वार्षिक रीपोर्ट
Annual Report

2015 - 2016

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

Annual Report

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सत्यमेव जयते
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सचिव, भारत सरकार
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स्वास्थ्य एवं परिवार कल्याण मंत्रालय
एवं
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Ministry of Health & Family Welfare
&
Director-General, ICMR

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MESSAGE

It is my pleasure to acknowledge the contribution of the entire team of scientists, scholars, technical personnel and administrative staff of the National Institute of Cholera & Enteric Diseases (NICED), Kolkata for collective effort to undertake various research activities as reflected in the Annual Report for the period 2015-2016.

The significant contribution of NICED in basic and applied research involving widespread coverage is clearly documented. The important areas of research that NICED scientists plan to conduct are to improve the quality of life for the mothers and children in the districts of West Bengal and also to lend timely help in the disaster prone areas of Sunderbans, following flood, cyclone in terms of early management and prevention of the spread of diseases. The diarrhoeal diseases surveillance program, presently in the second decade which made perceptible attempts towards innovative ideas, identification of emerging pathogens and early reporting to the State Health Department is commendable.

The institute has also set a mark in training manpower nationally, especially North-east states for improving laboratory diagnosis, management of diarrhoeal diseases and other diseases like sepsis, influenza and HIV.

The consistent progress of NICED in conducting collaborative research with both national and international organizations is highly appreciated. Like previous years, ICMR will extend their support to address any public health issues relevant to the national perspective as well as any emerging research interest.

(Soumya Swaminathan)

From the Director's Desk

The noteworthy contributions during the past five decades have earned the National Institute of Cholera & Enteric Diseases (NICED) an important place among premier research institutions in the country. The Diarrhoeal Disease Control Program and Oral Rehydration Therapy have been adopted by Department of Health & Family Welfare, Government of India. The active contribution to help State Health Department was achieved by implementation of the community outreach programs through evidence based research and striving to promote translational research whenever it was feasible.

The human resource development is considered to be of national importance and is achieved through doctoral programs, short term training programs, imparting training to health workers, care providers, organizing workshops for technical personnel, academicians, students etc. The scientific team of the institute also interact with national and international scientists through collaborative projects and have enabled the institute grow to its present status with state of the art infrastructure and financial support earned through various extramural research grants.

NICED has expanded its research activity to address important health issues governed by changing climatic factors, combating multidrug resistance, surveillance of respiratory illness, diagnosis of emerging infectious agents and implementation of community based programs in an attempt towards solving the HIV sociological behavior in different states of Eastern India.

A few noteworthy examples of translational research are development of improved diagnostic kits, indigenous vaccine development, studies on herbal formulations. The medical teams and technical personnel have provided timely assistance in outbreak situations in West Bengal as well as across the nation that were caused by diarrhoeal pathogens, unknown fevers etc. The recent contribution included understanding the high infant mortality rate in Mizoram.

The extramural funds from several national agencies as well as international agencies are gratefully acknowledged. The active collaborative interest in different aspects of research is being conducted towards a 'better tomorrow' and fulfilling the national goals.

The period under this report also witnessed several important outreach events that were celebrated by NICED such as International Yoga Day on 22 June 2015; Doctor's Day on 1st July, 2015; Sunderban Krishti Mela and Loko Sanskriti Utsab, Kultali, Basanti in December 2015 and NICED Foundation Day on 18 February 2016 with the oration lecture delivered by Dr. D.N. Guha Mazumder. NICED also participated in the One Day Exhibition organized by ICMR on the theme "Innovations in Health and Biotechnology" held in Rashtrapati Bhavan, New Delhi during Festival of Innovations (March 2016). Several children actively participated in a 'sit and draw competition' that was organized by NICED on under the theme "Clean environment leading to healthy life", at a local Club in Kolkata.

The constant endeavour of all the scientists, technical personnel, and administrative staff deserves special mention and my heartiest congratulations to one and all for being a great team to uphold the commitment of our institute towards the national interests.

Last but not the least, without the full support of ICMR Hqrs. it would not have been possible to undertake all the activities at NICED. Hence their support is gratefully acknowledged.

Dr. Shanta Dutta
Scientist-G & Director

CONTENTS

BACTERIOLOGY

Research carried out on cholera causing *Vibrio cholerae* strains clearly demonstrated that these strains are undergoing cryptic changes in the genome, which influence their virulence, speed of transmission and spread. The El Tor biotype *V. cholerae* strains carrying classical traits has emerged. A recent striking observation is the appearance of cationic antimicrobial peptide polymyxin B sensitive *V. cholerae* O1 strains isolated from cholera patients in Kolkata. Since the beginning of the seventh pandemic of cholera, the El Tor biotype has always been resistant to polymyxin B. However, for the first time starting from March 2013, polymyxin B sensitive El Tor strains totally replaced the resistant strains. This shift is an important event in the history of cholera after 1961 when El Tor vibrios first appeared.

Role of Sundarban mangroves, suggested as the cradle of cholera outbreaks, has been established as homeland of avirulent *Vibrio cholerae*. Planktonic attachment of *V. cholerae* is a survival strategy in adverse condition, whereas acquisition of toxin genes occurs during migration towards low saline inland system with favorable environmental factors. Seasonal *V. cholerae* dynamics have been identified in environmental settings of high saline mangrove and brackish water flowing inland with low saline condition.

Phage typing study on *V. cholerae* strains form different parts of the country were initiated at NICED since 1962 and today, this study is one of the mandates recognized by WHO to consider this Institute as a WHO collaborating centre for phage typing (diarrheal diseases research and training). Recently, 10 broad host range high lytic newer vibrio phages have been isolated from Kolkata belonged to podoviridae group as shown by EM study through Indo-UK collaborative project. These phages may be used as therapeutic against cholera infection, an alternative to antibiotic. This novel approach will be further pursued by utilizing cocktail phages in preclinical and clinical trials.

Typhoid being a serious public health problem, research on *Salmonella* Typhi, the causative agent for typhoid has revealed the antimicrobial resistance threat for treatment of typhoid. Various molecules subtyping techniques have been evaluated w.e.f identifying source and containment of the disease. Suitable herbal preparations are studied to develop alternative treatment modalities.

Immunogenicity and protective efficacy of heat killed multi-serotype *Shigella* immunogen in different animal models have been studied. A combination heat-killed immunogen of three different entero-invasive bacteria, *Shigella*, *Salmonella* and *Campylobacter* spp. has been developed. Sera of orally immunized rabbits with tri-valent heat-killed (TVHK) immunogen showed very high level of antibodies against all antigens till 120 days after last dose. Animal challenge studies revealed homologues protection in the vaccinated group. This trivalent heat-killed immunogen could be a low cost, simple, oral, non-living vaccine candidate in future.

Understanding of mucosal immune responses associated with natural *Shigella* infection is important to identify potential correlates of protection and to design effective vaccines. A study on circulating mucosal plasmablasts (ASC) producing specific antibodies against conserved invasive plasmid antigens (IpaC, IpaD20 and IpaD120) was carried out among shigellosis patients. These are now considered as potential candidate vaccines against shigellosis as evaluated in animal models. The ASC responses against Ipa antigens were developed in patients with recent onset shigellosis but such responses may not be protective and wane too rapidly and/or be of insufficient magnitude. These are consistent with the lack of cross-protection induced by natural *Shigella* infection.

The presence of mobile genetic elements has facilitated the spread of carbapenem- resistant genes. Mobilizable elements associated with carbapenemases in Enterobacteriaceae and *Acinetobacter* spp. isolated from septicemic neonates were investigated. The analysis revealed that in *Acinetobacter*, bla_{NDM-1} was organized in a composite transposon (Tn125) whereas in Enterobacteriacae the complete transposon could not be identified; only a remnant of the ISAba125 was systematically present. In Enterobacteriacae, bla_{NDM-1} was associated with different plasmid scaffolds, IncF type being the prevalent one.

Helicobacter pylori, a major cause of peptic ulcers and an early risk factor for gastric cancer is another research focus of this division. Studies on *H. pylori* demonstrated that strains harboring *babA2* from India are associated with increased virulence in in vitro study.

Scientists:

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Dr. R. K. Nandy, Scientist E
Dr. A. K. Mukhopadhyay, Scientist E
Dr. S. Basu, Scientist E
Dr. H. Koley, Scientist D

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Dr. Abhijit Sarkar
Dr. Subhasree Roy – Research Associate (ICMR)

Pre-doctoral Fellows

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Mr. Arindam Naha (SRF)
Ms Piyali Mukherjee (SRF)
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Mr. Suhrid Maiti, CSIR (JRF)
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Ms. Ushasi Bhaumik, DST Inspired (JRF)
Mrs. Madhumanti Halder UGC (JRF)
Ms. Sharmi Naha- DST-West Bengal (JRF)
Mr. Sounak Sarkar (JRF)

Ph D. Awarded:

Dr. Prasenjit Batabyal received Ph D. from University of Calcutta

Title of the Thesis: Characterization of *Vibrio cholerae* isolated from riverine and estuarine environs of South Bengal with special reference to ecological dynamics

Dr. Fatema Calcuttawala received Ph D. from University of Calcutta

Title of the Thesis: Molecular characterization of colicin determinants in clinical strains of *Shigella sonnei*

Dr. Abhishek Jaiswal received Ph D. from University of Calcutta

Title of the Thesis: A study on the efficacy of Vibriophage cocktail in reducing the intestinal *Vibrio cholerae* infection in animal model.

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

Principal Investigator : S. Dutta

Co-Investigators : S. Das, NICED; D.K. Paul, T. Ganguly, L. Misra, Dr. B.C. Roy PGIPS

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S.* Typhi), remains an unresolved public health problem in India. In recent years, increase in occurrence of fluoroquinolone (FQ) resistant *S.* Typhi isolates caused considerable inconvenience in selecting appropriate antimicrobials for treatment of typhoid. A total of 108 *Salmonella* Typhi isolates were collected from four different hospitals in Kolkata during April 2015 to March 2016. Multi-drug resistance (resistance to ampicillin, chloramphenicol, and co-trimoxazole) was found in 2 (1.9%) isolates harboring a non-conjugative non-IncHI1 plasmid (180kb), whereas resistance to nalidixic acid was observed in 105 (97.2%) isolates (Fig 1). Although 23 (21.3%) isolates were ciprofloxacin resistant, but decreased ciprofloxacin susceptibility (DCS) was noted in 82 (75.9%) isolates. None of the isolates were resistant to third-generation cephalosporin. One isolate (resistant to tetracycline, co-trimoxazole and streptomycin) harbored a conjugative IncN plasmid (50kb). Ampicillin and chloramphenicol resistance was mediated by bla_{TEM} and *catA1* genes respectively. Class 1 integron, was present in study isolates which possessed either one (dfrA7) or two (*dfrA15-aadA1*) gene cassettes conferring resistance to co-trimoxazole and streptomycin. Nalidixic acid resistant isolates with decreased susceptibility or resistance to ciprofloxacin had point mutation(s) in *gyrA* and *parC* genes of quinolone resistance-determining region (QRDR) (Table 1). A total of ten QRDR types were found. No mutation was noted in *gyrB*. Plasmid-mediated quinolone resistance (PMQR) determinants (*qnrA*, *qnrB*, *qnrD*, *qnrS*, *aac(6')-Ib-cr* and *qepA*) were also not found among the study isolates. In presence of the efflux pump inhibitor PAβN (40µg/ml), a 2 to 4-fold reduction in ciprofloxacin MICs was observed among susceptible, intermediate or resistant isolates (Table 2). No differences were detected in the transcription levels of efflux related genes acrB, ramA, marA and soxS, between ciprofloxacin susceptible, intermediate or resistant isolates. In addition, no mutations were detected in the regulatory regions (*acrR*, *ramR*, *ramA*, *marOR*, *marAB* and *soxRS*) of the AcrAB-TolC efflux system.

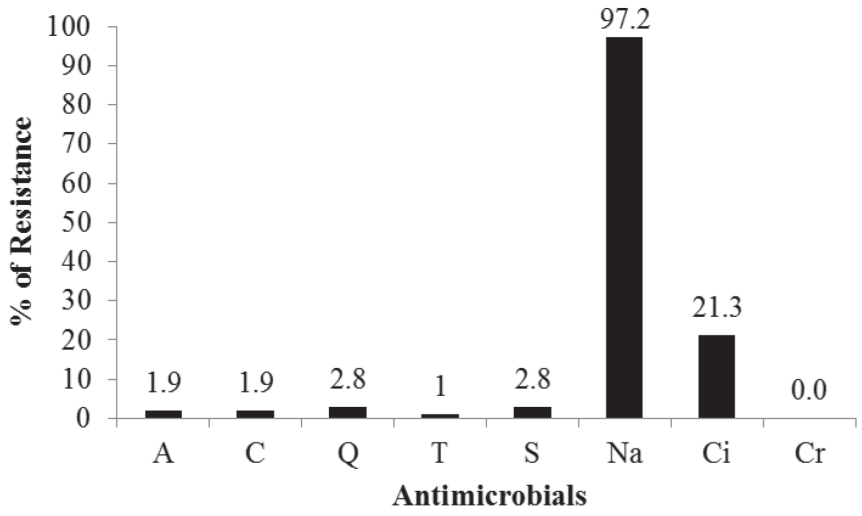


Fig 1 Antimicrobial resistance patterns in S. Typhi isolates (n=108)

A, ampicillin; C, chloramphenicol; Q, co-trimoxazole; T, tetracycline; S, streptomycin; Na, nalidixic acid; Ci, ciprofloxacin; Cr, ceftriaxone

Table 1 QRDR substitution profiles and types in *S*. Typhi isolates (n = 96)

QRDR type	QRDR substitution profile				No. of isolates (%)	Ciprofloxacin MIC range (µg/ml)
	GyrA	GyrB	ParC	ParE		
Q1	WT	WT	WT	WT	2 (2.1)	0.03-0.06
Q2	D87N	WT	WT	WT	1 (1.0)	0.25
Q3	D87Y	WT	WT	WT	2 (2.1)	0.25
Q4	S83F	WT	WT	WT	20 (20.8)	0.12-0.5
Q5	S83Y	WT	WT	WT	31 (32.4)	0.25-0.5
Q6	S83Y	WT	WT	L502F	1 (1.0)	0.5
Q7	S83F	WT	E84G	WT	3 (3.1)	1
Q8	S83F	WT	E84K	WT	1 (1.0)	1
Q9	S83F D87G	WT	S80I	WT	1 (1.0)	16
Q10	S83F D87N	WT	S80I	WT	34 (35.5)	16-32

QRDR, quinolone resistance-determining region; WT, wild type; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; N, asparagine; S, serine

Table 2 Mechanisms of fluoroquinolone resistance in a subset of S. Typhi Kolkata isolates (n=25)

Strain	Antimicrobial resistance profile[a]	MIC (µg/ml)[b]		PMQR mechanism[c]	Substitution(s) in the QRDR[d]				mRNA expression of efflux pump genes[e]			
		Na	Ci		GyrA	GyrB	ParC	ParE	*acrB*	*ramA*	*marA*	*soxS*
KOL-010	Susceptible	2	0.032 [0.016]	Absent	WT	WT	WT	WT	1.0	1.0	1.0	1.0
RGK-066	Na	128	0.125 [0.125]	Absent	S83F	WT	WT	WT	ND	ND	ND	ND
BCR-096	Na	128	0.25 [0.125]	Absent	D87Y	WT	WT	WT	ND	ND	ND	ND
BCR-049	A-C-Q-Na	≥256	0.25 [0.125]	Absent	S83Y	WT	WT	WT	1.5	1.8	0.6	1.1
BCR-052	Na	≥256	0.25 [0.125]	Absent	S83F	WT	WT	WT	ND	ND	ND	ND
KOL-025	Na	≥256	0.25 [0.125]	Absent	S83F	WT	WT	WT	ND	ND	ND	ND
BCR-043	Na	≥256	0.5 [0.25]	Absent	S83Y	WT	WT	WT	1.7	1.9	0.4	0.8
BCR-177	A-C-Q-Na	≥256	0.5 [0.25]	Absent	S83Y	WT	WT	L502F	0.8	2.5	1.0	1.6
KOL-009	Na	≥256	0.5 [0.25]	Absent	S83Y	WT	WT	WT	ND	ND	ND	ND
KOL-017	A-C-Q-Na	≥256	0.5 [0.25]	Absent	S83Y	WT	WT	WT	1.2	1.4	0.42	0.5
KOL-018	A-C-Q-Na	≥256	0.5 [0.12]	Absent	S83Y	WT	WT	WT	0.9	1.1	0.7	1.2
KOL-152	Na	≥256	0.5 [0.25]	Absent	S83F	WT	WT	WT	ND	ND	ND	ND
KOL-072	Na-Ci	≥256	1 [0.25]	Absent	S83F	WT	E84G	WT	0.9	1.1	0.5	0.4
KOL-073	Na-Ci	≥256	1 [0.25]	Absent	S83F	WT	E84K	WT	1.8	1.9	0.5	1.1
KOL-076	Na-Ci	≥256	1 [0.25]	Absent	S83F	WT	E84G	WT	0.8	1.6	0.9	0.9
KOL-106	Na-Ci-Of-Le	≥256	16 [8]	Absent	S83F;D87G	WT	S80I	WT	1.3	1.4	0.6	0.7
KOL-156	Na-Ci-Of-Le	≥256	32 [16]	Absent	S83F;D87N	WT	S80I	WT	0.1	1.5	0.9	0.7
KOL-162	T-Q-Na-Ci-Of-Le	≥256	32 [16]	Absent	S83F;D87N	WT	S80I	WT	0.4	2.6	1.6	0.5
KOL-112	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	1.1	1.2	0.7	0.4
KOL-116	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	0.8	1.4	0.6	0.6
KOL-148	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	0.7	1.0	0.8	1.2
KOL-170	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	1.1	1.5	1.0	1.1
KOL-174	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	1.4	1.5	0.9	0.6
BCR-191	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	0.9	1.6	0.8	0.9
BCR-211	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	1.3	1.0	0.4	0.7
KOL-132	Na-Ci-Of-Le	≥256	32 [8]	Absent	S83F;D87N	WT	S80I	WT	0.9	2.5	0.8	1.7

[a] A, ampicillin; C, chloramphenicol; Q, co-trimoxazole; T, tetracycline; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin; Le, levoflolxacin.
[b] Values in brackets are MICs in the presence of the efflux pump inhibitor PAβN at 40 µg/ml.
[c] PMQR, plasmid-mediated quinolone resistance.
[d] QRDR, quinolone resistance-determining region; WT, wild type; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; N, asparagine; S, serine.
[e] No substitution was detected in efflux pump regulator, but a single substitution was found in MarAB leading to S52N amino acid change.

