	Bengali	1	Students	118
College/Institution (Name – IIHT)			1	Students	39
Community Community members were engaged in health and hygiene related discussions and saplings were planted			2	Community residents	70
ID & BG Hospital 'How to remain healthy' was the topic and role of keeping our environment clean in this regard was discussed			1	Relatives of patients admitted in ID & BG Hospital	50
ICMR – NICED Cleaning and maintenance related activities were undertaken at NICED I, NICED II & JICA Buildings and participatory discussions were held on general hygiene			3	Staff and students	Most of the staff & students participated

OUTBREAK INVESTIGATIONS

Service provided by: Scientists of Bacteriology and Virology Division

On request from the State health referred samples from various State Govt. Hospitals were received and processed for diagnosis of dengue virus with the viral serotyping. All together around 12,000 odd samples have been screened and feedback results have been sent to the concerned department with intimation to the State Health Authority.

During the epidemic outbreaks of diarrhea which spread across different southern districts of West Bengal, microbial analysis and examination of samples of potable water sources, from different parts of West Bengal and reporting of results to the Govt. agencies, has been a routine activity of the environmental laboratory.

Water samples were received from different PHCs of N. 24 Parganas, Nadia and Hooghly, Howrah, Kolkata 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, 72 samples had been received from various sources of which 51 had been found to be positive for faecal coliforms and 27 for presence of *V. cholerae* (Table 13).

Table 13: District wise distribution of the Outbreak Water Samples, their respective sources and results from tests performed.

Sl No.	District	No. of samples received	Source					Culture Positive	PCR positive
			Tap	Tube well	Drinking water	Pond	Others		
1.	North 24 Parganas	55	6	38	1	7	3	53	32
2.	Nadia	1	1	0	0	0	0	1	1
3.	Hooghly	4	2	1	0	0	1	3	1
4	Howrah	8	0	6	0	2	0	6	5
6	Kolkata	4	3	0	1	0	0	2	2
	Total	72	12	45	2	9	4	65	41

Member of team for outbreak investigation: A. Chakrabarti

As nominated by the Director, as per Order No Z.28020/1/2017-EMR/AP; Dated 20th February, 2017. Dr A. K Chakrabarti, visited Chittoor district on 24th Feb, 2017 to investigate Seasonal Influenza /H1N1 outbreak till 01/03/2017. He was a member of the central investigation team and investigated an outbreak of Seasonal Influenza (H1N1) in Chittoor District Andhra Pradesh, India from Feb 24 to March 1, 2017. This visit was aimed to investigate the situation concerning the rising trend of the H1N1 in the state of Andhra Pradesh. The incidence of Influenza/H1N1 cases was investigated to detect the early warning signal and trend of Influenza /H1N1 cases. Health care delivery system of the district was assessed for detection and management of Influenza/H1N1 cases and technical support provided to prevent and control it.

Visited all the possible sites including District, Municipal and regional hospitals, medical colleges around the district and finally reported to DMHO, Chittoor District and Secretary, State Health Department of Andhra Pradesh at Vijayawada. This investigation was concluded with the report and recommendations in a meeting with State Health Secretary. Concerned authority of the state was suggested to take prophylactic measure.

An Outbreak of Foodborne infection caused by *Shigella sonnei* in West Bengal, India

Members of outbreak investigation team: F. Debnath, A. Mukhopadhyay, S. Dutta, G. Choudhury, R. Narayan Saha & Field support staff of Epidemiology division

A total of 19 cases with acute gastroenteritis (AGE) from Pakapol village of Bhangore II block, South 24 Parganas, West Bengal were admitted to the Infectious Disease & Beliaghata General Hospital, Kolkata after having food at a housewarming party. An epidemiological and microbiological investigation was carried out by NICED team to confirm the occurrence of foodborne outbreak; to describe it in terms of time, place and person; to determine the cause of outbreak; to recommend control measures.

We carried out a retrospective cohort study among the party attendees. We collected information by using institutionally developed AGE case search format. Stool specimens from twelve case patients were processed for isolation and identification of enteric pathogens by following standard methods.

Thirty-four people attended the party on 23rd November 2016 and had lunch together. Median incubation period for development of AGE cases from time of food consumption was 18.5 hours (IQR, 16.5 –22 hours). Overall attack rate was 73% (25/34), of which 76% (19/25) required hospitalization. All age groups were affected with 100% recovery rate. One served food item was significantly associated with the illness: tomato salad (RR = 4.14; 95% CI = 1.21–14.13). Eight stool specimens (67%; 8/12) were tested positive for *Shigella sonnei*. PFGE analysis showed that the recent outbreak *Shigella sonnei* strains were clonally related with the locally circulating strains in Kolkata.