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

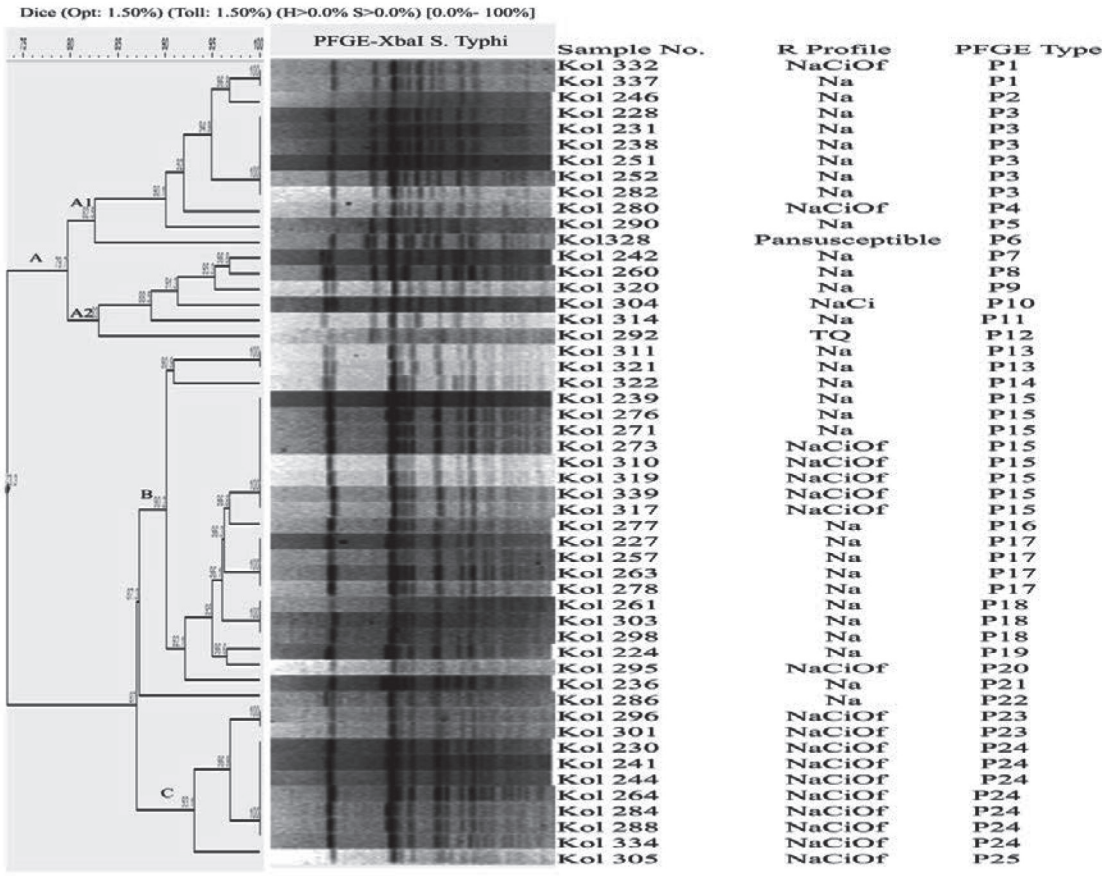
Principal Investigator : S. Dutta

Co-Investigators : S. Samajpati; M. Bhattacharya, D.K. Paul, B.C. Roy PGIPS

A total of 115 *S.* Typhi isolates were collected from 4 hospitals in Kolkata during Jan 2015-Dec 2015. All *S.* Typhi isolates were susceptible to amikacin, gentamicin, 3rd generation cephalosporins (cefixime, ceftazidime, cetriaxone). Only one (0.87%) isolate was multidrug resistant (MDR, resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole). Most of the isolates (112, 97.3%) were resistant to nalidixic acid followed by ciprofloxacin resistance (36, 31.3%) as per CLSI, 2015 guideline. Only 1 isolate (0.87%) was resistant to tetracycline and trimethoprim-sulfamethoxazole. Two (1.7%) isolates were pan-susceptible (susceptible to all tested antibiotics). Table 3 shows the percentage distribution of antimicrobial resistance in *S.* Typhi Kolkata isolates during 2015 (January to December). A total of five resistance profiles were observed among 115 isolates. 51 *S.* Typhi isolates with different antimicrobial profiles were analyzed by PFGE (Pulse field gel electrophoresis). Three major clusters (A-C) including 25 pulsotypes (P1 to P25) were found in the dendrogram of PFGE (Fig 2). The major clusters corresponded to nalidixic acid resistant and fluoroquinolone (FQ) resistant isolates. The sequence data of the six VNTR (Variable number tandem repeats) loci for 22 *S.* Typhi isolates from Kolkata was generated. The cluster analysis was performed by the UPGMA algorithm and a rooted tree was generated (http://minisatellites.u-psud.fr) (Fig 3). Two major clusters (A, B) including 22 MLVA types (M1-M22) were found in the dendogram of MLVA. Cluster A was divided into 2 subclusters, A1 and A2 including 7 nalidixic acid resistant isolates and one pansusceptible isolate. Cluster B included 2 subclusters B1 (M9, M10) and B2 (M11-M22). All MDR S. Typhi isolates and majority of fluoroquinolone resistant isolates belong to subcluster B2.

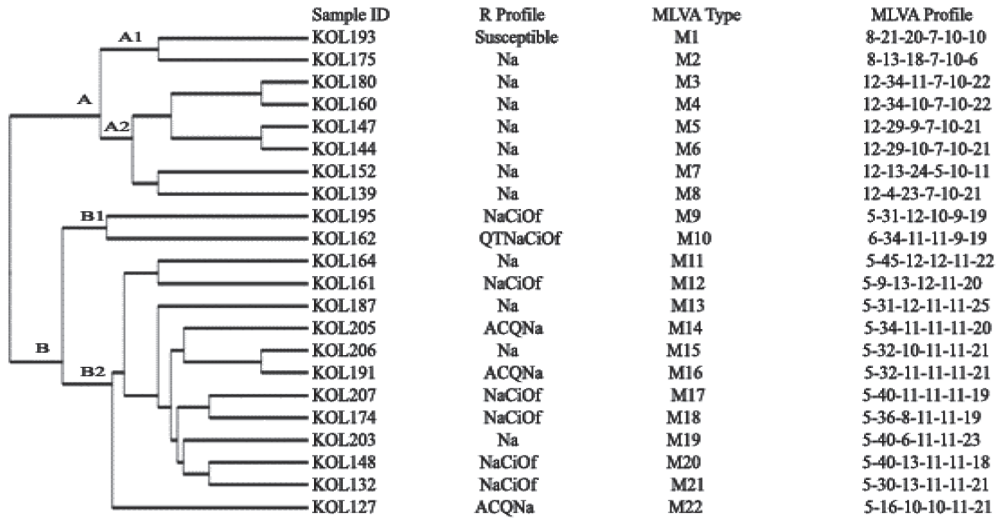
Table 3 Antimicrobial susceptibility patterns of S. Typhi Kolkata isolates 2015 (n=115)

to the Antimicrobial Agents	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Ampicillin	1 (0.87)	0	114 (99.1)
Chloramphenicol	1 (0.87)	0	114 (99.1)
Cotrimoxazole	2 (1.7)	0	113 (98.2)
Nalidixic acid	112 (97.3)	0	3 (26)
Ciprofloxacin	35 (34.4)	78 (67.8)	2 (1.7)
Ceftriaxone	0	0	115 (100)
Caftazidime	0	0	115 (100)
Cefotaxime	0	0	115 (100)
Cefixime	0	0	115 (100)
Amikacin	0	0	115 (100)
Gentamicin	0	0	115 (100)
Tetracycline	1 (0.87)	0	114 (99.1)
Ofloxacin	25 (21.7)	87 (75.6)	3 (26)
Azithromycin	0	0	115 (100)



PFGE Profile of 51 strains

Fig 2 Dendrogram showing the cluster analysis of 51S.Typhi isolates from Kolkata, India, 2015, by *Xba*I-PFGE. Band comparison was performed by using the Dice coefficient with 1.5% optimization (Opt) and 1.5% position tolerance (Tol). Pan-susceptible, susceptible to all 14 drugs tested; Q, co-trimoxazole; T, tetracycline; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin.



MLVA Profile of 22 *S.* Typhi strains

Fig 3 showing the cluster analysis of 22 S. Typhi isolates from Kolkata, India, 2014. Cluster analysis is performed by the UPGMA algorithm, and a rooted tree is generated (http://minisatellites.u-psud.fr). Pan-susceptible, susceptible to all 14 drugs tested; Q, co-trimoxazole; T, tetracycline; Na, nalidixic acid; Ci, ciprofloxacin; Of, ofloxacin

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

Principal Investigator : A. Palit

Physico-chemical Parameters:

Water temperature varied between 17.1°C to 37.2°C, with an elevated temperature in summer and comparatively lower in winter. Salinity varied greatly between the four (4) study sites. Highest salinity was recorded at Freserganj followed by the salinity at Kakdwip, the salinity at Diamond Harbour (0.0-2.4 PSU) was lower than the other two sampling stations. Salinity at Howrah site was too low to detect. Turbidity varied between 35 NTU to 550 NTU (at Howrah), 40 NTU to 900 NTU (at Diamond Harbour), 128 NTU -563 NTU at Kakdwip and 207 NTU – 484 NTU at Fresurganj.

Bacterial Preponderence:

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

(PSU – Practical Salinity Unit NTU – Nephelometric Turbidity Unit)

Diamond Harbour site was characterized with a higher tidal influence. Proximity to the sea mouth is the major contributing factor resulting in a clear visualization of tidal effect on the physico-chemical properties as well as on the predominance of different bacterial community. Thus a good variation of bacterial prevalence could be noticed along with the tidal changes (Fig 4).

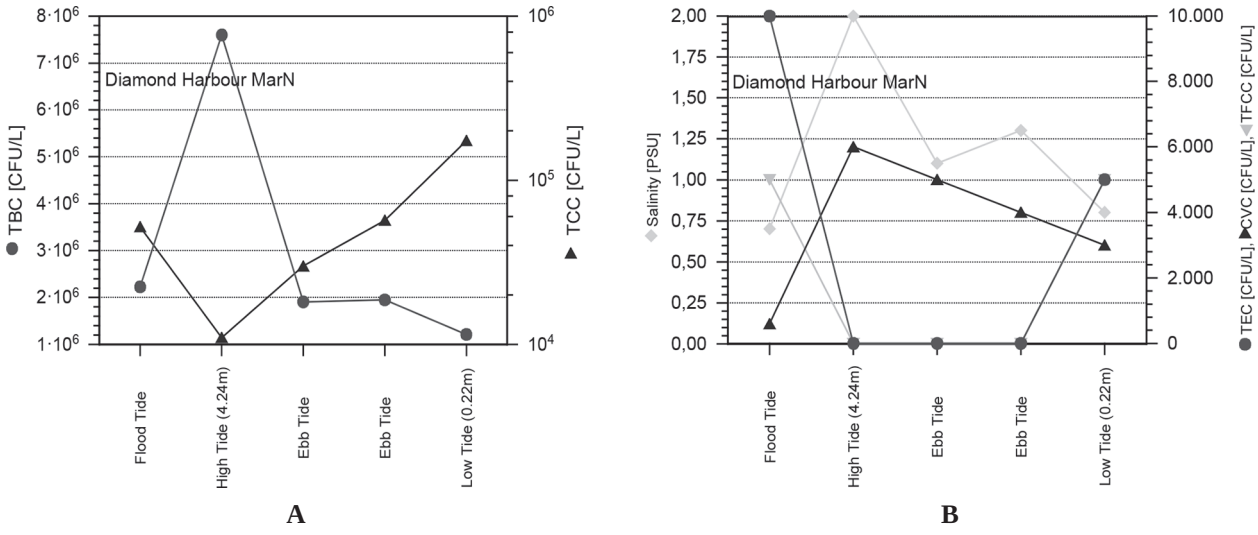


Fig 4 Tidal impact on bacterial as well as physico-chemical parameters

100 odd suspected Vibrio samples have been isolated, irrespective of study focus, *V. cholerae*, *V. parahaemolyticus* seems to be the most prevalent species among all other *Vibrio* organisms. *V. mimicus*, V. alginolyticus, V. *vulnificus* (other than *V. cholerae* and *V. parahaemolyticus*) has also been identified from different zones (Fig 5). Altogether 40 samples were positive for vibriophages. Vibriophage preponderance increased along with water temperature (during summer) and reached the peak during monsoon (Fig 6).

Fig 5 Seasonal abundance of enteric Vibrio species at all the sampling sites

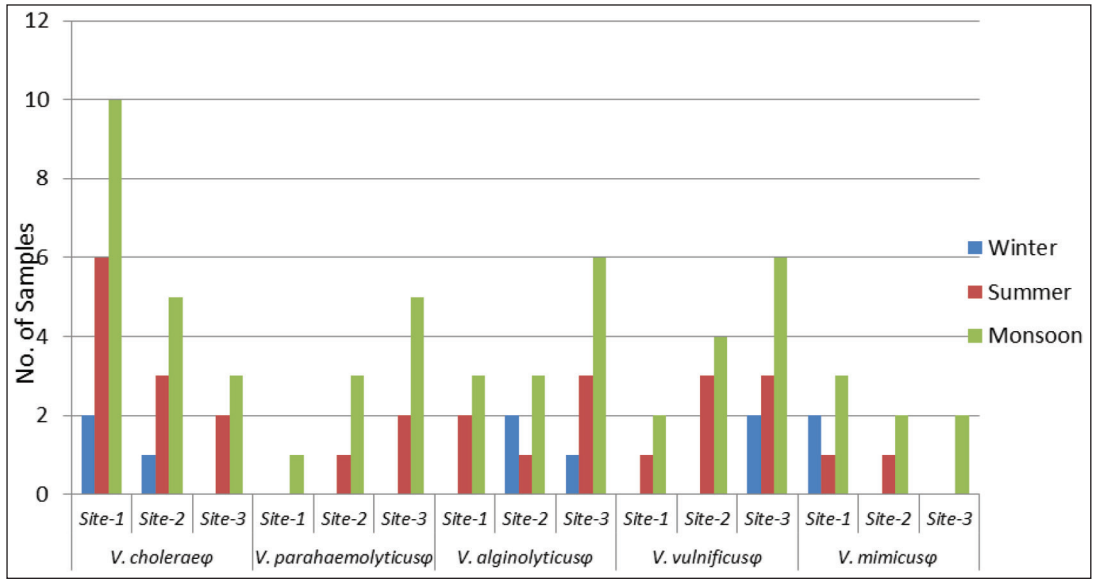


Fig 6 Seasonal abundance of species specific Vibriophages at all the sampling sites

Abundance of vibriophages in the riverine estuarine environment indicates a strong seasonal association, where it has been observed that all the phages showed their highest availability during monsoon period irrespective of the sites. Thus it can be hypothesised that alkaline pH along with higher load of organic debris enhances the propagation of the vibriophages during monsoon.

Thereby it is convincingly established that Gangetic riverine-estuarine aquatic ecosystem regulates the survival, distribution and transmission of diarrhoeagenic Vibrios from the saline habitat to inland fresh water riverine ecosystem, where it can adversely affect human health. Although a flowing aquatic ecosystem like river Ganges harbours different types of bacterial community, detection of entero-pathogenic Vibrios along with its phages, the first of its kind, through a year-long systematic surveillance indicates the role of Gangetic riverine-estuarine ecosystem, on sustained diarrhoeal disease transmission in south Bengal.

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

Principal Investigator : A. Palit

Results obtained so far:

Based on the year-long seasonal variation of physico-chemical properties at riverine-estuarine ecosystem, the study sites can be divided into three following categories:

High Saline Zone- The zone has been demarcated with a salinity range between 10 to 30 ppt. In the present study, Gosaba is such a site which can be categorised as high saline area (Fig 7).

Mid Saline Zone- The zone has been demarcated with a salinity range between 1 to 10 ppt. In the present study, Kakdwip and Diamond Harbour are such sites which can be categorised as mid saline area (Fig 7).

Low Saline Zone- The zone has been demarcated with a salinity range upto 1 ppt. In the present study, Howrah is such a site which is in low saline inland area. (Fig 7).

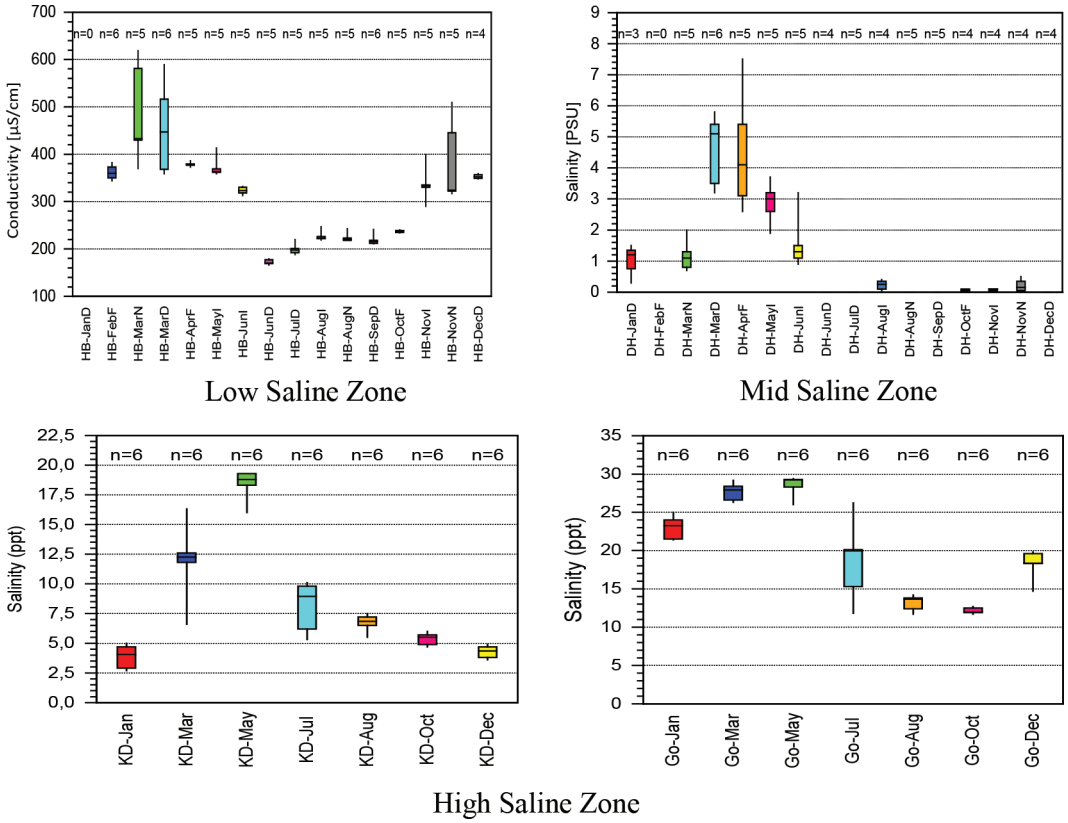


Fig 7 Seasonal variation of salinity at all the sampling sites

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 concentrations of SPM at sea-end stations are orders of magnitude greater suggesting that this part of the channel represent zone of estuarine turbidity maximum (ETM) [Table 4].

Since flood water discharge (within river track) is greatest during the South West monsoon there may be an increase in both stratification and strength of the inland riverine circulation.

SPM – Suspended Particulate Matter

The seaward moving dominant surface water river flow counteracts the landward moving bottom flow, intensified winds and wind-induced waves and tidal current activity.

V. parahaemolyticus could be isolated from 27/120 (22.5%) samples, being mostly prevalent in the high saline zone (Site III) (21/27), to lesser extent in Diamond Harbour (6/21) and was completely absent in 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. Irrespective of any organism or any site, monsoon seems to be the most favourable condition with maximum isolation rate achieved during the period.

Table 4 Physico-chemical properties of riverine-estuarine water samples

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

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

Principal Investigator : B. L. Sarkar

The strains of *V. Cholera*e isolated from patients, environment and outbreak sent to us from different institutes across the country (Table 5). A total of 342 strains of *V. cholerae* from 9 states including West Bengal were received from different endemic regions of the country during the current year for confirmation, serotyping, biotyping and phage typing at Vibrio Phage Laboratory. All the 342 (100 %) strains were confirmed as *V. cholerae* O1 biotype El Tor, was included in phage typing study. Majority of the strains belonged to Ogawa 337 (81.2 %) followed by Inaba5 (18.7%). These strains were grouped under Type 2 (95.6%) and 4 (4.3%) with the conventional scheme of Basu and Mukherjee. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (66.3%) followed by type 26 (7.6%), type 20 (4.3 %), type 25 (3.8 %) respectively. It has been shown that type 27 was the predominant phage type circulating in this country. For the last couple of years, *V. cholerae* O139 is absent as we did not receive any strains neither from Kolkata nor from any parts of the country.

Table 5 Biotype, Serotype and Phage type of V. cholerae strains received during the year 2015-16

State	No of Strain	Biotype		Serotype		Basu & Mukherjee			New Phage										
		El Tor	Classical	Ogawa	Inaba	T-2	T-4	UT	7	10	13	20	21	22	23	24	25	26	27
Andhra Pradesh	62	62	-	62	-	62	-	-	-	1	2	1	-	-	2	3	-	6	47
Delhi	5	5	-	5	-	5	-	-	-	-	-	-	-	-	-	-	-	-	5
Gujarat	68	68	-	65	3	65	3	-	3	-	3	-	4	5	-	-	4	7	42
Karnataka	1	1		1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Madhya Pradesh	22	22	-	20	2	20	2	-	-	-	-	3	-	1	-	-	-	2	16
Maharashtra	82	82	-	82	-	77	5	-	1	5	3	5	4	1	-	6	-	8	49
Punjab	63	63	-	63	-	61	2	-	2	-	3	4	1	-	5	-	7	-	41
Rajasthan	14	14	-	14	-	11	3		-	1	1	-	-	-	1	-	2	-	9
West Bengal	25	25	-	25	-	25	-	-	-	-	-	2	-	2	-	1	-	3	17
Grand Total	342	342	-	337	5	327	15	-	6	7	12	15	9	9	8	10	13	26	227
Total %	100	100	-	81.2	18.7	95.6	4.3	-	1.7	2.0	3.5	4.3	2.6	2.6	2.3	2.9	3.8	7.6	66.3

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

Principal Investigator : B. L. Sarkar

Co-Investigator : H. Koley

The Indo-UK collaborative project between NICED-UKERI, UK is completed. In and around Kolkata, a total of 55, O1 specific vibriophages were isolated. Later on, the restriction digestion (EcoRV) pattern of the phage DNA indicated only ten dissimilar phages. These ten phages also showed distinct plaque morphology compared to each other on nutrient agar plates. The *in vitro* lytic activity of these ten vibriophages was the maximum lytic activity observed. One step growth curve was also performed. The latent periods for the phage ranged between 10 and 15 min, and the estimated burst sizes ranged from 6 to 664. The morphometry of a subset of phage by transmission electron microscope showed that these phages belonged to the family Podoviridae. Additionally, genome sequencing, assembly and analysis and *in vivo* phage therapy trials in infant rabbits are underway in UK. All these ten vibriophages isolated were totally new and different from the previous *V. cholerae* phages routinely in use in our laboratory. These environmentally isolated phages are highly lytic and having broad host range. One of the outcomes of this project is sustainable towards the technology transfer from India (NICED) to UK on cholera phage research study. The use of these phages will lead to further research support to develop the idea further and into applied and translation work. Our results with these newer phages might be established as a milestone toward the therapeutic use of bacteriophages for cholera infection as an alternative to antibiotics. This novel approach will be further pursued by utilizing cocktail phages in preclinical and clinical trials. Our future strategy is to carry forward our mission by utilizing the cocktail phage in human volunteer study.

Title: Circulating gut homing (α4β7+) plasmablasts responses against Shigella surface protein antigens among hospitalized diarrheal patients

Principal Investigator : R. K. Nandy

Co-Investigators : A. Dey, International Vaccine Institute (IVI), Seoul, Korea; M.K. Bhattacharya, T. Ramamurthy, NICED; C. Czerkinsky IVI and 3IPMC Nice-Sophia Antipolis, Nice, France

Developing countries are burdened with *Shigella* diarrhea. Understanding mucosal immune responses associated with natural *Shigella* infection are important to identify potential correlates of protection and as such to design effective vaccines. We performed a comparative analysis of circulating mucosal plasmablasts producing specific antibodies against highly conserved invasive plasmid antigens (IpaC, IpaD20 and IpaD120) and two recently identified Pan-*Shigella* Surface Protein antigens (PSSP1 and PSSP2) common to all virulent *Shigella* strains. We examined blood and stool specimens from 37 diarrheal patients, admitted to the Infectious Diseases & Beliaghata General Hospital, Kolkata, India. The etiological agent of diarrhea was investigated in stool specimens by microbiological methods and real-time PCR. Gut homing (α4β7+) antibody secreting cells (ASCs) were isolated from patient blood by means of combined magnetic cell sorting and two-color enzyme-linked immunosorbent spot (ELISPOT) assay. Overall, 57% (21 out of 37 patients) and 65% (24 out of 37) were positive for *Shigella* infection by microbiological and real-time PCR assays, respectively. A higher frequency of α4β7+ IgG ASC responders against Ipa antigens was observed as compared to PSSP1 or PSSP2, regardless of *Shigella* serotype isolated from these patients (Fig 8). Thus, α4β7+ ASC responses to Ipa antigens may be considered as an indirect marker of *Shigella* infection. The apparent weakness of ASC responses to PSSP1 is consistent with the lack of cross-protection induced by natural *Shigella* infection. The finding that ASC responses to IpaD develop in patients with recent onset shigellosis indicates that such responses may not be protective or wane too rapidly and/or be of insufficient magnitude.

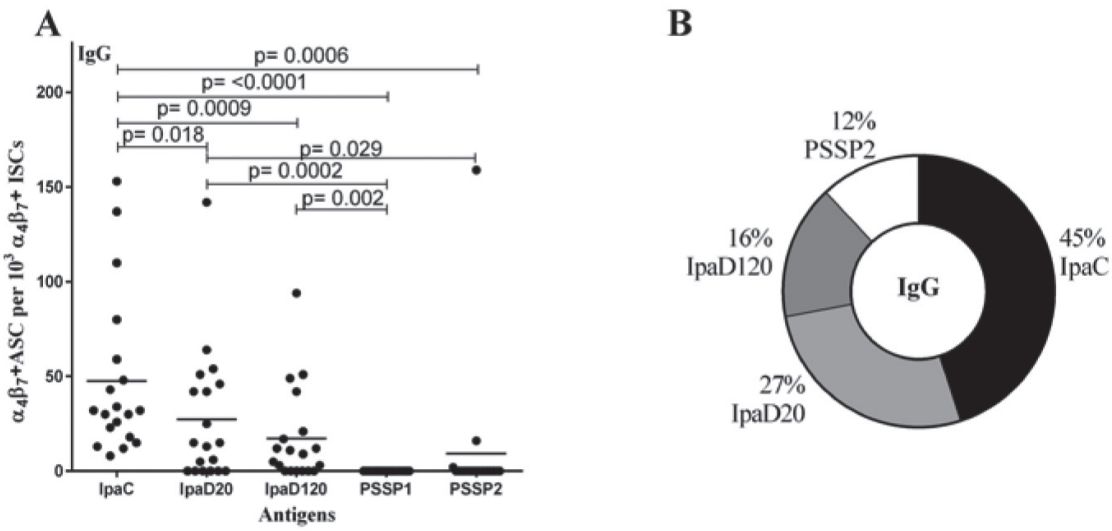


Fig 8 Gut homing ASC (α4β7+) responses determined by ELISPOT assays in IgG class-specific manner (A) and relative antibody class-specific proportion against different antigens (B) among shigellosis patients. Data were normalized for IgG antibody class and expressed per 103 gut homing immunoglobulin secreting cells (ISCs). Non-parametric paired t test were performed for statistical significance; bar represents mean value

Title: *Helicobacter pylori* strains harboring *babA2* from India are associated with increased virulence in *in vitro* study

Principal Investigator : A.K. Mukhopadhyay

Infection with *H. pylori* is associated with duodenal ulcer (DU) or gastric ulcer, gastritis, and gastric adenocarcinoma. About 65-70% of the Indian population is infected with *H. pylori*. 15-20% of overall infected population develop gastric or duodenal ulcer and less than 1% develop gastric adenocarcinoma. The babA2 gene along with the *cagA* and *vacA* of *H. pylori* has been considered as a risk factor for the disease outcome in certain populations. This study was aimed to understand the role of *babA2* of *H. pylori* with the background of *cagA* and *vacA* in disease manifestations in Indian sub population. A total of 114 *H. pylori* strains isolated from duodenal ulcer (DU) (n=53) and non-ulcer dyspepsia (NUD) patients (n=61) were screened for the prevalence of these virulence markers by PCR. The comparative study of IL-8 production and apoptosis were done by co-culturing the AGS cell line with *H. pylori* strains with different genotypes. Adherence assay was performed with *babA2* positive and negative strains. Two isogenic mutants of *babA2* were constructed and the aforesaid comparative studies were carried out. PCR results indicated that 90.6% (48/53), 82% (50/61) and 73.6% (39/53) strains from DU patients were positive for *cagA*, *vacA*, and *babA2* respectively, whereas the prevalence of these genes in NUD subjects was 70.5% (43/61); 69.8% (37/53), and 65.6% (39/61) respectively. Although adherence to AGS cells was comparable among strains with *babA2* positive and negative genotypes, but the triple positive strains could induce highest degree of IL-8 production and apoptosis, followed by the *cagA*$^-$/*vacA*$^-$/*babA2*$^+$ strains and triple negative strains, respectively (Fig 9 & 10). The wild type strains showed significantly higher IL-8 induction as well as apoptosis in *in vitro* than its isogenic mutant of *babA2* ((Fig 11 & 12). PCR study demonstrated that there was no significant association between the distribution of *babA2* genotype or of triple positive strains and disease outcome in this population. The adherence assay showed that there was no significant difference in the extent of adherence to AGS cells among *babA2* positive and negative strains. But the in vitro study indicated that the triple positive or even the *babA2* only positive strains are involved in increased virulence. The wild type strains also exhibited increased virulence compared to the *babA2* mutant strains. This inconsistency demonstrated that bacterial genotype along with host genetic polymorphisms or other factors play important role in determining the clinical manifestation of *H. pylori* infections.

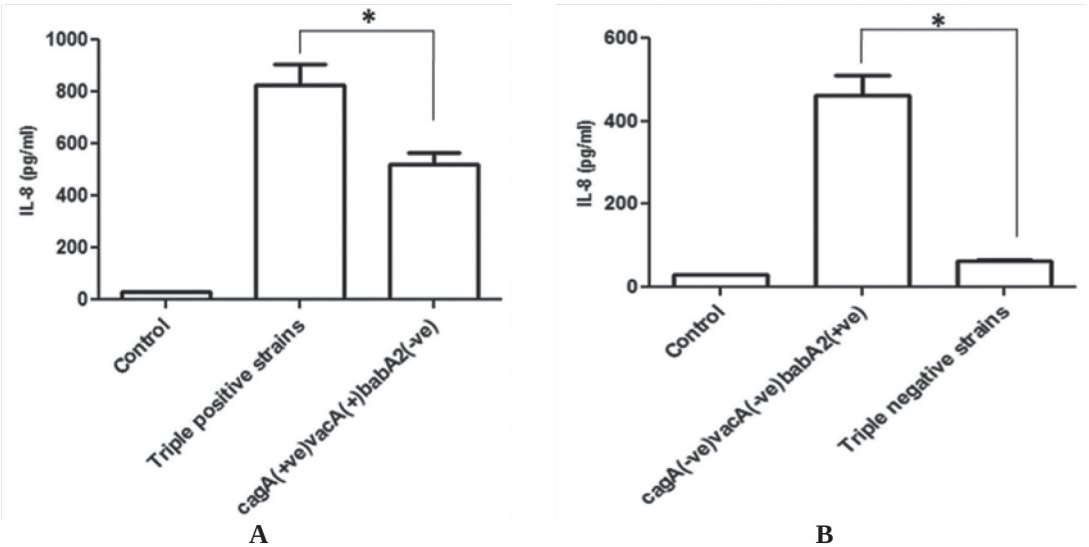


Fig 9 Triple positive strains and even the *babA2* positive only strains enhance IL-8 production in AGS cells.

In vitro IL-8 production from AGS cells co-cultured with randomly selected A) triple positive and *cagA*$^{+}$/*vacA*$^{+}$/*babA2*$^{-}$ strains, and B) *cagA*$^{-}$/*vacA*$^{-}$/*babA2*$^{+}$ and triple negative *H. pylori* strains (MOI is 100) for 8 h. IL-8 from culture supernatant was measured using ELISA as described in materials and methods. Data are expressed as mean ± standard error of mean (SEM) of 3 experiments in duplicates. **P*<0.05 as compared between groups.

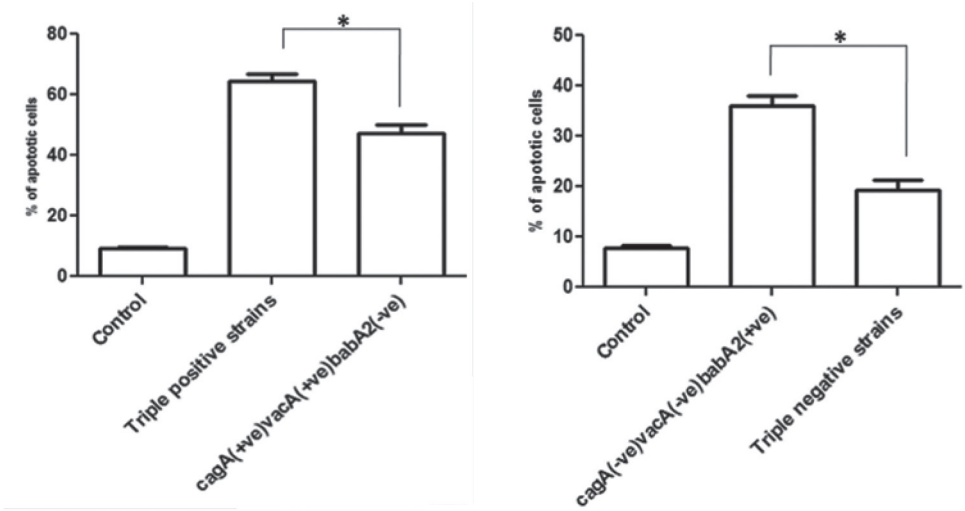


Fig 10 (a,b) Triple positive strains and even the *babA2* positive only strains cause more apoptosis in AGS cells

Graphical representation of % apoptotic cells (Sub G0 phase) infected with same group of strains (Like triple positive; *cagA*$^{+}$/*vacA*$^{+}$/*babA2*$^{-}$; *cagA*$^{-}$/*vacA*$^{-}$/*babA2*$^{+}$; and triple negative strains of *H. pylori* strains) were expressed as mean ± SEM.**P*<0.05 as compared between groups.This Figure is representative profile of at least three experiments.

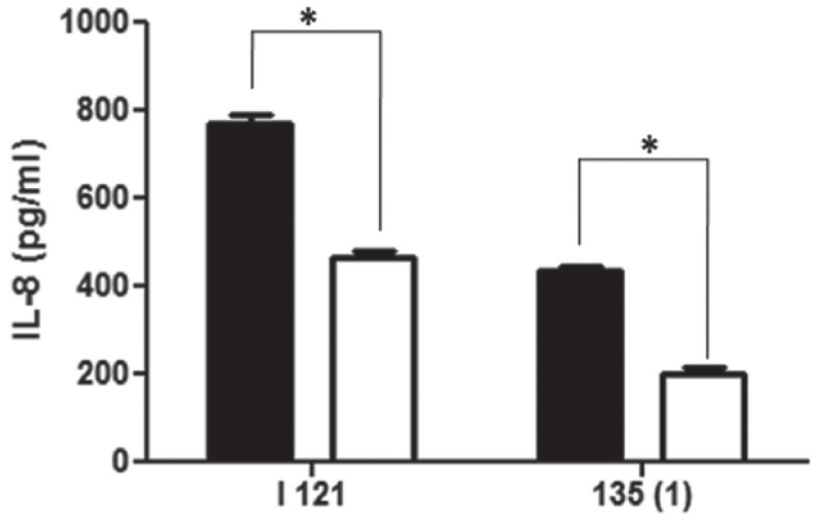


Fig 11 Wild type *H. pylor*i strains show higher IL-8 production than their isogenic mutant of *babA2* in AGS cells

IL-8 from culture supernatant was measured using ELISA as described in materials and methods. Data are expressed as mean ± standard error of mean (SEM) of 3 experiments in duplicates. Wild type is denoted with filled bar (■) and mutants denoted with empty bar (□). **P*<0.05 as compared between groups

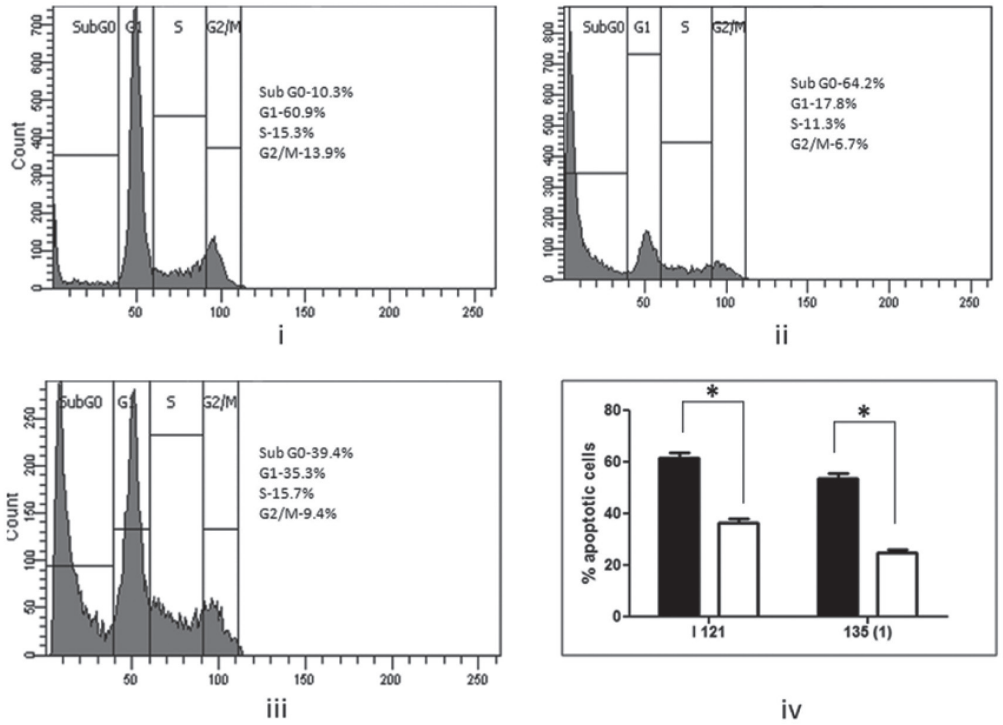


Fig 12 Wild type *H. pylori* strains cause more apoptotic cell death than their isogenic mutant of *babA2* in AGS cells

Apoptosis of AGS cells (5i worked as controls) co-cultured with different genotypic variant i.e. 5ii) wild type I-121 strain; and 5iii) isogenic *babA2* mutant of I-121 strain for 24 h (MOI is 100), stained with propidium iodide and analysed by flow cytometry. Figures 5i-iii is only representative profile of at least three experiments carried out with I-121 strain and its *babA2* mutant. (5iv) Graphical representation of percentage of apoptotic cells (sub G0 phase) infected with two wild type and their babA2 mutant strains were expressed as mean ± SEM. Wild type is denoted with filled bar (■) and mutants denoted with empty bar (□). *P<0.05 as compared between groups.