We confirmed that the food borne outbreak was caused by *Shigella* sonnei and leftover raw tomatoes kept

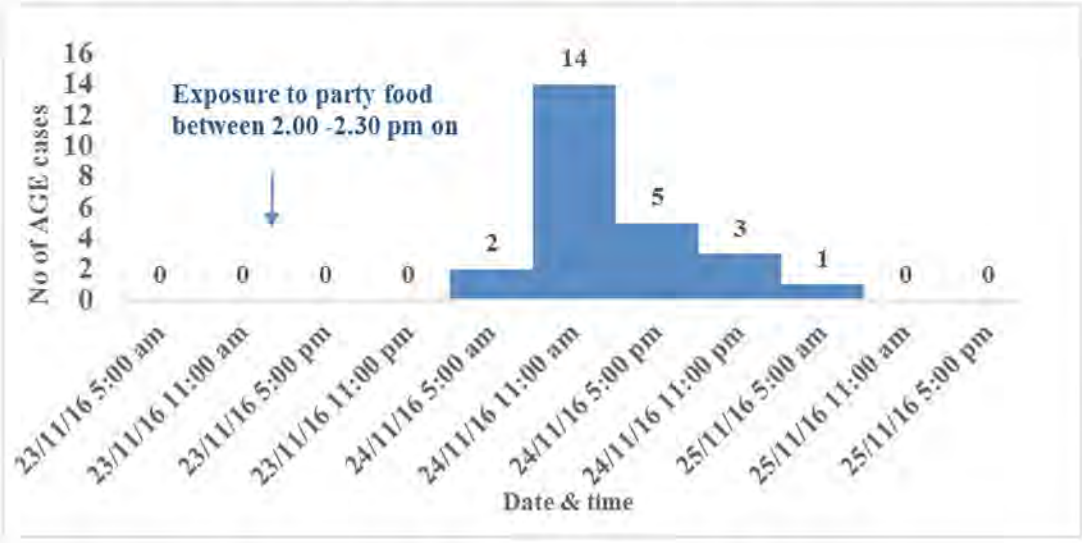


Fig 4: Distribution of Acute Gastroenteritis Cases, food borne outbreak, Pakapol Village, Bhangore II, South 24 Parganas, West Bengal, India, 23rd – 25th November 2016

ICMR – NICED team visiting the affected family, Pakapol Village, Bhangore II block, South 24 Parganas, November, 2016

Dengue fever outbreak investigation in Baranagar Municipal area, 2016

Members of outbreak investigation team: F. Debnath & field staff of Epidemiology division

During second week of August 2016, ICMR – National Institute of Cholera & Enteric Diseases, Kolkata was requested by North 24 Parganas District Health authority to carry out an epidemiological investigation at Baranagar Municipality in response to reporting of increased number of fever cases. As a response, ICMR-NICED carried out a thorough epidemiological investigation combining three approaches: 1) record review from local health authority 2) Door to door case search in ward no. 1; 3) Case search at in/out patient department of Baranagar State General Hospital and confirmed existence of dengue fever outbreak at Baranagar Municipal area which is adjacent to Kolkata by comparing the monthly attack rate of dengue during that period to the previous three years. The outbreak started in the last week of July 2016 and continued till second week of December , 2016 (Fig 5). Total 1660 confirmed dengue cases (Over all attack rate = 7 per 1000) and two deaths (case fatality = 1 per 1000) were reported in this outbreak (Table 1). ICMR – NICED, tested 212 blood specimens from probable dengue case patients of that area for confirmation through NS1 ELISA/MAC ELISA test and 163 blood specimens turned out to be dengue cases. Out of these 163 dengue cases, serotyping was done for 63 cases and DEN 1 (65%,41/63) was the major circulating serotype.

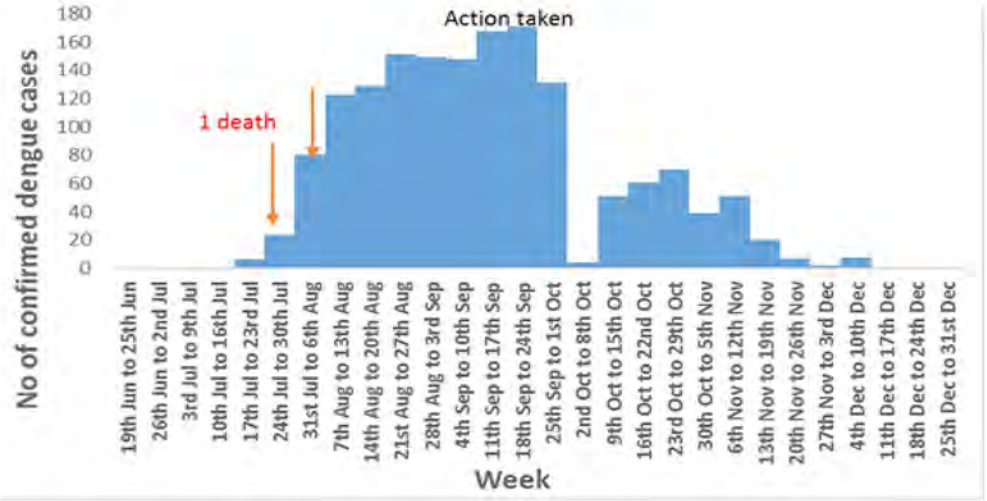


Fig 5: Distribution of dengue cases, Baranagar Municipality, July – December 2016

During training of the health workers of Baranagar Municipality before starting house to house active case search for dengue fever

FLAGSHIP PROGRAMMES-SWACHH BHARAT CAMPAIGN:

Campaign regarding Swachh Bharat was conducted which included several activities in the community by involving the residents of the urban slums through Voluntary Cleanliness Programme in the community. Other than this there were public seminars organized at the clubs and non-government organization in the localities. There was a Tree Plantation Programme organized in collaboration with the ward 66 Councillor and his office. To promote good health through clean environment there was community based activity organized by identifying areas to throw garbage and stop indiscriminate garbage disposal. Other community seminars included topics on personal hygiene, hand washing and diarrhoeal management at the community level

Tree Plantation program in urban slums of Kolkata

Voluntary cleaning activities involving local residents

TRAINING AND EXTENSION:

The Division of Training & Extension is an important and integral part of the National Institute of Cholera and Enteric Diseases (NICED). The division was established with the aim to cater to various needs of a scientific institute. A scientific institute cannot prosper solely depending on its research activities alone. To prosper in a holistic manner, a research institute should also engage in several activities that include workshops, seminars, symposium and training to create a noble scientific work force. The Division of Training and Extension at NICED is bestowed upon with the responsibility of proper and successful management of the afore-mentioned activities.

The Division has conducted many seminars and workshops successfully and has also arranged for symposiums and undertaken training programmes to generate proper scientific workforce.

Among the many seminars and workshops held during the last year NACO workshops have been organized throughout the year. Also STH workshop has been organized for training on identification of soil transmitted helminths. Seminars were organized by scientists and research scholars on weekly basis on different aspects of their latest research. NICED foundation day was observed and prestigious Dr. S. C Pal oration lecture was successfully organized by this division in 2017. This year, the lecture was given by Hon'ble Secretary DHR and DG, ICMR, Dr. Soumya Swaminathan. The division also arranged Science Day lecture and also organized a Good Clinical Practice (GCP) training program in March 2017. The division was also very much involved in organizing the very big event Hackathon in West Bengal in collaboration with ICMR HQ. NICED is expanding with time and with it the Division of Training and Extension is also growing in responsibilities and duties. The division, like the previous times, will engage with the activities of arranging scientific programmes in the future and will conduct them successfully. Newer facilities and infrastructures will be infused into this division and the division will continue to be an important and integral part of NICED, conducting its responsibility successfully and helping NICED prosper in every field possible.

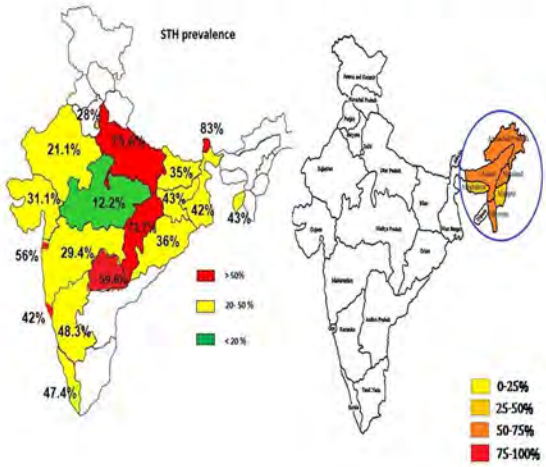
Teaching and Training Sessions in Uttar Pradesh: STH, Methods, identification, egg counting, & data maintenance, Biosafety

Identification of STH in Field in Chattisgarh

Discussion with CMOH in Nagaland

Identificatin of STH in Field in Tripura

Deworming Day, in Nagaland



Trainings conducted

- EQAS workshop & NABL Accreditation for State Referral Laboratories was held during 20-21 September 2016 at ICMR-NICED. Technical Officer & M.T (Lab) from Assam, A & N Islands, Jharkhand, Meghalaya, Mizoram & Odisha participated in the training program.

- Regional Training of Trainers for eastern region was organized by RI (E) and held at ICMR-NICED during 4-5 January 2017. HSS Program Officer, Technical Officers, TSU representative from NACO, officers from NIHFW, RI officers, SST members and SACS personnel participated in the training.

- EQAS workshop & Panel sera distribution for SRLs was held at ICMR-NICED on February 23, 2017. Technical Officer & M.T (Lab) from Assam, A & N Islands, Jharkhand, Meghalaya, Mizoram & Odisha participated in the workshop.

- HSS laboratory personnel training for eastern region was organized by RI (E) and held on 24th February 2017 at ICMR-NICED. Laboratory In-Charges, Technical officers and laboratory technicians from HSS testing labs attended the training program.

- STH workshop has been organized for training on identification of soil transmitted helminths.

- Seminars were organized by scientists and research scholars on weekly basis on different aspects of their latest research.

- On occasion of observation of Swacch Bharat Campaign several lectures by eminent doctors and scientists were arranged.

- Institutional Ethical Committee meetings.
- Scientific Advisory Committee meetings.
- Posters have been prepared and demonstrated in different State Govt. events on Enteric diseases, diagnostics, treatment and prevention.
- NICED Foundation day was observed and prestigious Dr. S. C Pal oration lecture was successfully organized by this division in 2017. This year, the lecture was given by Hon'ble Secretary DHR and DG, ICMR, Dr. Soumya Swaminathan.
- The division also arranged Science Day lecture.
- GCP training program in March 2017.
- The division was also very much involved in organizing the very big event Hackathon in West Bengal in collaboration with ICMR HQ.
- A training workshop on Laboratory Diagnosis of Emerging Viral Diseases was conducted on March 28-29, 2017 at ICMR-NICED.