Title: Polymyxin B sensitive El Tor Vibrio cholerae O1: another major shift towards the classical biotype

Principal Investigator : A.K. Mukhopadhyay

The epidemiology of global cholera, especially in Africa and Asia, has shown periodic subtle changes. The recent epidemic of cholera in Haiti, a previously cholera naïve Caribbean country, affected more than half a million people with around 8000 deaths and brought this ancient menace in the frontline of the public health program. This life-threatening disease is caused by *Vibrio cholerae*, which has more than 200 serogroups. However, only two serogroups O1 and O139 cause epidemic and pandemic cholera. Based on certain phenotypic and genetic characteristics, *V. cholera*e O1 is classified as classical and El Tor biotypes, which differ biochemically and clinically. Classical strains typically cause a more severe disease, while El Tor strains involve less severe and sometimes even asymptomatic cases. However, El Tor strains appear to have increased fitness in the environment, which may be why they have largely replaced classical strains as the cause of disease in recent years. As a result, the classical biotype is believed to be extinct while the El Tor is the currently prevailing one. The division into the classical or El Tor biotype is centred on several laboratory tests. Among the phenotypic traits that distinguish between the two biotypes, polymyxin B sensitivity(50 U) is very reliable and is considered as one of the most stable phenotype in the biotyping scheme. Current research has shown that V. cholerae strains are undergoing cryptic changes in the genome, which influences their virulence and rapidity in transmission and spread. In the past decade, El Tor biotype carrying classical traits was emerged.

A recent striking observation is the appearance of cationic antimicrobial peptide polymyxin B sensitive *V. cholerae* O1 strains isolated from cholera patients from June 2012 in Kolkata, India. A total of 255 strains isolated between 2003 and 2014 in Kolkata were tested for this study. Since the start of the seventh pandemic of cholera, the El Tor biotype has always been resistant to polymyxin B. However, for the first time starting from March 2013, polymyxin B sensitive El Tor strains totally replaced the resistant strains (Fig 13).The results of MIC using E-test for polymyxin B confirmed their susceptibility to this antibiotic (Fig 14). In this assay, the El Tor strain (VC073) was highly resistant to polymyxin B, displaying a MIC of 96 µg/ml, whereas the newer variant strains in Kolkata showed a drastic reduction in resistance. The results also showed that these strains contained Haitian variant *ctxB* (*ctxB7*), which is similar to the classical cholera toxin. Our earlier studies identified many new attributes of Haitian *V. cholera* variant strains in Kolkata since 2003. Here, we report the emergence of El Tor strains producing classical CT that have lost a very important El Tor biotype marker and acquired a vital classical biotype characteristic probably altering the regulatory mechanisms of lipid A modification machinery in *V. cholerae*. This shift is an important event in the history of cholera after 1961 when El Tor vibrios first appeared. The new variant strains need to be carefully monitored using an active holistic surveillance system with respect to their clinical and epidemiological manifestations.

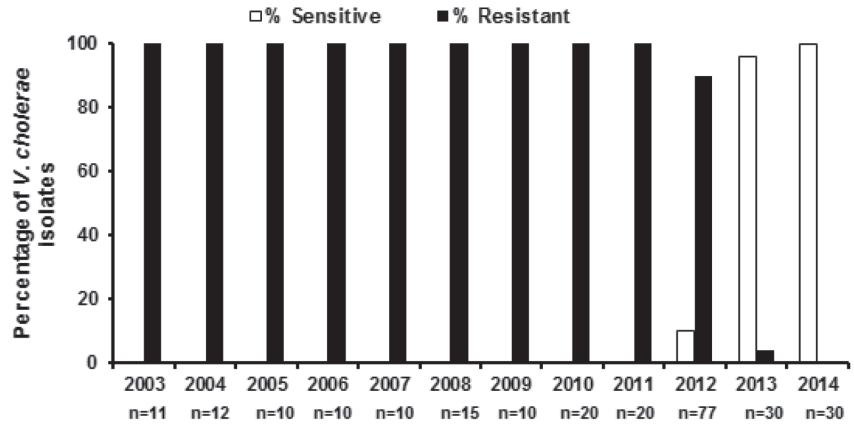


Fig 13 Isolation profile of Polymyxin B sensitive strains in Kolkata: Occurrence of Polymyxin B susceptibility pattern in Kolkata *Vibrio cholerae* O1 El Tor variant strains from 2003 to 2014. A total of 255 strains were tested during the study period and "n" denotes the number of strains tested in each year. Polymyxin B sensitive strains first appeared in Kolkata during June 2012. The first strain, which was isolated during January 2013, was resistant and then all the tested strains isolated during 2013 and 2014 were sensitive to Polymyxin B, which was considered as important biotyping marker for the classical strains.

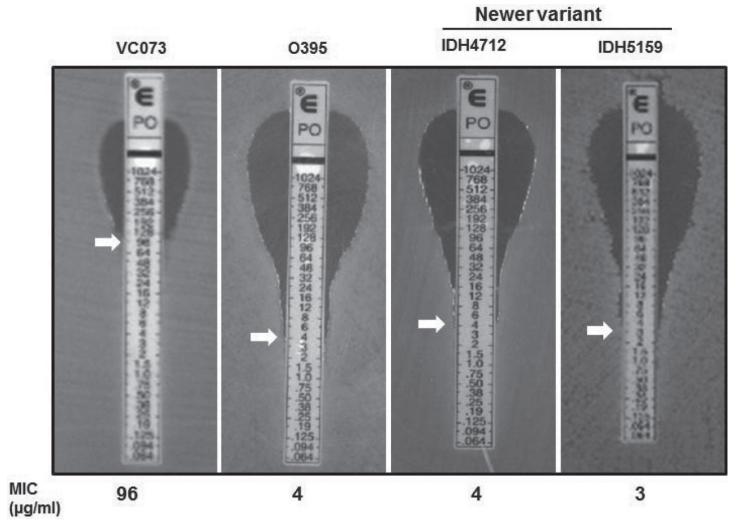


Fig 14 The MIC of Polymyxin was determined in El Tor, classical and Newer El Tor variant strains and the MIC value is indicated with a white arrow. Polymyxin B sensitivity was displayed by Newer El Tor variant strains which is the characteristic feature of classical strains. Data are representative of three biological repetitions

Title: Mobilizable elements in carbapenem-resistant isolates

Principal Investigator : S Basu

Co-Investigators : S. Datta, S. Chatterjee, S. Mitra, NICED; T. Som, S. Mukherjee, Department of Neonatology, Institute of Postgraduate Medical Education & Research, SSKM Hospital, Kolkata

Carbapenem resistance has emerged as a major impediment in the treatment of neonatal infections. The dexterity with which carbapenem-resistant determinants move from one species to another, is due to the presence of mobile genetic elements. Mobilizable elements associated with carbapenemases in Enterobacteriaceae and *Acinetobacter* spp isolated from septicemic neonates were investigated. Transfer of carbapenem resistance was successful in the different species. In Enterobacteriaceae, carbapenem resistance was due to NDM-1 (New Delhi Metallo-β-lactamase-1) whereas in *Acinetobacter*, oxacillinases (OXA-23-like, OXA-58-like and OXA-51-like) and NDM-1 were responsible.

In Enterobacteriaceae, blaNDM-1 was associated with different plasmid scaffolds (IncFII, IncL/M, IncN, IncR, IncHIB-M/FIB-M), IncF type being the prevalent one. Genetic structures surrounding blaNDM-1 showed its association with at least a remnant of ISAba125 at its 5'-end and bleMBL at its 3'-end. The spread of NDM-1 was not related to class 1 integron which possessed resistance determinants against trimethoprim (dfrA12, dfrA1, dfrA5), streptomycin (aadA2, aacA4) and rifampicin (arr-3). RFLP pattern showed that three isolates possessed the same FII/FIIs plasmid; two of these three isolates were from a single neonate implying interspecies genetic transfer of blaNDM-1.

Contrary to Enterobacteriaceae, in *Acinetobacter*, blaNDM-1 was organised in a composite transposon between two copies of ISAba125 in the isolates irrespective of the species (Fig 15). The dissemination of the blaNDM-1 in *Acinetobacter* spp is likely linked to Tn125 in diverse clones of the isolates. OXA-23-like gene and OXA-58-like genes were linked with ISAba1 and ISAba3 respectively. Integrons were variable in sequence but not associated with carbapenem resistance as was also seen in Enterobacteriaceae.

This analysis of the mobilizabale elements in Enterobacteriaceae and *Acinetobacter* spp has revealed that in *Acinetobacter*, blaNDM-1 is organised in a composite transposon (Tn125) whereas in Enterobacteriaceae the complete transposon could not be identified, only a remnant of the ISAba125 was systematically present.

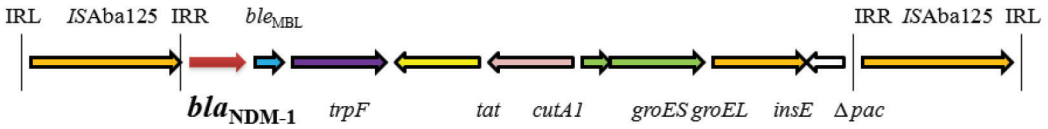


Fig. 15A

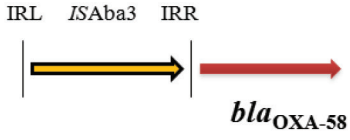


Fig. 15B

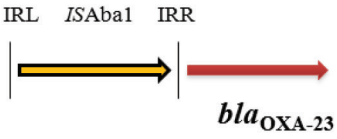


Fig. 15C

Fig 15 (a) Schematic representation of Tn125 carrying blaNDM-1 gene in a representative transconjugant. Genes and their transcription orientation are indicated by arrows. The lengths of the target genes and their exact location of the target genes are not to scale. Gene names are abbreviated according to the corresponding proteins: cutA1 for divalent cation tolerance protein; groES, groEL for heat-chaperonin protein; insE for ISCR21 of tnpA family. Δ pac for truncated phospholipid acetyltransferase. IRL and IRR are for inverted repeat left and right, respectively. (b) Diagram showing the linkage between blaoxa-58-like with ISAba3. (c) Diagram showing the linkage between blaoxa-23-like with ISAba1

Title: Development of a cost-effective vaccine candidate with outer membrane vesicles of a tolA-disrupted Shigella boydii strain

Principal Investigator : H. Koley

Our previous studies on outer membrane vesicles based vaccine development against shigellosis, revealed the inability of Shigella to release significant amount of vesicles naturally, during growth. Disruption of tolA, one of the genes of the Tol-Pal system of Gram negative bacterial membrane, has increased the vesicle release rate of a *Shigella boydii* type 4 strain to approximately 60% higher (Fig. 16). We also noticed the vesicles, released from tolA-disrupted strain captured more OmpA protein and lipopolysaccharide, compared to the vesicles released from its wild type prototype. Six to seven weeks old BALB/c mice, immunized with 25µg of three oral doses of the vesicles, released by tolA mutant, conferred 100% protection against lethal homologous challenge through nasal route, compared to only 60% protection after the same dose of wild type immunogen. Mice, immunized with the vesicles from tolA-mutant, manifested significant secretion of mucosal IgG and IgA (Fig 17). A sharp and significant response of pro-inflammatory cytokines (TNF-α, IL-6, IFN-γ) were also observed in the lung lavage of these groups of mice, within 6h post challenge; but at 24h, these inflammatory cytokines showed the sign of subsidence and the system was taken over by the release of anti-inflammatory cytokines (IL-4 and IL-10). Studies with naïve peritoneal macrophages, proved further, the potency of these vesicles to stimulate nitric oxide and TNF-α, IL-12p70, IL-6 and IL-10 productions in-vitro. The ability of these vesicles to trigger polarization of CD4(+) T cells toward Th1 adaptive immune response, had also been observed along with the presence of anti-inflammatory cytokines in the system. Our study demonstrated, the vesicles from tolA-disrupted Shigella were able to suppress Shigella-mediated inflammation in the host and could balance between inflammation and anti-inflammation, promoting better survival and health of the infected mice. Outer membrane vesicles from tolA-mutant, could be a potential cost-effective vaccine candidate against shigellosis.

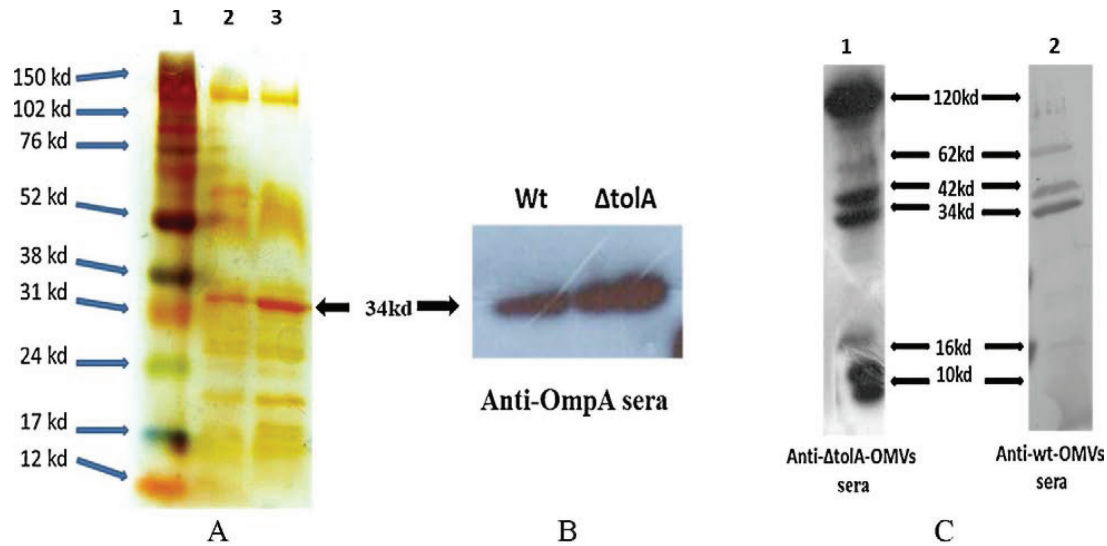


Fig 16 (A) SDS–PAGE of OMVs, stained with 0.1% silver nitrate solution. Lane 1, 2 and 3 are the molecular weight marker, wt-OMVs and ΔtolA-OMVs respectively. The band at 34 kDa position was very prominent in lane 3. To confirm this 34 kDa is OmpA protein, Western blot of these two types of OMVs was carried out against anti-OmpA sera. Part (B) was showing a representative picture of this immunoblot. Lane Wt and ΔtolA were representing wt-OMVs and ΔtolA-OMVs respectively. It clearly showed the oversecretion of OmpA protein by ΔtolA-OMVs. Part (C) showed the representative Western blots of the whole cell lysates of BCH612 wild type strain against anti-ΔtolA-OMVssera (Panel 1) and anti-wt-OMVs sera (Panel 2), respectively

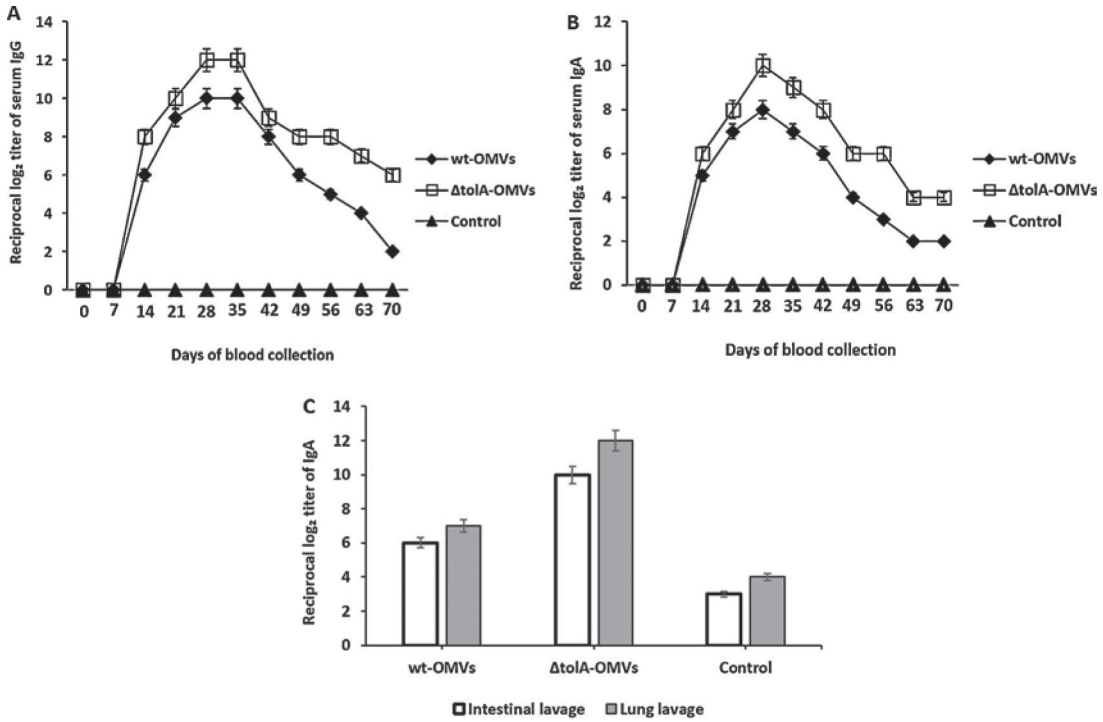


Fig 17 Graphical representation of the secretion of IgG (A) and IgA (B) in mouse sera. Blood was collected on the days indicated along the horizontal axis. Both the immunoglobulins started to respond from day 14 after first immunization, with a peak on day 28 and decreased gradually with time but remained above the level of detection till 10 weeks. Control sera showed a baseline response of these immunoglobulins and the difference between control and immunized on each day was found to be statistically significant (P value < 0.005). The response in tolA-OMVs sera were significantly higher compared to wt-OMVs sera (P value < 0.005). Data represented here are the mean ± SD of three independent experiments. (C) sIgA in mucosal fluids like lung and intestinal wash, was measured by ELISA before and after immunization. TolA-OMVs immunization helped to secrete significantly greater sIgA in both lung and intestine, compared to wt-OMVs immunized group (P value < 0.005). Control group of mice showed basal level response. Data represented here are the mean ± SD of three independent experiments

Awards/Honours Received

S. Dutta

- Delivered the Platinum Jubilee Lecture on in the section of Medical Sciences (including Physiology) at the 103rd Indian Science Congress to be held at University of Mysore, Mysuru during January 3-7, 2016
- Member of Scientific Advisory Committee, NIPER, Kolkata
- Member of Research Advisory Committee, Institute of Environmental studies and Wetland Management, Dept. of Environment, Govt. of West Bengal
- Expert Core Group Panel on Rare Disease Initiative by NIPER, Kolkata
- Recruitment board member of UPSC
- Nominated Principal Member of the drinks and drinking water sectional committee FAD 14 of Bureau of Indian Standards since 2012.
- Principal member of evaluation of Immunobiologicals sectional committee FAD 15 of Bureau of Indian Standards for since 2015.