EQAS workshop at NICED

HSS Lab personnel training at NICED

Seminar lectures at ICMR-NICED delivered by invited speakers during 2016-2017:

1. Dr. Anindito Sen, Scientist and Application Specialist in Biological Sciences, JEOL LTD., Ohtemachi, Tokyo delivered a talk on "Electron microscopic studies of the therapeutic effects of nano medicines on breast cancer cells" on 8th July, 2016

2. Prof. (Dr.) Kim Jungryong, MD, PhD, Director, G.S. Corporation Hospital & Consultant, National Medical Center, South Korea and Ex-Researcher, School of Tropical Medicine, Kolkata delivered a talk on "Small journey for Neglected Tropical Diseases" on 12th July, 2016

3. Dr. Arindam Mondal, Assistant Scientist, Mehle Lab, Department of Medical Microbiology & Immunology, University of Wisconsin Madison, Madison delivered a talk on "Hijacking host kinases to regulate the balance between mRNA & genomic RNA synthesis during influenza virus infection" on 26th July, 2016

4. Dr. Arunansu Talukdar, Professor, Medicine Department, Medical College Hospital, Kolkata delivered a talk on "Dengue from a clinician's perspective" on 19th August, 2016

5. Dr. Punyasloke Bhadury, Assistant Professor, Indian Institute of Science Education and Research (IISER, Kolkata) delivered a talk on "Microbial diversity in Sundarbans mangrove ecoregion- insights into bacterioplankton with pathogenic potential" on 30th September, 2016

6. Dr. Salvador Almagro-Moreno, Assistant Professor, Burnett School of Biomedical Sciences, University of Central Florida, USA delivered a talk on "Ecological and evolutionary approaches to unravel the emergence of pandemic *Vibrio Cholerae*" on 12th November, 2016.

7. Prof. Timothy Walsh, Professor of Medical Microbiology and Antibiotic Resistance, School of Medicine, Cardiff University, UK delivered a talk on "Global AMR: when two worlds collide" on 6th December, 2016.

8. Professor Mrinalini C. Rao, Department of Physiology and Biophysics College of Medicine, University of Illinios at Chicago, USA delivered a talk on "The Yin and Yang of Bile Acid Action: A Conundrum in the Colon?" on 6th January, 2017.

9. Dr. Arindam Moitra, Associate Prof, NIBMG, Kalyani delivered a talk on "Next Generation Human Genetics and its impact on the knowledge and practice of medicine" on 7th February, 2017.

10. Dr. Raja C. Mugasimangalam of Genotypic Technology delivered a talk on "Applications of the Magical Nanopore Sequencing in Ultrafast Metagenomics, Plant, Insect, Fungal, Bacterial and Viral Sequencing and Diagnostics" on 9th February, 2017.

11. Koushik Roy, Postdoctoral Scholar, Signalling Systems Lab, University of California, Los Angeles USA delivered a talk on "A step to predict immune response" on 24th February, 2017.

12. Amitabha Mukhopadhyay, Postdoctoral Scholar, Department of Biochemistry and Molecular Biology, The University of Chicago, USA delivered a talk on "Challenging pseudo-bipolar divisions in cancer cells with extra centrosomes" on 17th March, 2017.

13. Dr Tapash Chandra Ghosh, Senior Professor, Bioinformatics Centre, Bose Institute delivered a talk on "Determinants of Protein Evolutionary Rates: Theories and Controversies" on 24th March, 2017.

EXTRAMURAL PROJECTS

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	2014-2017
Title:	Generation of Culture-differentiated Innate Memory CD Cells with Toll-like Receptor Expression and Responsiveness to Patho gen/Danger-associated Molecules
PI:	Dr. T. Biswas
Funding Agency	DBT
Duration	2014 to 2017
Title:	Seasonal dynamics of enteropathogenic bacteria in Gulf of Khambat, Gujrat: its impact on health of coastal population
PI:	Dr. A. Palit
Funding Agency	Ministry Of Earth Science
Duration	Three years
Title:	A situation analysis of health facility preparedness for screening and treat ment of syphilis among antenatal clinic attendees in India (It is a multi-state project with West Bengal, Haryana, Tamilnadu, Maharastra and Assam)
PI:	Dr. K. Sarkar
Funding Agency	National AIDS Control Organization (NACO)
Duration	Nine month; The project is scientifically approved but the budget part is being reviewed by NACO
Title:	Establishing a network of population based influenza surveillance platform for elderly persons in India
PI:	Dr. K. Sarkar
Funding Agency	CDC, Atlanta, USA, through AIIMS, New Delhi
Duration	Nine month initially; would be extended further
Title:	Understanding HIV discordant/concordant relationship in familial, societal & program context in India - a multi-site qualitative study to inform intervention development
PI:	Dr. S. Panda
Funding Agency	National AIDS Control Organization (NACO)
Duration	10 months
Title:	Surveillance of enteric viruses with special reference to non-Rotaviral diarrhea (OUP 3-8)
PI:	Dr. T. Krishnan
Funding Agency	AMED through Okayama University, Japan
Duration	2015-2018
Title:	Apoptosis and molecular targeting therapy in cancer by microbial proteases
PI:	Dr. A. Pal
Funding Agency	DST-SERB
Duration	2017-2020

Title:	Studies on a novel virulence factor *YghJ* in Gram negative pathogens causing neonatal septicaemia
PI	Dr. A. Pal
Funding Agency	ICMR
Duration	2016-2018

Title:	External Quality Assurance for HIV testing
PI:	Dr. M. K. Saha
Funding Agency	National AIDS Control Organization
Duration	2002 – 2019

Title:	HIV Sentinel Surveillance
PI:	Dr. M. K. Saha
Funding Agency	National AIDS Control Organization
Duration	2008-2019

Title:	Evaluation of diagnostic kits for HIV, HBV and HCV
PI:	Dr. M. K. Saha
Funding Agency	National AIDS Control Organization
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 Organization
Duration	2012 – 2019

Title:	Counseling and Testing for HIV, Blood Borne Infections and STIs
PI:	Dr. M. K. Saha
Funding Agency	WBSAP&CS
Duration	2012 – 2019

Title:	Molecular assay for HIV-1 Plasma Viral Load
PI:	Dr. M. K. Saha
Funding Agency	National AIDS Control Organization
Duration	2015 – 2019

Title:	Molecular characterization of HIV for drug resistance mutations among infant using dried blood spot sample
PI:	Dr. M. K. Saha
Funding Agency	ICMR
Duration	2015 – 2018
Title:	Evaluation of impact of antiretroviral therapy under National AIDS Control Program in India.
PI:	Dr. M. K. Saha
Funding Agency	National AIDS Control Organization
Duration	2017-19
Title:	A prospective longitudinal study on changes of inflammatory cytokine levels among HIV infected subjects in an Eastern Indian hospital
Co-PI:	Dr. M. K. Saha
Funding Agency	WB DST
Duration	2015 - 2017
Title:	The interplay of climate and non-climate factors in determining the risks and predicting outbreaks of waterborne diseases.
Project Coordinator	Dr. S. Dutta
PI	Dr. A. Deb
Co-P.I	Dr. A. Palit
Co-Investigators	Dr. F. Debnath, Dr. A. De
Funding Agency:	Department of Science & Technology, Govt. of India
Duration:	3 years (2017-2020)
Title:	Exploration of the biologic basis for underperformance of oral Polio and Rotavirus vaccine in India
PI-Lab, PI-Epidemiol	Dr. R. K. Nandy, Dr. S. Kanungo
Funding Agency	International Vaccine Institute (IVI), South Korea
Duration	2012- 2016
Title:	Gastro Intestinal Tract Pathogen Repository (GTPR)
Coordinator	Dr. S. Dutta
PI:	Dr. R. K. Nandy
Co-PI	Dr. H. Koley
Funding Agency	Indian Council of Medical Research (ICMR)
Duration	2014- 2016
Title:	Retrospective analysis on the evolutionary aspects of *Vibrio cholerae*
PI:	Dr. A. K. Mukhopadhyay
Collaborator	Dr. Masatomo Morita
Funding Agency	NIID, Japan
Duration	2013-2018

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	2015-2018

Title:	Changing pattern of the *Vibrio cholerae* strains in India along with the antimicrobial resistance and its relationship with pathogenesis for better management of cholera
PI:	Dr. A. K. Mukhopadhyay
Collaborator	Dr. Hemanta Koley and Dr. Santasabuj Das
Funding Agency	AMED, Japan
Duration	2015-2020

Title:	Role of *Helicobacter pylori* Tumour Necrosis Factor Alpha inducing protein (Tip Alpha) in causing gastro duodenal diseases including gastric cancer
PI:	Dr. A. K. Mukhopadhyay
Collaborator	Dr. Rajashree Das (Amity University)
Funding Agency	ICMR
Duration	2017-2020

Title:	Exploratory study to standardize PCR tests on paraffin sections to detect *Helicobacter pylori* and compare with other detection tests.
PI:	Dr. A. K. Mukhopadhyay
Collaborator	Dr. R. Sukanya (NCDIR, Bengaluru)
Funding Agency	ICMR
Duration	2017-2019

Title:	Evaluation of available rapid diagnostic tests for cholera
PI:	Dr. A. K. Mukhopadhyay
Collaborator	Dr. S Dutta (NICED); Dr. T. Ramamurthy and Dr. B. Das (THSTI, Faridabad)
Funding Agency	BMGF through THSTI
Duration	2016-2018

Title:	Biomedical Informatics Center of ICMR, 2nd Phase of Task-force Project (IRIS ID: 2013-1551G).
PI:	Dr. S. Das
Funding Agency	Indian Council of Medical Research
Duration	Five years/ 2013-2018

Title:	Studies on therapeutic peptides against human *Salmonella* infections as drugs and vaccine adjuvants (OUP-4)
PI:	Dr. S. Das
Funding Agency	Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) of the Japan Agency for Medical Research and Development (AMED)
Duration	Five years/ 2015-2020