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

- Diagnostic Microbiology and Infectious Diseases (DMID)
- J of Antimicrobial Chemother (JAC)
- J of Applied Microbiology (JAM)
- J of Medical Microbiol
- BMC infectious diseases-a BMC series journal,
- PLoS One, PLoS Neg Trop Dis

- International J of infectious Diseases (IJID)
- Indian Journal of Medical Research (IJMR)
- Reviewer of STS, ICMR proposals; DST Proposal

Invited International Editorial Board member of following journals:

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

MoU signed between:

- MoU between Bengal Chambers of Commerce and Industry (BCCI) and NICED on 15th January, 2016 (signing Authority Mr. Ambarish Dasgupta, President, BCCI and Dr. Shanta Dutta, DIC, NICED)
- Expert Core Group Panel on Rare Disease Initiative – attended one meeting on 11th January, 2016 at IICB, Kolkata – signed MoU between NIPER and NICED

A. Palit

- Member, Drinking Water sectional Committee, FAD 25, Bureau of Indian Standards, Ministry of Consumer affairs, Food and Public distribution, GOI, 2010-11.
- Member, Water purification system sectional committee, MHD 22, Bureau of Indian Standards, Ministry of Consumer affairs, Food and Public distribution, GOI, 2010-11.
- Member, Water purification system subcommittee, FAD 14:2, Bureau of Indian Standards. Ministry of Consumer Affairs, Food and Public Distribution. GOI, 2015.

Acted as an invited reviewer for following International journals:

- Epidemiology and Infection
- Journal of Infection
- Current Science
- Journal of Medical Microbiology
- Ecological Indicators

S. Basu

- Acted as reviewer for Antimicrobial agents and Chemotherapy, Journal of Antimicrobial Chemotherapy, Frontiers in Microbiology, Journal of Global Microbial Resistance, Microbial Drug Resistance, Journal of Medical Microbiology , Indian Journal of Medical Research, BMC Microbiology.
- Participated in formulating the Guidelines for breast milk storing and dispensing for babies admitted at health facilities as an expert. This is an initiative of the Child Health Division, Ministry of Health and Family Welfare, Govt. of India.

A.K. Mukhopadhyay

- Elected Fellow of the West Bengal Academy of Science and Technology in the year 2015 for his contribution in the field of “Molecular Microbiology”

Conferences/Seminars/Workshops/Trainings Attended/Organised

S. Dutta

- Attended 38th National Conference of Indian Association of Medical Microbiologists (Microcon) held at Jaipur from 15-19 October 2014 and delivered a talk titled “Evaluation and comparison of performances of diagnostic PCRs for typhoid fever.”
- Attended 10th Annual Conference of Indian Association of Medical Microbiologists (West Bengal Chapter) held at Medical College and Hospital, Kolkata on 19 & 20 Sept 2015 and participated as judge for reviewing the oral presentations in the award category session.
- On invitation, attended 60th IPHACON 2016 on 6th March, 2016 at Dehradun, Uttarakhand to chair a session on “Cholera Vaccines” and to become chief guest in the valedictory session of the conference.

- Guest of Honour at the 59th Annual Conference of Indian Public Health Association (West Bengal Chapter), held on 12th December, 2015 at GLT Hall, Medical College & Hospital, Kolkata.
- Participated in the National Task Force (NTF) Meeting on "Laboratory containment of Wild Poliovirus" on 14 July 2015 at Nirman Bhawan, A wing, New Delhi
- Participated in a meeting on "Establishment of Bio-repository in ICMR institutes" at National Institute of Research in Tuberculosis, Chennai on 15 July 2015 and presented the status of NICED GTPR (Gastrointestinal Tract Pathogen Repository)
- Participated in a symposium by Central Calcutta Society for advancement of Human Development & research on diarrhoeal Diseases and its modern perspectives on 21 July 2015 and delivered a lecture on "Challenges in preventive and control of Salmonellosis"
- Attended review meeting for technical progress of Viral Research Diagnostic Laboratory (VRDL) on 23 July 2015 at ICMR HQ.
- Participated in the consultation meeting of the National task Force (NTF) on Poliovirus Containment held on 29 July 2015 at ICMR Hqrs. New Delhi
- Attended the workshop on "Updated national Guidelines on Clinical management of dengue" by Health Dept., Kolkata Municipal Corporation held on 1 August 2015 at Uttam Mancha, Kolkata
- Attended 6th Regional Technical Advisory Group meeting on Kala-azar during 28th to 30th September, 2015 at the Hotel DeSovrani, Salt Lake City, Kolkata
- Guest Speaker in the National Seminar on "Cholera and Enteric Diseases' – A centenary tribute to great scientist Dr. S.N. De" on 3rd October, 2015 at CSIR-Central Glass and Ceramic Research Institute, Kolkata
- Attended UK-India workshop on new diagnostics and therapeutics to tackle antimicrobial resistance held on 12-13th October, 2015 at Hotel Taj Mansingh, New Delhi
- Participated in the 2nd Cholera Expert Group Meeting at the 4th Floor meeting room, Global Health Strategies, New Delhi on 14th October 2015.
- Delivered a talk on "Vaccine and vaccination" at the Foundation workshop on Clinical and Laboratory Medicine Research at the Guwahati Medical College, Guwahati on 4th November, 2015.
- Special Guest of Honour in the Valedictory program at the 2nd National convention of the Society for Ethnopharmacology, India on "Integrated Approaches for Promotion and Development of Herbal Medicine" organized by School of Natural Product Studies, Jadavpur University on 6th December, 2015 at Triguna Sen Auditorium, Jadavpur University, Kolkata.
- Guest of Honour at the 59th Annual Conference of Indian Public Health Association (West Bengal Chapter), held on 12th December, 2015 at GLT Hall, Medical College & Hospital, Kolkata.
- Participated First Scientific Advisory Committee meeting of National Institute of Pharmaceutical Education and Research (NIPER)-Kolkata on 15th December, 2015.
- Nominated by Vice Chancellor, Jadavpur University to act as Member of the Selection Committee for appointment to the post of Assistant Professor/Associate Professor in Pharmaceutical Technology to be held on 22nd to 23rd December, 2015 from 11-00 a.m. in the committee Room No.II of the University.
- Participated in the Sundarban Kristi Mela O Loko Sanskriti Utsab organized by Kultali Milon Tirtha Society on 24th December, 2015 which was held during December 20- 29, 2015, Kultali
- Became member of the Expert Core Group Panel on Rare Disease Initiative – attended one meeting on 11th January, 2016 at IICB, Kolkata – signed MoU between NIPER and NICED
- Nominated by DG, ICMR from ICMR side to have discussion on the research initiative to be included in MoU between IIT, Kharagpur and ICMR on 12-13 January, 2016 at IIT, Kharagpur
- Participated in the Panel Discussion held in the Symposium on Rare Disease Initiative jointly organized by All Indian Institute of Hygiene and Public Health and Calcutta School of Tropical Medicine held on 20-21 January, 2016 at IICB, Kolkata
- Attended the Japan-India Bilateral meeting on the collaborative research projects "Laboratory based collaborative network of infectious diseases in Asia" held during 25-27th January, 2016 at Tokyo, Japan
- Attended international seminar on infectious diseases at NIID, Tokyo during Jan 25-27 2016.
- As a member of the teams of external evaluation for hospital infection control, visited Regional Institute of Medical Sciences (RIMS), Imphal vide Ministry's order No.Z.28015/51/2014-G.I(H)(Pt.) on 31st January and 1st February, 2016.
- Visit to Krish Biotech Research Pvt. Ltd., Kalyani on 3rd February, 2016

- As a nominated member attended Screening Committee meeting for the post of Scientist-E (Pathophysiology), NICED, Kolkata held on 5th February, 2016 at ICMR Hqrs., Delhi
- Meeting with delegates from UN Food and Agricultural Organization (FAO) on 10th February, 2016 at NICED
- Expert group meeting on current global scenario of cholera with special reference to West Bengal on 14th February, 2016 at NICED
- Organized Pharmacovigilance seminar of NIPER on 22nd February, 2016 at NICED.
- Attended the conference on Antimicrobial resistance (AMR) organized by MoH & FW, GoI with support from the WHO during 23-25th February, 2016 at the Ashok Hotel, New Delhi
- Convened a meeting of Pharmacoepidemiology and pharmacovigilance committee at NICED on 25th February, 2016.
- FAO meeting on 1st March, 2016 at NICED with FAO experts on AMR
- On invitation form M/o Health and Family Welfare, attended WHO sponsored workshop on Antimicrobial Resistance at New Delhi on 23-25 March 2016.
- On invitation, Participated in the WHO workshop on "Pharmaco-epidemiology and Pharmacovigilance" held at NIRT, Chennai on 4th & 5th March 2016 and presented on "Role of NICED in the IPC
- To discuss project proposal convene a meeting with Executive Medical Officer, Borough-III, Kolkata Municipal Corporation on 21st March, 2016.
- As an invited member attended Selection Committee meeting for the award of ICMR-PDF held on 22nd March, 2016 at ICMR Hqrs.

A. Palit

- Imparted training in public health services to medical microbiologists of Bankura Sammilani Medical College students (September, 2015)
- Nominated, invited and participated in the Workshop on Sources of Environmental Pollution in India : Influence of Municipal Solid Waste and Biomass Burning on Air Quality and the Microbiome of the Ganges (25-26 October, 2015) at IIT, Kanpur
- Invited & participated in a PANEL DISCUSSION on "Microbiome of the Ganges on 26th October, 2015 at IIT, Kanpur with participants from IIT, Kanpur & Roorkee, NEERI (CSIR), Nagpur, Georgia Tech Institute, USA etc.

B.L. Sarkar

- Attended 4th Annual Conference on Molecular Pathology Association of India (MPAI) at Tata Medical Centre, Kolkata on Feb 14-15, 2015.
- Delivered lecture in one day seminar entitled "Development of a bacteriophage-based biocontrol technology for the treatment of cholera" organized by British Council, New Delhi jointly with DST-UKIERI on Sep 21, 2015.
- Invited to participate in an interactive session towards the collaboration between CCRH-NICED and also presentation was made entitled "NICED, Cholera and Cholera Bacteriophage" at New Delhi on Dec 10, 2015.
- Invited/acted as Board of examiner of Microbiology Department at North Bengal University on Jan 14 & 15, 2016.

R.K. Nandy

- Joined as guest faculty and presented "Immune responses to Vaccine in developing countries" in 15th International Advanced Course on Vaccinology in Asia-Pacific Regions; organized by International Vaccine Institute (IVI), Seoul, Korea during May 11-15, 2015.
- Invited talk "ABCD of genetics: needed for clinicians" at PULMOCON-15, organized by Institute of Pulmocare and Research, Kolkata during October 10-11, 2015.
- Poster presentation at United States-Japan (US-Japan) Cooperative Medical Sciences Program (CMSP) 50th anniversary conference followed by 18th International Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim"; organized by National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH), USA during January 11-15, 2016.
- Invited talk "Challenges and prospects of Rotavirus vaccines" 60th National Annual Conference organized by Indian Public Health Association (IPHACON-2016) at Dehradun, India during March 4-6, 2016.

A.K. Mukhopadhyay

- Invited to attend the NICED-NIID joint bilateral meeting regarding the HMSC cleared collaborative project on "Laboratory based collaboration network of infectious diseases in Asia" and to the International Seminar on Infectious Diseases at the National Institute of Infectious Diseases (NIID), Tokyo during January 25th-27th 2016. Dr. Mukhopadhyay presented the progress of his project on "Retrospective analysis on the evolutionary aspects of Vibrio cholerae".
- Invited by National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) to attend the United States-Japan (US-Japan) Cooperative Medical Sciences Program (CMSP) 50th anniversary celebration followed by the 18th International Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim" and also to present his research work at the "Cholera Panel Meeting" under US-Japan CMSP; during January 11-15, 2016 at Bethesda, Maryland, USA.
- Participated and presented on "Studies on the Evolution of El Tor variant V. cholerae in India" in the 2nd Meeting of the Global Task Force on Cholera Control (GTFCC) Working Group on Laboratory Methods for Cholera Surveillance jointly organized by Translational Health Science and Technology Institute (THSTI), Faridabad, India and Department of Pandemic and Epidemic Diseases, WHO, Geneva, Switzerland during 26-27 November 2015 at Faridabad, India.
- Delivered an invited talk on "Multidrug resistant Campylobacter and campylobacteriosis: an Indian perspective" in the workshop organized by the Eastern Regional Station of Indian Veterinary Research Institute (IVRI) under the theme "Antibiotic Resistance: A Threat to Livestock and Public Health" held at Belgachia, Kolkata during September 5, 2015.

S. Basu

- 10th International Symposium on the Biology of Acinetobacter 2015. Greece, 3-5th June, 2015. Carbapenem resistance in Acinetobacter spp. causing neonatal sepsis over 8 years period:emphasis on NDM-1. Chatterjee, S, Saswati Datta, Shravani Mitra, Tapas Som, Rajlakshmi Viswanathan, Sulagna Basu.
- Microcon, Pondicherry, India, 25-29 November, 2015. Exploring the versatility of septicemic Escherichia coli with respect to virulence, carbapenem resistance and transmission of resistant-determinants. Saswati Datta, Subhasree Roy, Rajni Gaind, Suchandra Mukherjee, Sulagna Basu. (Oral Presentation)
- Microcon, Pondicherry, India, 25-29 November, 2015. Plasmid mediated fluoroquinolone resistance in neonatal sepsis causing Klebsiella pneumoniae isolates. Shravani Mitra, Saswati Datta, Tapas Som, Sulagna Basu. (Poster)
- 17th International Congress on Infectious Disease, Hyderabad, India, 2-5 March, 2016. Emergence, spread and exchange of blaNDM-1 gene among Enterobacteriaceae in septicaemic neonates. Saswati Datta, Shravani Mitra, Somdatta Chatterjee, Sulagna Basu.(Datta S received a travel award to attend the conference)
- 17th International Congress on Infectious Disease, Hyderabad, India, 2-5 March, 2016. Carbapenem-resistant Acinetobacter species: An emerging nosocomial superbug. Somdatta Chatterjee, Saswati Dutta, Sulagna Basu. (Chatterjee S received a travel award to attend the conference)
- 17th International Congress on Infectious Disease, Hyderabad, India, 2-5 March, 2016. Correlation of β-lactam resistance with over expression of efflux pumps among neonatal septicaemic isolates of *Acinetobacter baumannii* from India. Roy, S. and Basu,S. (Roy S received a poster award and a travel award to attend the conference)
- Attended UK-India workshop on New Diagnostics and Therapeutics to tackle antimicrobial resistance, New Delhi, 12-13 October 2015
- Attended- Combating Antimicrobial Resistance, Public Health Challenge and Priority organized by Ministry of Health and Family Welfare, Government of India and World Health Organization, New Delhi, 23-25 February, 2016.

H. Koley

- Next Generation OMVs Based Shigella Vaccine, Hemanta Koley, Soma Mitra, Ritam Sinha, Priyadarshini Mukherjee, Debaki Ranjan Howlader, Ushasi Bhaumik and Dhrubajyoti Nag. Presented at 103rd Indian Science Congress, January 04-07, 2016, Mysore, India.
- Outer membrane vesicles: A novel particle of next generation Shigella vaccine. Hemanta Koley. 8th Indo Global Summit and Expo on Vaccines, Therapeutics & Healthcare (VTH-2015), November 02-04, 2015 HICC, Hyderabad, India.
- Development of a universal Shigella vaccine based on virulence gene expression Hemanta Koley. January, 2016, National Institute of Infectious Diseases, Japan.

BIOCHEMISTRY

The Division of Biochemistry primarily focuses on in-depth understanding of the molecular mechanisms of host pathogen interaction using biochemical and biophysical 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 has already begun to provide a greater understanding of the complexity of bacterial pathogenesis and aim to translate the knowledge in developing novel therapeutic intervention strategies against enteric infections in near future. Further, know-how gathered is being applied to establish molecular tools for detection of virulence markers in pathogenic strains.

Scientist:

Dr. N.S. Chatterjee

Staff:

Mr. R. Naik, Technical Assistant (till 29th Feb 2016)

Post-Doctoral Fellow:

Dr. Epshita Chatterjee

Pre-Doctoral Fellow:

Ms. Moumita Mondal, SRF
Ms. Anusuya Debnath, SRF
Mr. Sudipto Mondal, SRF
Ms. Rhishita Chourashi, SRF
Mr. Debjyoti Bhakat, JRF
Mr. Suman Das, JRF

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, utilizes chitin as the sole carbon and nitrogen source and remains associated with the chitinous exoskeletons of zooplankton. Presently, our studies are directed towards the understanding the chitin utilization pathway in *V. cholerae* in environmental survival, horizontal gene transfer and pathogenesis. *V. cholerae* exochitinase ChiA2 plays a key role in acquisition of nutrients by chitin hydrolysis in the natural environment as well as in pathogenesis in the intestinal milieu. We demonstrate the importance of ChiA2 in horizontal gene transfer in the natural environment. We found that the expression of ChiA2 and TfoX, the central regulator of *V. cholerae* horizontal gene transfer, varied with changes in environmental conditions like pH, salinity, temperature, presence of chitin. The activity of ChiA2 was also dependent on these conditions. We observed maximum expression and activity of ChiA2 at 20°C, pH 5.5, and 100 mmol/L salinity in the presence of chitin. The same condition also induced TfoX expression and was favorable for horizontal gene transfer in *V. cholerae*. ChiA2 was upregulated and maximally active to produce a significant amount of (GlcNAc)2 from chitin. The same environmental condition also induced TfoX expression, followed by its translational activation by the $(GlcNAc)_2$ produced, leading to efficient horizontal gene transfer.

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 factor CS6 of ETEC is a prevalent one and is a vaccine target. CS6 has two structural subunits (CssA and CssB) and forms a-fimbrial protein on the ETEC surface. CS6 exists as variants in different isolates. CS6 is assembled via the chaperone-usher pathway. During this period of investigation, the role of specific N- and C-terminal amino acid residues of CssA and CssB in the functional assembly using different molecular biology and biochemical techniques were unveiled. We identified the functionally important residues using alanine scanning mutagenesis. Each mutant was tested for binding with Caco-2 cells and the results were compared with the surface expression of mutant CS6. In this assay, many mutants were not expressed on the surface while some showed reduced expression. It appeared that some, but not all, of the residues in both the N and C termini of CssA and CssB played an important role in the intermolecular interactions between these two structural subunits as well as chaperone CssC. Our results demonstrated that T20, K25, F27, S36, Y143 and V147 were important in the CssA stability, probably through interaction of CssC. We also found that I22, V29 and I33 of CssA and G154, Y156, L160, V162, F164 and Y165 of CssB were responsible for CssA-CssB intermolecular interaction. In addition, some of the hydrophobic residues in the C-terminal of CssA and N-terminal of CssB were involved in the stabilization of higher order complex formation. Overall, the results presented here might help in understanding the CS6 assembly pathway and predict its overall structure.