Title:	Studies on immune responses elicited by candidate peptide vaccines and polysaccharide-peptide conjugate vaccines against *Salmonella enterica* serovars Typhi and Paratyphi infections
PI:	Dr. S. Das
Funding Agency	Council of Scientific and Industrial Research, Government of India
Duration	Three years/ 2015-2017

Title:	Designing inhibitors of interactions between bacterial Leucine-rich Repeat (LRR)-containing effector proteins with E3 ubiquitin ligase activities and their host targets as novel anti-infective agent (Medical Innovation Fund)
PI:	Dr. S. Das
Funding Agency	Indian Council of Medical Research
Duration	Two years/ 2015-2016

Title:	Study of mechanism of probiotic action in persistent diarrhea in children caused by enteroaggregative *E.coli* – using a mouse model (GIA/49/2014-DHR)
PI:	Dr. S. S. Das
Funding Agency	Department of Health Research, Government of India
Duration	Three years/ 2014-2017

Title:	Genetic variations of *Giardia lamblia*
PI:	Dr. S. Ganguly
Funding Agency:	NIID, Japan
Duration:	Three years (2016-2018)

Title	National Rotavirus Surveillance Network - Referral Lab Eastern India
PI	Dr. M. Chawla Sarkar
Co-PI	Dr S. Panda Dr Nupur Ganguly, Institute of Child Health, Kolkata Dr Chandradipa Ghosh, Vidyasagar University, WB
Funding Agency	ICMR
Duration	Four years/ Sept 2013-Dec2016

Title:	Screening of small molecules with antiviral activity as adjunct therapy for viral diarrhea
PI:	Dr. M. Chawla Sarkar
Co-PI	Dr. H. Koley
Funding Agency	Okayama University, Japan
Duration	Five years / 2015-2020

Title:	Enhancing Biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of high-risk group viral patho gens causing viral hemorrhagic fevers and respiratory infections.
PI:	Dr. M. Chawla Sarkar
Co-PI	Dr. S. Panda, Dr. A. Deb
Funding Agency	CDC, USA
Duration	Five years / 2015-2020

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	Assessing drug resistance in Enterobactericae causing neonatal sepsis in North-East India: resistance mechanisms and transmission
Co-PI	Dr. S. Basu
Funding Agency	ICMR
Duration	2015-2018

Title:	Burden of antibiotic resistance in neonates from developing societies, (BARNARDS)
PI (Indian Counterpart):	Dr. S. Basu
Funding Agency:	Cardiff University, Bill and Melinda Gates Project
Duration:	2017-2018

Title	Development and evaluation of a heat killed multi-serotype oral *Shigella* vaccine
PI	Dr. H. Koley
Co-PI	Dr Keinosuke Okamoto
Funding Agency	Okayama University, Japan
Duration	Three years 2016 to 2019

Title	Development of a *Shigella* vaccine based on virulence gene expression
PI	Dr. H. Koley
Co-PI	Dr J. Mitobe
Funding Agency	NIID, Japan
Duration	Six years/ 2012-2018

Title:	Development of nanoparticle or microparticle adjuvanted subunit oral vaccine against poultry salmonellosis.
PI:	Dr. H. Koley
Co-PI	Dr S. Tamuly, Assam Agricultural University, Khanapara
Funding Agency	DBT-Delhi
Duration	Three years November 2016-2019
Title	Studies on Immunogenicity and protective efficacy of multi-serotype OMV's of circulating multidrug resistant bacteria in chicken model: A novel approach to develop a candidate vaccine for farm.
PI	Dr. H. Koley
Co-PI	Dr A. Mukhopadhyay
Funding Agency	WB-DST
Duration	Three years December 2016-2019
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 Rota virus Gastroenteritis in Healthy Indian Infants
PI	Dr. S. Kanungo
Funding Agency	PATH Vaccine Solutions, USA
Duration	Three Years/ 2014-2017
Title	Exploration of the Biologic Basis for under-performance of Oral Polio and Rotavirus Vaccine in India
PI	Dr. S. Kanungo, Dr. R. K. Nandy
Funding Agency	International Vaccine Institute
Duration	2012-2016
Title	Anti-diarrheal mechanism of Zn: Effect on epithelial ion transport and tight junction function
PI	Dr Mirajul Hoque Kazi
Funding Agency	DBT
Duration	2013-2016

PUBLICATIONS

1. Adewale AK, Pazhani GP, Abiodun IB, Afolabi O, Kolawole OD, Mukhopadhyay AK, Ramamurthy T. Unique Clones of *Vibrio cholerae* O1 El Tor with Haitian Type ctxB Allele Implicated in the Recent Cholera Epidemics from Nigeria, Africa. PLoS One.2016 Aug 1;11(8):e0159794.

2. Ali M, Debes AK, Luquero FJ, Kim DR, Park JY, Digilio L, Manna B, Kanungo S, Dutta S, Sur D, Bhattacharya SK, Sack DA. Potential for Controlling Cholera Using a Ring Vaccination Strategy: Re-analysis of Data from a Cluster-Randomized Clinical Trial. PLoS Med. 2016 Sep 13;13(9):e1002120.

3. Aoun J, Hayashi M, Sheikh IA, Sarkar P, Saha T, Ghosh P, Bhowmick R, Ghosh D, Chatterjee T, Chakrabarti P, Chakrabarti MK, Hoque KM. Anoctamin 6 Contributes to Cl- Secretion in Accessory Cholera Enterotoxin (Ace)-stimulated Diarrhea: an essential role for phosphatidylinositol 4,5-bisphos phate (pip2) signaling in cholera. J Biol Chem. 2016 Dec 23;291(52):26816-26836.

4. Arya BK, Bhattacharya SD, Sutcliffe CG, Kumar Niyogi S, Bhattacharyya S, Hemram S, Moss WJ, Panda S, Saurav Das R, Mandal S, Robert D, Ray P. Impact of *Haemophilus influenzae* Type B Con jugate Vaccines on Na-sopharyngeal Carriage in HIV-infected Children and Their Parents From West Bengal, India. Pediatr Infect Dis J. 2016 Nov;35(11):e339-e347.

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College of Medicine & J.N.M. Hospital
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Kolkata- 700106

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National Institute of Cholera & Enteric Diseases
P-33 CIT Road, Scheme-XM, Beliaghata,
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Dr. Mamta Chawla Sarkar, Scientist-E	Member
Mr. Tapan Kr. Saha, Administrative Officer	Liaison Officer
Mr. Sunil Bernard, Private Secretary	Member
Mr. Pradip Bose, Assistant	Member
Mr. Vishwanath Besra, Assistant	Member
Mr. Sudhir Omesh, TO-A	Member

SC/ST Cell

Dr. Malay Kr. Saha, Scientist-F	Chairperson/Liaison Officer
Dr. Amit Pal, Scientist-F	Member
Mr. Pradip Bhadra, Section Officer	Member
Ms. Arpita Sarbajna, TO-A	Member
Mr. Vishwanath Besra, Assistant	Member

Grievance Cell

Dr. Tapas Biswas, Scientist-G	Chairman
Dr. Sulagna Basu, Scientist-E	Member
Mr. Tapan Kumar Saha, Admin. Officer	Member
Mr. Vishwanath Besra, Assistant	Member
Mr. Somesh Chandra Bhunia, TO-A	Member
Mr. Sudhir Omesh, TO-A	Member
Mr. Pradip Kr. Bose, Assistant	Member Secretary

Complaints Committee on Sexual Harassment of Working Women

Dr. Triveni Krishnan, Scientist-F	Chairperson
Dr. Mamta Chawla Sarkar, Scientist-E	Member
Dr. Sulagna Basu, Scientist-E	Member
Mr. Tapan Kr. Saha, Admin. Officer	Member
Smt. Arpita Sarbajna, Technical Officer-A	Member

Institutional Biosafety Committee (IBSC):

Dr. Amit Pal, Scientist-E	Chairman
Dr. Nabendu Sekhar Chatterjee, Scientist-E	Member Secretary
Dr. Asis Kr. Mukhopadhyay, Scientist-E	Member
Dr. Mamta Chawla Sarkar, Scientist-E	Member
Dr. Rupak Bhadra, Sr. Principal Scientist, Division of Infectious Diseases and Immunology, CSIR-IICB, Kolkata	External Expert
Dr. Suman Kanungo, Scientist-D	Medical/Biosafety Officer
Dr. Parimal Karmakar, Prof. Dept. of Life Science and Biotechnology, Jadavpur University, Kolkata	DBT Nominee

Library Committee:

Dr. Shanta Dutta, Scientist-G	Chairperson
Dr. Kamalesh Sarkar, Scientist-F	Working Chairperson
Dr. N.S. Chatterjee, Scientist-E	Member
Dr. Mamta Chawla Sarkar, Scientist-E	Coordinator
Dr. Suman Kanungo, Scientist-C	Member
Administrative Officer	Member
Mrs. Saheli Samanta, ALIO	Member

Purchase Committee:

Dr. Tapas. Biswas, Scientist-G	Chairperson
Dr. Byomkesh. Manna, Scientist-F	Member
Dr. Amit Pal, Scientist-F	Member
Dr. Malay Kr. Saha, Scientist-F	Member
Dr. Ranjan Kr. Nandi, Scientist-E	Member
Dr. Pallavi Indwar, Scientist-B	Member
Mr. Gopal Chandra Das, Assistant	Member

Web Committee:

Dr. Shanta Dutta, Director	Chairperson
Dr. Ranjan Kumar Nandy, Scientist-E	Co-opt Member
Dr. Sandipan Ganguly, Scientist-E	Co-opt Member
Mr. Sunil Bernard, Private Secretary	Member
Mrs. Saheli Samanta, ALIO	Coordinator

Vigilance Officer:

Dr. N.S. Chatterjee, Scientist-F

Staff List:

Director's Secretariat

Shri Sunil Bernard, Private Secretary
Shri Nitya Gopal Sutradhar, Technician - B

Office of the Administrative Officer

Shri Subodh Karmakar, Administrative Officer (Till Aug 2016)
Shri Tapan Kumar Saha, Administrative Officer (From Sep 2016)
Mrs. Renu Jaiswal, Lower Division Clerk
Shri Jhantu Malakar, MTS (General)
Shri KH. Ibomcha Singh, MTS (General)
Shri Omkar Lal, MTS (General)