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 B C Roy Children's Hospital, 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 interactions of 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 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 the intestinal immune responses and modulation of immune response by indigenous probiotic bacteria in the animal models of inflammatory bowel diseases.

Scientists:

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

Staff:

Mr. A. Pal, Technical Officer- A
Ms. P. Bhowmick, Technical Officer A
Mr. K.G. Saha, Technician B
Mr. S. Routh, Technician B
Mr. S. Turi, MTS
Mr. S. Dey, MTS

Postdoctoral fellows:

Dr. Ayan Lahiri - Research Associate (CSIR project)
Dr. Amita Barik - Scientist II (ICMR project)

Ph.D. Students

Mr. Nirmalya Dasguta - SRF (ICMR)
Mr. Bhupesh Kumar Thakur - SRF (ICMR)
Ms. Pujarini Datta - SRF (DST-INSPIRE)
Mr. Asim Biswas - SRF (CSIR)
Ms. Atri Ta
Mr. Sayan Das - SRF (CSIR)
Ms. Rimi Chowdhuri - Technical Assistant (Okayama University Project)
Mr. Rahul Shubhra Mondal - Scientist I (ICMR project)
Mr. Krishnendu Das - JRF (ICMR)

Ph.D. Awarded:

Dr. Nagaraja Theeya received Ph D. from University of Calcutta, Kolkata.

Thesis Title: A study on the role of bacterial protein kinases and phosphatases in the pathogenesis of enteric infections

Title: Hospital based surveillance for diarrhoeal diseases

Principal Investigator : M.K. Bhattacharya

Co Investigator **:** S.S. Das, P. Indwar NICED; Principal B.C Roy Post Graduate Institute of Paediatric Sciences (BCRPGIPS); Principal Infectious Diseases and Beliaghata General Hospital

From April 2015 to March 2016, a total of 1211 fecal specimens were collected from every 5th patient admitted with acute watery diarrhea at Infectious Diseases Hospital (IDH), Kolkata (during 24 hours a day on 2 randomly selected days per week) for etiological analysis (~5.77% of admitted patients). In case of B.C Roy Post Graduate Institute of Paediatric Sciences (BCRPGIPS), 796 specimens were collected (every 5th systematic sample from OPD patients- Monday to Friday) (~20% of total OPD patients). Type of diarrhea at presentation in IDH and BCRPGIPS were watery (84.7% vs. 74.5%), bloody (0.3% vs. 1.0%) and semi-solid (15.0% vs. 24.5%). In ID & BG Hospital 0.0% under-five children presented with severe dehydration and 100% with some dehydration. However, in B.C. Roy Hospital these values are 0% and 0.8% respectively.

In children below 5 years of age, isolation of rotavirus was 43.0% in ID Hospital whereas 31.8% in B. C. Roy Post Graduate Institute of Paediatric Sciences. *Vibrio cholerae* O1 (8.3%) and *Campylobacter* spp. (1.7%), *Vibrio fluvialis* (1.2%) were more in the IDH. In the BCRPGIPS, prevalence of adenovirus (6.0%), *C. jejuni* (5.5%), enteroaggregative *Escherichia coli* (1.2%) and *Shigella* spp. (5.2%) were high. Vibrios remained susceptible for most of the fluoroquinolones. In both the hospitals, most of the *Shigella* strains were highly resistant to fluoroquinolones but were susceptible for ceftriaxone and partially for azithromycin. NDM-type carbapenemase were detected in 27 strains of *V. fluvialis* strains isolated from 2015-2016. All these NDM-positive strains were susceptible to azithromycin. Weekly reports were sent to Govt. and other agencies for control and improvement of patients' care and treatment regime accordingly to the drug susceptibility patterns were suggested.

Title: A dietary metabolite, butyrate regulates TLR5 expression in the intestine through differential DNA binding of Sp1 and Sp3 transcription factors

Principal Investigator : S.S. Das

Toll-like receptor 5 (TLR5) expression in the intestinal epithelial cells (IECs) is critical to maintain health, as underscored by multiple immunological and metabolic disorders in mice genetically engineered for IEC-specific TLR5 knockout. However, the regulation of physiological TLR5 expression in the gut is incompletely understood. We showed that dietary fibre-derived butyrate, a short chain fatty acid, transcriptionally upregulates TLR5 in the IECs and augments flagellin-induced immune response. Both basal and butyrate-induced expression is regulated by differential binding of Sp1/Sp3 to the two GC-box sequences of TLR5 minimal promoter. Butyrate activates different protein kinase C isoforms, which dephosphorylate/acetylate Sp1 by serine/threonine phosphatases and phosphorylate Sp3 by ERK-MAPK. This resulted in the replacement of Sp1 by Sp3 at the TLR5 promoter, leading to enhanced p300 recruitment and histone acetylation, which activate transcription (Fig 18). The above findings may be exploited for the development of novel therapeutics against intestinal diseases targeting TLR5.

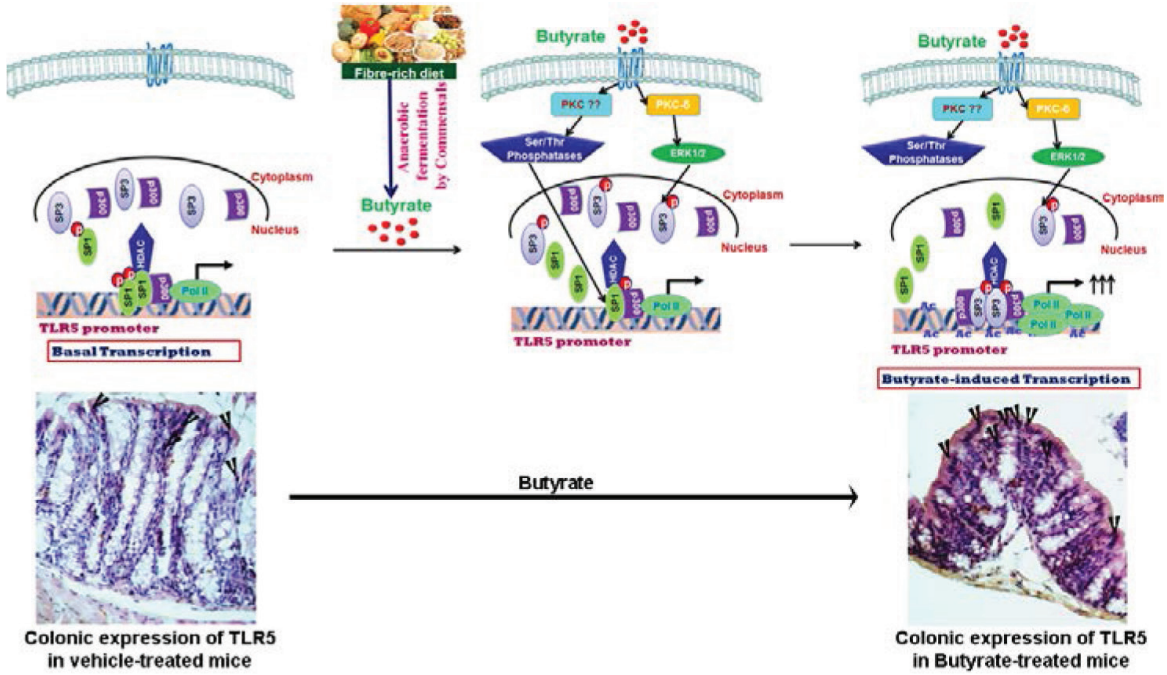


Fig 18 Mechanistic model representing the regulation of physiological expression of TLR5 in the colonic epithelial cells. The model shows that butyrate, a dietary metabolite transcriptionally induces the expression of TLR5 by regulating post-translational modifications and DNA binding of Sp1/Sp3 through the activation of serine/threonine phosphatases and ERK-MAPK by two different PKC isoforms

Title: Immunogenicity and protective efficacy of a candidate protein subunit vaccine against *Salmonella enterica* serovar Typhi (*S.* Typhi)

Principal Investigator : S.S. Das

Co Investigators : H. Koley, K.K. Banerjee

Infection caused by *S.* Typhi is a major threat to public health worldwide. The most widely-used typhoid vaccine in India, which is derived from Vi-polysaccharide fails to impart long-term protection and is poorly immunogenic in young children. We have shown that T2544, an outer membrane protein of S. Typhi, confers protective immune response in both mice and humans following immunization and natural infection, respectively. Immunization with purified T2544 induces humoral as well as cell-mediated immunity. Antibody-dependent cellular cytotoxicity (ADCC) assays showed effective lysis of *Salmonella*-infected EL4 cells opsonized with T2544-antibodies by NK cells. T2544-specific cytotoxic T lymphocytes (CTLs) were capable of lysing *Salmonella*-infected EL4 cells *in vitro* (Fig 19A) as well as in the immunized mice (Fig 19B). Effector and memory B cells against T2544 were detected in the immunized mice by ELISPOT assays (Fig 19C), in addition to the candidate vaccine being able to drive T cell proliferation (Fig 19D). In summary, T2544-based vaccine is a better alternative to the currently available Vi-polysaccharide vaccine.

Clinical Research: S. S. Das

Hospital-based surveillance of diarrhoeal diseases and enteric fever in children – Stool/rectal swab and/or blood is collected from the patients attending the OPD of Dr. B. C.Roy Post Graduate Institute of Paediatric Sciences Kolkata and sent to NICED laboratories for the diagnosis of 26 enteric pathogens including bacteria, viruses and protozoa.

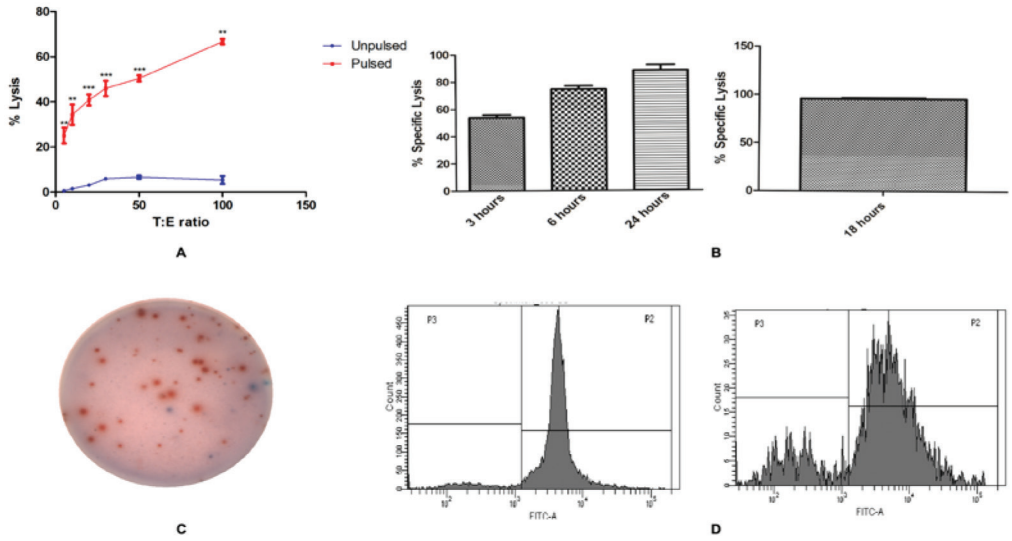


Fig 19 (A) ELISPOT assay showing T2544-specific B cells secreting IgA (blue spots) or IgG (red spots). (B) *In vitro* CTL assay: lysis of *S.* Typhi-infected EL4 cells by Cytotoxic T Lymphocytes (CTL) isolated from T2544-immunized mice. (C) *In vivo* CTL assay. (D) T cell proliferation assay: upon co-culturing with unpulsed (left) or T2544-pulsed (right) dendritic cells

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 didn't show presence of adenovirus. All the patients presented with convulsion of 2-3 episodes and were discharged within 5-6 days.

Awards/Honours Received

P. Indwar

- 3rd Prize in Fifteenth Foundation Training Programme for Scientists and Technologists Sponsored by Department of Science and Technology New Delhi.

Conferences/Seminars/Workshops/Trainings Attended/Organised

M.K. Bhattacharya

- Attended the Investigator Meeting for ROTA:03/12 study being conducted at Mumbai on 20-21, May 2015.
- Attended the 60th National Annual Conference of Indian Public Health Association (IPHACON 2016), Himalayan Institute of Medical Sciences, Dehradun - March 4th - 6th, 2016

S.S. Das

- Dr. S. Das delivered the Plenary lecture at the "New avenues in Microbiology and Biotechnology: Challenges and prospects" organized by the Department of Microbiology, West Bengal State University, Barasat on March 18-19, 2016.
- Dr. S. Das delivered an Invited lecture at the "Frontiers in Modern Biology-2015" organized by The Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Kolkata held on December 5-6, 2015.

- Sayan Das, Ph.D. student presented a poster at the 8th Indo-Global Summit and Expo on Vaccines, Therapeutics and Health Care held in Hyderabad on Nov 2nd to 4th, 2015. (Received Best Poster Award).
- Dr. S. Das delivered an Invited lecture at the Immunocon, 2015 (42nd Annnual Meeting of the Indian Immunology Society), held at Rajendra Memorial Research Institute of Medical Sciences, Patna on October 9-11, 2015.
- Dr. S. Das delivered Invited lecture at the Thematic Unit of Excellence on Computational Materials Science at S. N. Bose National Centre for Basic Sciences, Kolkata on September 29, 2015.
- Organized workshop entitled "Interactive session on medical informatics: to make most out of clinical data through computational approaches" at NICED on July 6, 2015 (attended by 25 participants).
- Organized workshop entitled "Molecular modelling & drug designing" at NICED on October 13-14, 2015 (attended by 12 participants).
- Organized workshop entitled "Application of Statistics in Clinical Research" on October 15-16, 2015 (attended by 22 participants).
- Dr. S. Das taught at the Refresher Course in Life Sciences for College and University Teachers held at the Academic Staff College, Calcutta University (Rajabajar Campus) on 8th April, 2015.
- Rimi Chowdhury, Ph.D. student attended the "Protein Interactions and Networks Workshop" organized by from Wellcome Trust, UK at Hinxton, UK (received travel support from Wellcome Trust and DST, India).
- Taught Medical Microbiology Paper to the Msc Microbiology (4th Semester) students of the University of Calcutta.

P. Indwar

- Attended the Fifteenth Foundation Training Programme for Scientist and Technologists Sponsored by Department of Science and Technology at Indian Institute of Public Administration New Delhi From 23rd Nov 2015 - 12th Feb 2016.
- Attended Global R&D Summit 2015 at Vigyan Bhawan New Delhi 7th - 8th Dec 2015.

DATA MANAGEMENT

Biostatistics involves the theory and application of statistical science to address public health problems and for further study on biomedical research. The department's research in statistical methods and interdisciplinary collaborations with other departments provides many opportunities of exploration of research data and its participation.

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

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

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 National hospital based Rotavirus surveillance network in eastern zone of India and Integrated Diseases Surveillance Project (IDSP) and international collaborators like International Vaccine Institute, Korea, and PATH vaccine solutions.

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

There is a future plan 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.

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

Scientists:

Dr. B. Manna, Scientist 'F'

Conferences/Seminars/Workshops/Trainings Attended/Organised

B. Manna

- Delivered a lecture on "Pathogen specific diarrhoeal diseases scenario: story from NICED" in scientific forum at NICED, Kolkata on 22nd June 2015
- Delivered a lecture on "Basic concept of research" for under graduate students of National Institute of Homeopathy on at NICED, Kolkata on 21st August 2015
- Delivered lecture on "Does vaccine coverage vary on oral vs. injectable vaccine? – Evidence from a large community based two clinical trials in Kolkata, India" in IPHCON-2016 at Dehradun, UP on 5th March, 2016.

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 JRF)

EPIDEMIOLOGY

The Epidemiology Division of the institute conducted clinical trials, undertook a number of community-based research studies with translation potentials and investigated disease outbreaks and important public health problems. Furthermore, the scientists of the division actively took part in health campaigns of national interest. The research activities included evaluation of a re-assortant pentavalent rotavirus vaccine, assessment of the prevalence of malnutrition and anemia among school children in West Bengal, understanding the interplay between health system responsiveness and care-seeking patterns for common childhood illnesses, and testing a training module to improve management practices of non-qualified care providers in the urban slums of Kolkata. The scientists also investigated the problem of astonishingly high infant mortality in the southernmost district of Saiha in Mizoram. In addition, they looked into one food-borne outbreak of diarrhea in North 24-Parganas district of West Bengal. The staff and scientists of the division also routinely conducted awareness programs and other activities under the "Swachh Bharat" program among the school children and residents in the slum areas of Kolkata. Moreover, in a festival in the Sundarbans area, NICED established a stall to apprise the rural population about the Council's activities of public health importance.

Scientists:

Dr. K. Sarkar, Scientist-F
Dr. S. Panda, Scientist-F
Dr. A. Deb, Scientist-E
Dr. S. Kanungo, Scientist-C
Dr. F. Debnath, Scientist-B

Staff:

Ms. S. Manna, Technical Officer- A
Mr. R. Saha, Technical Officer-A
Mr. C. Mandal, Technical Assistant
Mr. A. Chakraborty, Technician- C

Predoctoral Fellows:

Mr. T. Mahapatra

Title: Assessment of malnutrition & anaemia among primary & upper-primary school students of all districts of West Bengal

Investigators **:** K. Sarkar, S. Kanungo

The study revealed a state-wise prevalence of malnutrition of 26.1% & 19.6% in primary & upper-primary students respectively. Another 28.6% & 26.1% of the studied population were `At High Risk' of developing malnutrition (3rd to <10th percentile weight & height in respect to age & sex) among primary & upper-primary students. State-wide Ideal Nutritional status was 17% & 23.5% among primary & upper-primary students. Prevalence of anaemia was 29% & 19% among primary & upper-primary students. Malnutrition was higher in rural students than their urban counterpart and more in boys than girls. But prevalence of anaemia was higher among girls than boys. Problem of exclusive stunting was 6.9% & 5.7% and that of exclusive under-weight was 6.4% & 4.6% among primary and upper-primary students respectively. Both under-weight and stunting was present to the tune of 12.8% & 9.3% in primary & upper-primary students respectively. The study revealed that state-wide prevalence of malnutrition was 26.1% & 19.6% in primary & upper-primary students respectively. Another 28.6% & 26.1% of the studied population were `At High Risk' of developing

malnutrition (3rd to <10th percentile weight & height in respect to age & sex) among primary & upper-primary students. State-wide Ideal Nutritional status was 17% & 23.5% among primary & upper-primary students. Prevalence of anaemia was 29% & 19% among primary & upper-primary students. Malnutrition was higher in rural students than their urban counterpart and more in boys than girls. But prevalence of anaemia was higher among girls than boys. Problem of exclusive stunting was 6.9% & 5.7% and that of exclusive under-weight was 6.4% & 4.6% among primary and upper-primary students respectively. Both under-weight and stunting was present in the tune of 12.8% & 9.3% in primary & upper-primary students respectively. (Fig 20)

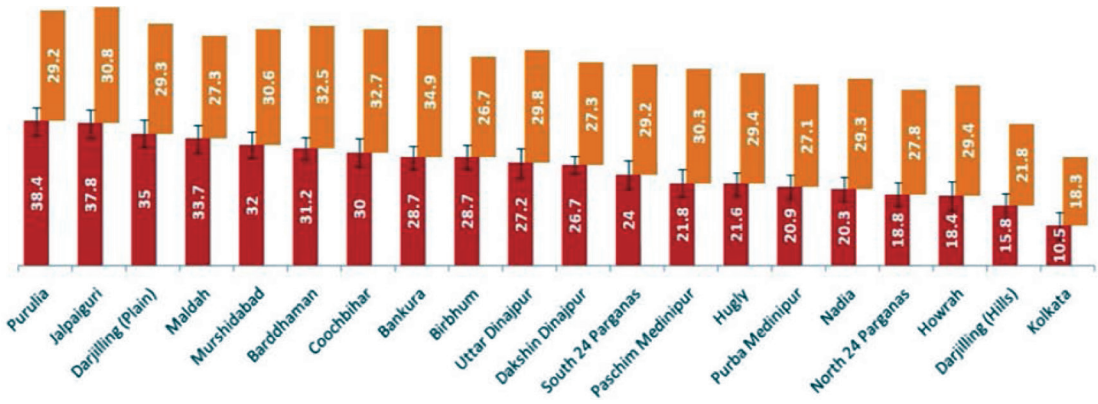


Fig 20 District-wise malnutrition (bottom bar) & at high risk (top bar) scenario of primary students

Following above, a series of Dissemination workshops were carried out to sensitize the district machineries to improve the quality of Cooked Mid Day Meal Programme activities. Sixteen out of twenty districts of West Bengal was already covered in collaboration with District authorities.

Title: High Infant mortality in southern district of Mizoram – a field investigation by NICED

Investigators : S. Panda, S. Dutta, A.K. Deb, M. K. Chakrabarti, Director-in-charge NICED; C. Hnichho, H. Thapi Phosa, K. Rakhu, S. Lalfakawma Fanai, District Health Services, Saiha Mizoram

NICED conducted a community based investigation in Saiha, the southernmost district of Mizoram, at the behest of the Indian Council of Medical Research, undersecretaries of Government of India and Honorable member of Rajya Sabha to investigate factors associated with high infant mortality in the district. The infant mortality rate recorded in the district during 2014-2015 (113 per 1000 live birth) was 3 times higher than the estimated rate for the whole State and the Country. Dr Samiran Panda served as the leader of the investigating team and organized community consultation engaging district health officials, local youths and staff of the National Health Mission (NHM). In the process a socio-culturally appropriate investigation tool was developed. While Dr. Alok K Deb, Dr. Shanta Dutta, and Dr. Manoj Chakrabarti from NICED played various roles in the team, Dr. Chhaihlo Hnichho, Dr. Hli Thapi Phosa, Dr. Khaila Rakhu, Dr. Samuel Lalfakawma Fanai, from the District Health Services of Saiha, Mizoram and Ms. Mary Vanlalpeki from the NHM, Saiha constituted crucial technical workforce. Two proximate attributes of infant deaths namely 'low birth weight of children', and 'raw areca nut (*kuhva*) intake by mothers while pregnant' were identified through this investigation. Another factor associated with infant death was 'child delivery at home'. The report generated by NICED was well accepted by the State level stakeholders as well as the Council and indicated future course of interventions.

Community consultation

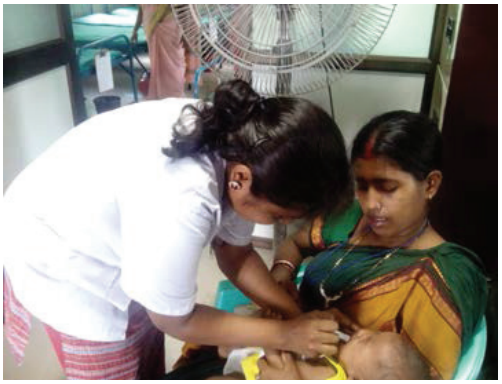
Title: Phase III, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Live Attenuated Bovine-Human Rotavirus Reassortant Pentavalent Vaccine (BRV-PV) Against Severe Rotavirus Gastroenteritis in Healthy Indian Infants

Principal Investigator : S Kanungo

Co-Investigators **:** M.K. Bhattacharya, B. Manna, A.K. Mukhopadhyay, R.K. Nandy, M. Chawla Sarkar, NICED; D Paul, Professor, and MSVP, BC Roy Post Graduate Institute of Paediatric Sciences, Kolkata

The Serum Institute of India, developed the live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine for oral vaccination against human rotavirus gastroenteritis in healthy infants, to evaluate vaccine efficacy in 2014. Infants in several regions of India who were representatives of different demographic, climatic and sociocultural factors were enrolled. Total sample size was 7500 and vaccine and placebo allocation ratio was 1:1. NICED is a part of this large multi centric phase III trial which aimed to evaluate the safety and efficacy of the pentavalent vaccine developed by Serum Institute of India. The work was initiated in Aug 2014. 700 children aged below 6 weeks were recruited and dosed after consenting as per DCGI norms. The dosing was completed in March 2016.

Post dosing surveillance is ongoing at field level to detect the gastroenteritis cases and other adverse events at the earliest. Field clinics were set up since initiation of the projects, manned by physicians and health workers.



Subject receiving intervention in study clinic

Subjects being followed up in clinic

All the other adverse events other than diarrhoea were also identified, treated and documented as per protocol. The diseased subjects are followed up till the resolution of the episode. The serious adverse events that occurred till date have been managed and reported to the regulatory authorities within stipulated timeline. Within this period (1st April 2015 to 31st Mar 2016) total of 1038 cases of Acute Gastroenteritis / diarrhoeal episodes were detected. A total of 510 subjects (73%) suffered from gastroenteritis in their first year of life. The study is ongoing.

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 main objective of the study was to assess the impact of a multi-component intervention in improving non-qualified practitioners' diarrhea related knowledge and practice to provide appropriate clinical management of diarrhea in urban slums of Kolkata. A multi-component educational intervention was designed to improve the knowledge of non-qualified allopathic practitioners of the urban slums of six administrative wards of Kolkata municipal area, 140 such practitioners who were chosen randomly from an exhaustive list of practitioners in the study area and interviewed at baseline, were provided with six modules of educational materials on symptoms and spread of diarrheal diseases, cholera (disease as a whole), prevention and management of diarrheal diseases along with oral rehydration solution 6 months' time span. After a gap of 2 months between February and April, 2015, 124 practitioners (16 were lost to follow-up due to migration out, death, sickness etc.) who were available for the post-intervention interview were administered the same questionnaire. Mean overall and domain specific knowledge scores for spread, management of diarrhea and ORS increased significantly (Fig 21) (at α=0.05) after intervention compared to baseline. Intervention was associated with significant betterment of knowledge regarding diarrheal spread and management, ORS and overall knowledge [a OR=4.31(2.63-7.07); p<.0001]. Based on stratified analyses, intervention seemed to work better among those practitioners who were practising for 10 years or more [OR=3.15(1.85-5.36)], those having no prior training [OR=4.75(2.24-10.05)] and pharmacists [OR=8.27(3.33-20.52)].

Now field activities were on to evaluate the retention of knowledge after six months and exit interviews are ongoing among the patients attending these practitioners.

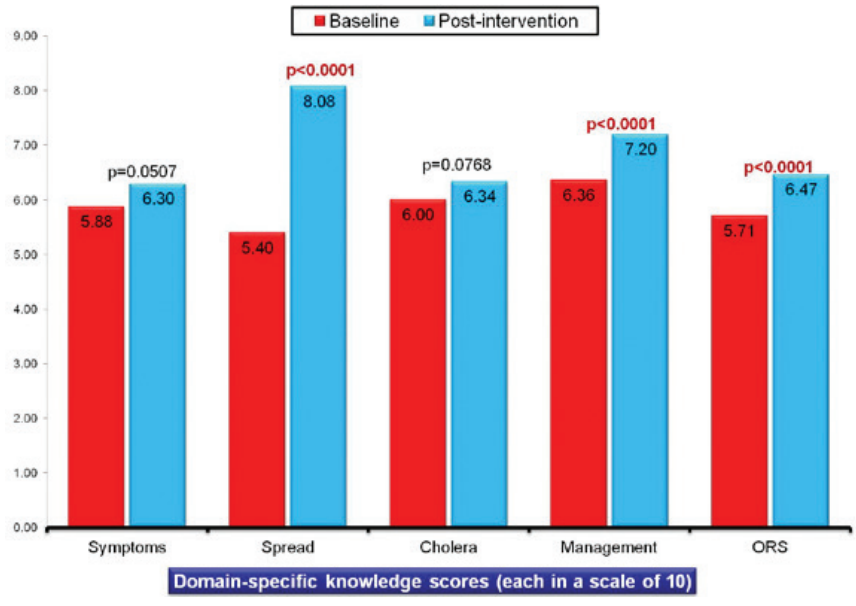


Fig 21 Role of the intervention in improving knowledge of diarrhea among practitioners of urban slum of Kolkata

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 is an ongoing intramural work, with 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 behaviour of caregivers regarding ailments. Exploration of possible interrelationship among these estimates and their variations across the strata of socio-demographic aspects among under-5 children is also planned. The study has got two components, quantitative as well qualitative one.

Using open-ended guidelines (customized adoption from WHO) 3 Focus Group Discussions (FGD, one in each ward) were conducted in ward no. 58, 59 and 66, involving five eligible consenting adult caregivers of under-5 children of each age-strata (0-11, 12-23, 24-59 months) randomly selected from the initially visited 50 households for each focus group discussion. It showed that most important factor for the selection of practitioners (whether qualified or non-qualified or pharmacists), availability of the practitioners and cost of treatment (especially the fees of the physicians) (Table 6). Severity of the ailment was the most common reason of visiting a qualified practitioner. Cost, waiting time and non-welcome behaviour were the most common barriers for visiting the qualified practitioners for each ailment. Lack of attention (self-perceived) and waiting time were the experienced problems in Government sector. A cross-sectional study was carried out among the caregivers of under-five children where 345 subjects were interviewed in each age strata (0-11, 12-23 & 24-59 months) three times within a year. It showed that fever accompanied by cough and cold was the most commonly perceived morbidity among participating under-five kids followed by diarrhea. While more than 30% visited non-qualified practitioners for the ailment of their under-5 kids only 20% sought care in the Government hospitals (Fig 22). The study is ongoing

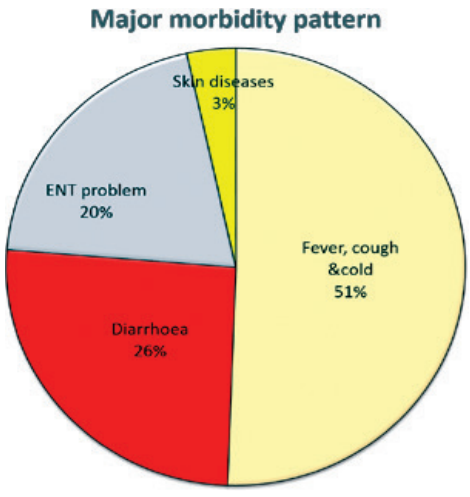


Fig 22 Morbidity pattern among under-five children as perceived by their caregivers in urban slums of Kolkata

Table 6 Health-seeking pattern among participating caregivers of under-five children in the urban slums of Kolkata

Variables	Categories	Frequency (n = 903)	Percentage
Health-care seeking	Qualifies Doctor's chamber (not in a hospital)	407	45.07
	Non-Qualified Doctor's chamber (not in a hospital/pharmacy)	276	30.56
	Government Hospital	183	20.27
	Private Hospital/Norsing Home	25	2.77

Title: Measuring competency of the peripheral health workers in detection & management of common syndromic conditions: A cross-sectional study in North 24 Parganas district of West Bengal, India

Principal Investigator : F. Debnath

Background

In this study, we measured competency level of the peripheral female health workers for detection and management of diarrhoea, acute respiratory tract infection, fever, malaria and factors associated with inadequate competency in North 24 Parganas district of West Bengal, India through an analytical cross sectional study as hardly any information is yet available.

Methods

We sampled 272 peripheral female health workers from a population of 738 through simple random sampling method. Four validated clinical vignettes and structured questionnaire were used for data collection. We calculated overall competence score (%) by combining marks obtained in all four conditions and described in quartiles. We also calculated 95% Confidence interval [CI] for proportions. Overall competence score (%) ≥ 75% was defined as adequate competency and < 75% as inadequate competency. We calculated crude and adjusted odds ratio with 95% CI to determine independent factors associated with inadequate competency. Approval from Institutional Human Ethics Committee of National Institute of Epidemiology, India was obtained.

Result

Combining all four conditions, 68% (185) of 272 study participants scored ≥ 75% (95% CI, 62% - 74%). In detection and management of acute respiratory tract infection and fever, 43% and 46% scored ≥ 75%. Factors found independently associated with inadequate competency were experiencing a stock out of 2 to 3 drugs in last month (Adjusted OR 1.9; 95% CI, 1.1 - 3.5), received training in IMNCI ever (AOR 2.42; 95% CI, 1.43 –4.12).

Conclusion

More than two thirds of the peripheral female health workers of North 24 Parganas were adequately competent to detect and manage said conditions. Ensuring uninterrupted drug availability at health sub center, quality in service trainings is important for being competent in detection and management.

Title: Dengue fever investigation at Baranagar Municipality of North 24 Parganas, West Bengal, 2015

Investigator : F. Debnath

Background

In November 2015, a death due to fever and increased number of fever cases were reported from Baranagar Municipality of North 24 Parganas district of West Bengal, India. We investigated the outbreak with the following objectives: to confirm existence of an outbreak, to describe it in terms of time, place and person, to determine the cause of outbreak and to recommend control measures.

Methods

We collected information on number of dengue cases and deaths for the year 2012 – 14 from district public health office. We collected information on date of onset, serological reports, outcome of all the confirmed dengue cases from January to November'15. We also collected information on date of onset, clinical features, contact history, awareness related to dengue fever and its mode of spread of all the suspected dengue cases

during our active case search survey during December 2015. We sent the blood samples of suspect dengue cases for serological confirmation. We did environmental and entomological surveys.

Results

671 cases were reported from 14th of September 2015 till 16th of December 2015. After that no more cases were reported. Out of these, 54% (363) were female and the attack rate was 3/1000 population. Attack rate in the age group of five years and less was 1/1000 population, among 17 to 40 years of age group was 3/1000 population and in the age group of > 60 years it was 2/1000. 94% had headache, 90% had myalgia, 64% had arthralgia, 45% had body rash, 52% experienced retro-orbital pain, 39% had abdominal pain, one patient had loose stool. Nobody experienced bleeding from any site of the body. Case fatality was 3/1000 dengue cases. 612 cases were positive for Non Structural Antigen 1 ELISA test (NS1 ELISA). House Index, Container Index, and Breteau Index in ward no 1 was 6%, 3% and 7% respectively. In ward no 4, House Index, Container Index, and Breteau Index was 3%, 1% and 3% respectively. In ward 27, House Index, Container Index, and Breteau Index was 4%, 2% and 4% respectively.

Conclusion

The fever outbreak in Baranagar was probably due to dengue virus infection.

Conferences/Seminars/Workshops/Trainings Attended/Organised

K. Sarkar

- Attended Indian Railway Public Health Conference, held at Chittaranjan, Burdwan, West Bengal, on 22 January 2016 and presented our work titled `Malnutrition scenario of school children of West Bengal' as oral presentation.
- Following completion of district-wise assessment of malnutrition & anaemia among primary and upper-primary school students, it was planned to conduct a series of workshops at all district to sensitize the district authority and various other stake holders about existing situation of malnutrition in school children and actions to be taken for improving their malnutrition scenario.

Objectives of the workshop:

1. To sensitize the District authority & other stake holders of Mid Day Meal Programme about existing scenario of malnutrition and various dimensions of it.
2. To sensitize the authority of its existing water & sanitation status in various districts.
3. Necessary actions to be taken for improvement of malnutrition scenario by strengthening school meal programme including health component of it

NICED conducted series of one-day workshops at 16 districts to sensitize the district authorities and other relevant personnel involved in implementing school meal programme. District authority such as District Magistrate, Additional District Magistrate, SDOs, BDOs, Mid Day Meal supervisors etc. and school teachers (involved in Mid Day Meal Programme) participated at this workshop. Details of existing scenario of malnutrition status & its influencing factors were explained through oral presentation & face to face interaction. An audio-visual presentation (based on the study conducted by NICED) was made & used for this purpose. Future activities for further improvement of nutritional scenario were also discussed at length. Existing scenario of health and water & sanitation status of district schools was also presented and emphasis was given for its improvement. Need for training of school teachers for early detection of malnutrition by physical anthropometry and establishment of nutrition surveillance system involving district schools were also suggested where NICED could play its role as a coordinating institute.

List of districts where one-day Dissemination Workshop on Malnutrition were already conducted

Sl. No.	Districts	Held on	Remarks
1	Kolkata	13-Aug 2015	Well attended workshop
2	North 24 Parganas	24-Aug 2015	Well attended workshop
3	Howrah	25-Aug 2015	Well attended workshop
4	Birbhum	28-Aug 2015	Well attended workshop
5	Nadia	1-Sep 2015	Well attended workshop
6	South 24 Parganas	4-Sep 2015	Well attended workshop
7	Murshidabad	16-Sep2015	Well attended workshop
8	Jalpaiguri	29-Sep2015	Well attended workshop
9	Alipurduar	29-Sep 2015	Well attended workshop
10	Coochbehar	30-Sep 2015	Well attended workshop
11	Hooghly	5-Oct 2015	Well attended workshop
12	Burdwan	9-Oct 2015	Well attended workshop
13	North Dinajpur	24-Nov 2015	Well attended workshop
14	Malda	26-Nov 2015	Well attended workshop
15	Siliguri	8-Dec 2015	Well attended workshop
16	Darjeeling Town	9-Dec 2015	Well attended workshop
17	Bankura	11-Dec 2015	Well attended workshop

A Deb

- Attended the NACO NDAP Dissemination Workshop organized by NACO / Population Council in New Delhi on September 30, 2015.
- Attended the Dissemination Workshop of Reports from National IBBS and HIV Sentinel Surveillance 2014-15 organized by NACO in New Delhi on February 9, 2016.
- Attended Pharmacovigilence Seminar organized by NIPER, Kolkata at NICED on February 22, 2016.