Accounts Section

Shri G. C. Das, Assistant
Shri Pradip Kumar Bose, Assistant
Shri Dipak Kumar Gayen, Upper Division Clerk

Cash Section

Shri Chinmoy Kumar Naskar, Assistant
Shri Ranjit Biswas, Upper Division Clerk

Store Section

Shri Pradip Bhadra, Section Officer
Shri Sanjoy Banerjee, Assistant
Shri Ratan Chowdhury, Assistant
Shri Santanu Sen, Personal Assistant
Shri Prosenjit Guha, Upper Division Clerk
Shri Tapan Kumar Paul, MTS (General)
Shri Abhishek Banerjee, Telephone Operator

Personnel Section

Shri S. K Das, Private Secretory
Shri Shyamalendu Ghosh, Assistant
Shri Kishore Sharma, Lower Division Clerk

Establishment Section

Shri Pijush Kanti Ghosh, Section Officer (Till Aug 2016)
Shri Pradip Bhadra, Section Officer (From Sept 2016)
Shri Arup Chandra, Upper Division Clerk
Mrs. Minoti Bhattacharya, MTS (General)

Pension Section

Shri Vishwanath Besra, Assistant
Shri Shyamal Bandyopadhyay, Assistant

Despatch Section

Shri Gobindo Kundu, Upper Division Clerk
Shri Guru Charan Tudu, Technician - B

Library

Smt S. Samanta, Assistant Library Information Officer
Shri T. Pal, Library Information Assistant
Shri M. Chakraborty, Technical Assistant
Shri Bhaskar Roy, Technician-B

Training & Extension

Shri A. Jana, Technician-B
Shri A. K. Roy, Technician - B
Shri S. Adhikari, MTS (General)

Maintenance, Instruments & Equipment Section

Shri P. K. Ghoshal, Maintenance Engineer, Technical Officer-B (Eng. Support)
Shri A. R. Das, Care-taker
Shri S. K. Dey, Technical Assistant
Shri A. K. De, Technician-B
Shri K. Dey, Technician-B
Shri B. Mandi, Technician B
Shri B. Das, MTS
Shri M. L. Dosad, MTS
Shri S. Mullick, MTS
Shri S. Hazra, MTS
Shri A. Das, MTS

Shri B. Moshi, MTS
Shri D. Turi, MTS
Shri R. Hela, MTS
Ms. B. Hela, MTS
Shri A. Paramanik, MTS
Shri A. Seal, MTS
Shri S. Maiti, MTS

Media Section

Shri A. K. Saha, Technician-B
Shri K. Roy, Technician-B
Shri K. Ghoshal, MTS
Shri S. Mondal, MTS
Shri V. K. Singh, MTS

Animal Section

Shri K. C. Paramanik, Technical Officer - A
Shri K. C. Tudu, Technician-C
Shri S. Hari, Technician-B
Shri P. Turi, MTS
Shri R. Hazra, MTS
Shri S. R. Balmiki, MTS

Vehicle Section

Shri D. K. Saha, Driver (Spl. Grade)
Shri S. K. Das, Driver (Grade I)
Shri D. K. Chowdhury, Driver (Grade I)
Shri H. P. Das, Driver (Grade I)
Shri M. Ali Khan, Technician-C (Eng. Supp.)
Shri A. K. Dutta, Driver (Grade II)
Shri S. Das, Driver (Grade II)
Shri S. K. Ghosh, Driver (Ordinary Grade)
Shri D. Dey, Driver (Ordinary Grade)

Scientists Associated with ICMR-NICED

Dr. A. Ghosh, J.C.Bose Distinguished Chair Professor, National Academy of Sciences, India
Dr. M. K. Chakrabarti, ICMR Emeritus Scientist
Dr. A. N. Ghosh, ICMR Emeritus Scientist
Dr. M. K. Bhattacharya, ICMR Emeritus Scientist
Dr. B. L. Sarkar, ICMR Emeritus Scientist

Employees transferred to/joined ICMR-NICED during 2016-2017

Sl.No.	Name and designation	Division	Date of transfer/joining
1.	Dr. Aloke Kumar Chakraborty, Scientist - D	Virology	19.07.2016 (From NIV Pune)
2.	Dr. Pranab Chatterjee, Scientist - B	Epidemiology	27th January, 2017

Retired Employees of the Institute during 2016-2017

"Farewell dear Friends"

Sl.No.	Name and designation	Division	Date of Retirement
1.	Dr. Mihir Kumar Bhattacharya, Scientist - F	Clinical Medicine	30.06.2016
2.	Mr. Subhodh Karmakar, Admin. Officer	Administraton	31.08.2016
3.	Mr. Pijush Kanti Ghosh, Section Officer	Administraton	31.08.2016
4.	Dr. Banwarilal Sarkar, Scientist - F	Bacteriology	31.01.2017
5.	Mrs. Swapna Manna, Technical Officer - A	Epidemiology	28.02.2017

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

P-33, CIT Road, Scheme-XM, Beliaghata, Kolkata-700 010
Tel.: 033-2363-3373 ● Fax: 033-2363-2398
Conference Call: 033-2363-9782