S. Kanungo

- As faculty and oral presentation on Current Epidemiology of Cholera & disease burden in Indian Subcontinent" at the 4th Meeting of the Initiative against Diarrhoeal and Enteric diseases in Asia (IDEA) at New Delhi India on 30 March -2nd April 2015
- Participated and represented the institute in Sundarban Kristi Mela o Loko Sanskiti Utsab held on 20-29th Dec 2015, in Kultali, South 24 Parganas and disseminated horizon of work of the institute and explained the importance of cleanliness, personal hygiene in prevention of diarrhea and cholera
- Poster Presentation titled Cholera vaccination in endemic areas: time to redefine dosing interval" at the "Cholera Panel Meeting" under United States-Japan (US-Japan) Cooperative Medical Sciences Program (CMSP) 50th anniversary celebration and 18th International Conference on "Emerging Infectious Diseases (EID) in the Pacific Rim" held from 11th - 15th January 2016 at Bethesda, MD, USA, invited by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH).
- Oral Presentation titled "Disease impact of rotavirus in India: Current Updates and nuances of vaccination " at the 60th National Annual Conference of Indian Public Health Association, Dehradun from 4th-6th March 2016
- As resource person, deliberation of oral lecture as resource on "Ebola Virus Disease (EVD)- Global Concern" at the Dept. of Physiology, University of Calcutta, India in refresher course in Life Sciences (thrust area : Integrative Life Sciences : The Destiny), on 6th April 2015.

IMMUNOLOGY

The intestinal epithelium covering the luminal surface is continually exposed to commensal bacteria. Although the commensals are not immunological "self", the intestinal immune system tolerates them resulting in a symbiotic relationship. Reduction of inflammatory reactions by mechanisms including down-regulation of TLRs is adopted for maintenance of the healthy intestine. The homeostasis of the intestinal niche is transiently altered by pathogenic bacteria such as Shigella dysenteriae type 1 or enterohaemorrhagic *Escherichia coli* that penetrate the colonic epithelial cells causing local infection before spreading. Coordinated effort of the epithelial cells equipped with pattern recognition receptors that include toll-like receptors (TLRs) and mucosal immune system is required for exclusion of the invading bacteria. Porin and LPS of Gram-negative bacteria are the two major constituents of the outer membrane to which convalescent Abs are generated in humans after infection. Porins have been categorized as ligands primarily for TLR2, while LPS is recognized by TLR4 and its co-receptor MD-2. The outer membrane pore-forming proteins co-express TLR1 or TLR6 with TLR2 on APCs for bridging innate signaling with adaptive immune responses. Porins are surface exposed, strongly immunogenic and antigenically related, the features make them ideal to investigate frontline defense during host-pathogen interactions. Signaling initiated by TLRs are extremely important to display the adjuvant action of a molecule such as porin to boost the host defense. Study of immunopotentiating activity of porin showed the protein skew adaptive immune response through NF-κB expressing chemokines and type 1 cytokines. Porin-pulsed antigen-presenting cells (APCs) proliferates T cells leading to transition of naïve T cells into effector Th1 cells. Since TLR activation may lead to spontaneous autoimmunity, it is necessary for an adjuvant to adjust the responses for proper immunity. However, TLR-ligands are frequently chosen as candidates for vaccine or adjuvant development because they can robustly coordinate innate signaling and adaptive immune responses. Adjuvant action of porin was noticed to drive systemic immunity, which is considered a benchmark for a successful adjuvant. Our work shows porin differentially regulated splenic marginal zone (MZ) and follicular zone (FO) B cell responses. The protein up-regulated TLR2 and -6 and stimulated the activation and co-stimulatory molecules on FO B cells skewing the cells toward TLR-dependent type-1 cytokine response. In contrast, porin could not up-regulate the TLRs and activate MZ B cells. These cells responded to porin by expressing toll-interacting protein (TOLLIP), the TLR2 and -4 signaling inhibitor along with stimulation of the intracellular pathogen recognition receptor NLR caspase recruitment domain containing protein 5 (NLRC5). The $CD1d^{hi}$ MZ B cells released IL-10 unequivocally demonstrating regulatory B cell feature. Immunization with porin also resulted in transient IL-10 expression by the $CD19^{+}CD21^{hi}$ B cells prior to plasma cell formation. Moreover, the plasma cells developed from the B-2 cell subsets show marked variation in generation of immunoglobulin subclasses. Our work highlights porin, a TLR2 and -6 specific immunogen can trigger variation in immune response, the inter-connectedness of which governs the ultimate successful outcome. Porin shows the diverse but decisive role it can play by distinctly modulating MZ and FO B cell responses both *in vitro* and *in vivo*. The adjuvant action of the protein supports on one hand plasma cell and Ab repertoire formation and on the other hand adjusts the responses through counter regulation. These observations will be helpful to understand the complexity of multi-faceted responses generated by the immune system not only as a measure to counter PAMP but also for providing heightened immunity inclusive of checks and controls. Next, a cell culture system was developed to generate innate memory $CD8^{+}$ T cells from $CD4^{+}CD8^{+}$ double positive thymocytes that differentially recognize pathogen-associated and danger associated molecules with promise of therapeutic use particularly during sepsis.

Scientist:

Dr. T. Biswas, Scientist G

Research Scientist

Dr. R. Biswas

Staff:

Mr. S.K. Shaw, Technician-B

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

Pre-Doctoral Fellows:

Ms. Debolina Sinha, UGC-SRF

Mr. Amlan Kanti Ghosh, UGC-SRF

Title: Cytokine Regulation of Porin Stimulated B-1a Cell

Principal Investigator : T. Biswas

Porin-treatment of B-1a cells resulted in up-regulation of the key type 1 cytokine IL-12 with IL-10 remaining unaffected indicating switching towards proinflammatory response. Further, we determined whether porin could actually reprogram the naturally IL-10-producing B-1a cells for an inflammatory phenotype. Therefore, we determined the secreted levels of representative T_h1 cytokines. Quantification of an array of proinflammatory cytokines by ELISA from five-day-old culture supernatants showed 145, 138 and 40 pg release of IL-12 p70, IFN-γ and TNF-α, respectively per million of porin-treated B-1a cells (Fig. 23). We have found TLR2 as the primary pattern recognition receptor that associates with TLR6 upon porin stimulation. Knocking down of the key TLR, TLR2 by using antisense oligonucleotides depleted IL-12, IFN-γ and TNF-α by 90, 92 and 72%, respectively, as compared to the non-transfected cells (Fig. 23).

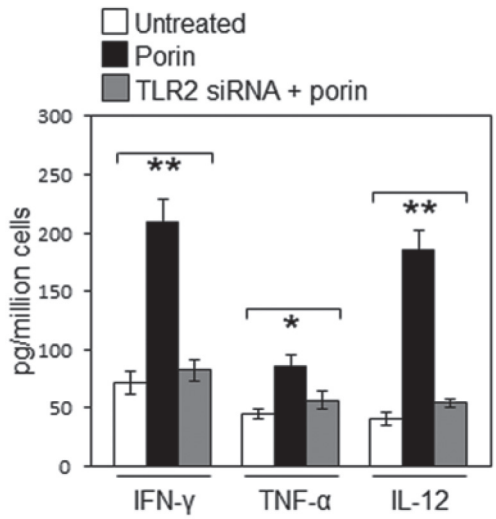


Fig 23 Quantification of porin-induced cytokine release of B-1a cells by ELISA. B-1a cells were treated with and without porin, or pre-incubated with TLR2 siRNA prior to addition of porin. The cell-free supernatants were analyzed for IFN-γ, TNF-α and IL-12 after 5-day of culture. Data represent mean ± SEM of six independent experiments, each done in triplicate. *p<0.05, **p<0.005

Title: Generation of Culture-differentiated Innate Memory CD8 Cells with Toll-like Receptor Expression and Responsiveness to Pathogen/Danger-associated Molecules

Principal Investigator : T. Biswas

Nonconventional innate memory $CD44^+CD122^+Eomes^+PLZF^+CD8^+$ T cells were derived in culture from $CD4^+CD8^+$ double positive (DP) thymocytes of normal BALB/c and C57BL/6 mice. These culture-differentiated cells express toll-like receptor (TLR)4 and release interferon (IFN)-γ and interleukin (IL)-10 under steady-state

conditions. We showed that the TLR4-ligand lipopolysaccharide (LPS) stimulates the TLR and up-regulates IFN-γ skewing the cells toward type 1 polarization. In presence of LPS these cells also express suppressor of cytokine signalling (SOCS)1 and thus suppress IL-10 expression (Fig. 24).

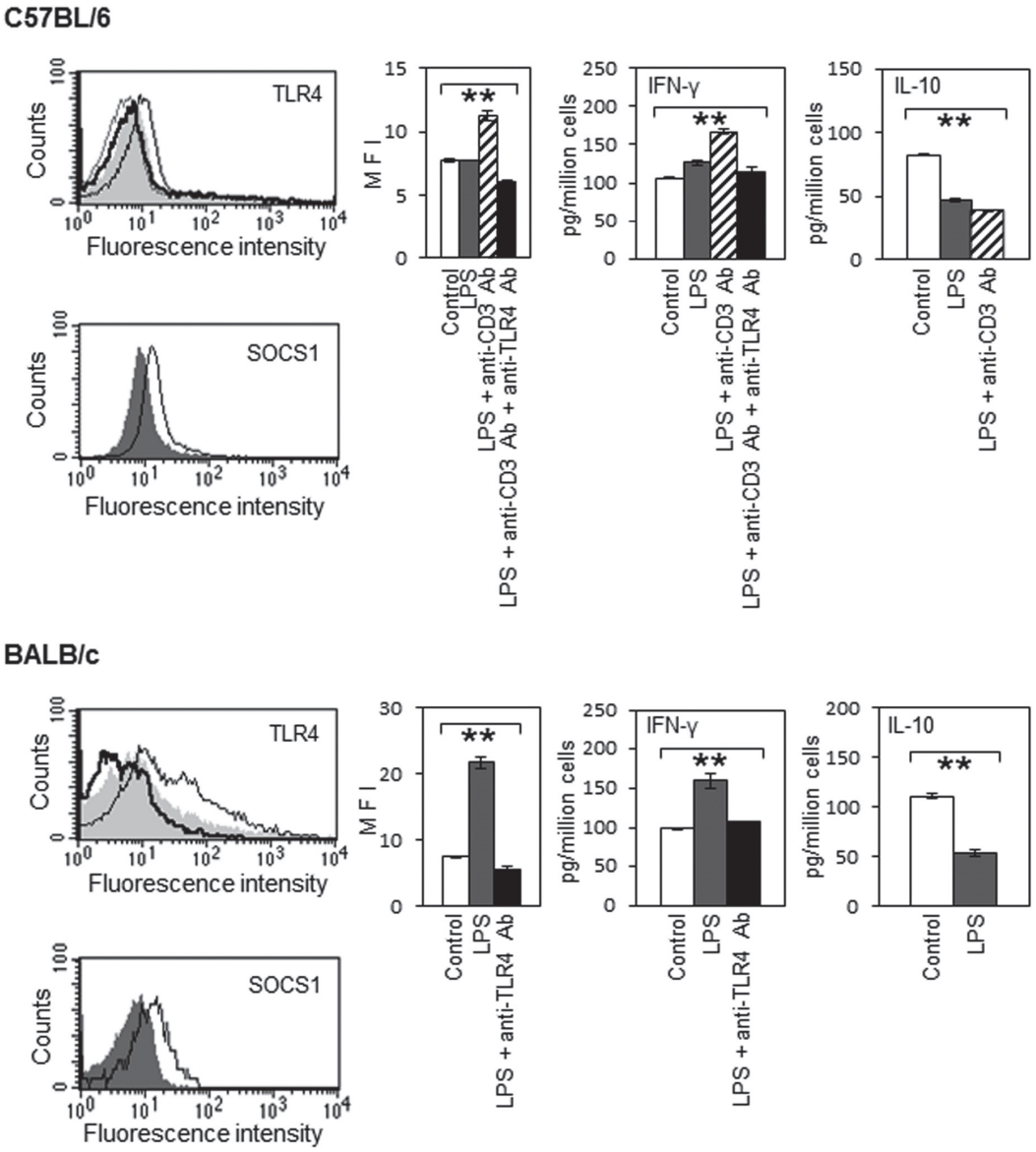


Fig 24 Effect of LPS on TLR4, IFN-γ, IL-10 and SOCS1 expression by culture-differentiated $CD8^+$ T cells. TLR4 expression in untreated (shaded), LPS (gray line), LPS plus anti-CD3 mAb (thin black line) and LPS plus anti-CD3 mAb with anti-TLR4 Ab (thick black line) treated cells from C57BL/6 mice is shown. Similarly, stimulation of TLR4 in untreated (shaded), LPS (thin black line) and LPS plus anti-TLR4 Ab (thick black line) treated cells from BALB/c mice is demonstrated. LPS-induced SOCS1 expression (black line) over untreated control (shaded) is indicated in both mice strains. The release of IFN-γ and IL-10 in presence and absence of LPS were quantified by ELISA at 72 h. The cells from C57BL/6 mice were cultured in anti-CD3 mAb coated plates. The data shown are representative of three independent experiments. The bar diagrams show mean ± SEM of three separate experiments. $^{**}p<0.005$

Conferences/Seminars/Workshops/Trainings Attended/Organised

T. Biswas

- Adjudicated Ph. D thesis & viva voce at PGIMER, Chandigarh (2015).
- Immunology theory question paper setter for M.Sc. examination at the Department of Human Physiology and Community Health, Vidyasagar University (2015).

PARASITOLOGY

Parasitic infections continue to be a serious health problem throughout the globe. 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 Helminths (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 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 New TRICOMBO Copro ELISA kit for simultaneous detection of three parasites. The Division of Parasitology was able to perform 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

Post Doctoral Fellows:

Dr. Prasanta Saini

Pre Doctoral Fellows:

Mr. Dibyendu Raj (SRF),

Mr. Sumallya Karmakar (SRF),

Ms. Rituparna Sarkar (JRF)

Ph D Awarded:

Dr. Avik Kumar Mukherjee received Ph D. from Jadavpur University

Title of the thesis: Characterization of local isolates of common enteric parasites in Kolkata with special reference to *Giardia lamblia*

Dr. Koushik Das received Ph D. from Calcutta University

Title of the thesis: Characterization of local isolates of *Entamoeba histolytica* in India and their pathogenic variability.

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

Principal Investigator : S. Ganguly

The pathogenesis of intestinal infections with *Giardia lamblia* remains elusive (Roxstrom-Lindquist *et al.*, 2006). This protozoan parasite is not invasive and no conventional toxin has been identified (Morrison HG *et al*., 2007). Any mechanism that supports its ability to remain in the host small intestine or to resist host defenses may be viewed as a virulence factor. Here our present data showing release of some *Giardia* enzymes, previously only characterized as being involved in the intracellular energy metabolism of *Giardia*.

Several previous studies have searched for secreted proteins correlated to disease and colonization by *G. lamblia*. The VSPs that cover the entire trophozoite surface (Nash TE *et al*., 2002), were originally identified as spontaneous excretory–secretory proteins (Nash TE *et al*., 1983; Nash TE *et al*., 1985). A 58 kDa nonVSP protein, reported to localize to the surface of *G. lamblia* P-1 strain (Kaur H *et al*., 2001), was found in the culture supernatant of trophozoites incubated in serum-free medium (Kaur H *et al*., 2001; Shant J *et al*.,

2002). Moreover, several unknown proteins with masses ranging from 15 to 225 kDa as well as un-identified proteins with cysteine-type protease activity have been reported in supernatants of *Giardia* -host cell co-cultures (Rodriguez-Fuentes GB *et al*., 2006; Jimenez JC *et al*., 2000; Jimenez JC *et al*., 2000).

This is the first time giardial ADI have been shown to be present in Giardia culture supernatants (Fig 25). These typically cytoplasmic proteins have been found to be surface-exposed or secreted in other organisms. We were not able to detect any signal sequences in the proteins but now there are many examples of secreted proteins in eukaryotes without signal sequences (Nickel W *et al*., 2005). Arginine deiminase, is involved in arginine metabolic pathway (Table 7). This is an unusual, bacterial-like pathway, not present in human host cells. Trophozoites use ADI to actively metabolize arginine for energy, thus depleting arginine from the growth medium. Arginine depletion is known to induce apoptosis in human cell lines (Philip R *et al*., 2003) and human giardiasis patients show an increased rate of apoptosis of intestinal epithelial cells (Troeger H *et al*., 2007). This has been suggested to be a major disease mechanism (Buret AG, 2007). Further experiments will show if ADI is involved in this induction of apoptosis.

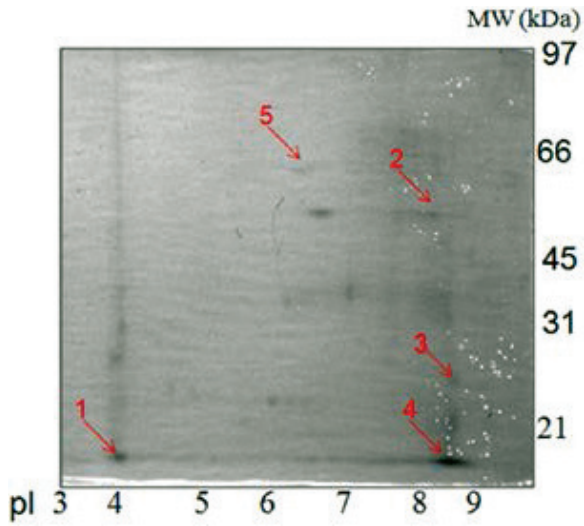


Fig 25 Precipitated culture supernatant proteins from 30 min incubations of *Giardia lamblia* under H_2O_2 stress. Identified *Giardia* proteins are indicated by 1-5 and the identities of the proteins are described in Table 1. Unmarked spots failed to be identified. The figure shows representative silver stained 2D electrophoresis gel. However, identification was performed from coomassie stained gel.

Table 6 Health-seeking pattern among participating caregivers of under-five children in the urban slums of Kolkata

Putative protein name	Spot no.	pI	MW (kDa)	Expect value	Identity (accession no.)
Hypothetical protein	1	4.2	9.3	9.5E-0.6	GLP15_1196
Hypothetical protein	2	9.6	59.5	2.4E-10	GL50803_3925
Hypothetical protein	3	8.3	24.4	1.6E-12	DHA2_5161
Hypothetical protein	4	8.0	12.4	3.9E-07	GL50803_39094
Arginine deiminase	5	6.2	66	8.5E-06	Gl50803_112103

Many intracellular pathogens are killed by NO, but the role of NO in controlling infections of extracellular pathogens is not well established (Eckmann L *et al*., 2002). Interestingly, the NO levels in intestinal epithelial cells have also been shown to be important in the regulation of adsorption/secretion of water (Kukuruzovic R *et al*., 2002), suggesting that it could be associated with symptoms of giardiasis. The secreted ADI of *Giardia* might reduce the levels of intestinal arginine further and lower the NO production by IECs. In support of this hypothesis, recombinant mycoplasmal ADI reduced NO production in human cells (Dillon BJ *et al*., 2002; Noh EJ *et al*., 2002). Therefore, arginine consumption and NO reduction define a novel cross talk between Giardia as a non-invasive pathogen and the host intestinal epithelium.

Title: Differential pathogenesis of Giardia: Role of Giardia virus

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.

Array analysis and RT PCR validation

The hybridized slides were scanned using ScanArray® software in the scanner and more than 200 clones were identified that show 2 folds or higher times up regulation or down regulation than the control set. The result was cross checked twice and also by dye swapping. The clone numbers that matched in all the cases have been chosen for further sequencing analysis. From the array analysis some interesting gene candidates were selected for Real time PCR validation (Fig. 26A, 26B, 26C). Transcriptional regulation due to stress shows that it affects the parasite cell massively and can change several physiological activities. This has helped to generate basic knowledge about some pathways controlling the survival and evolution of this human enteric parasite.

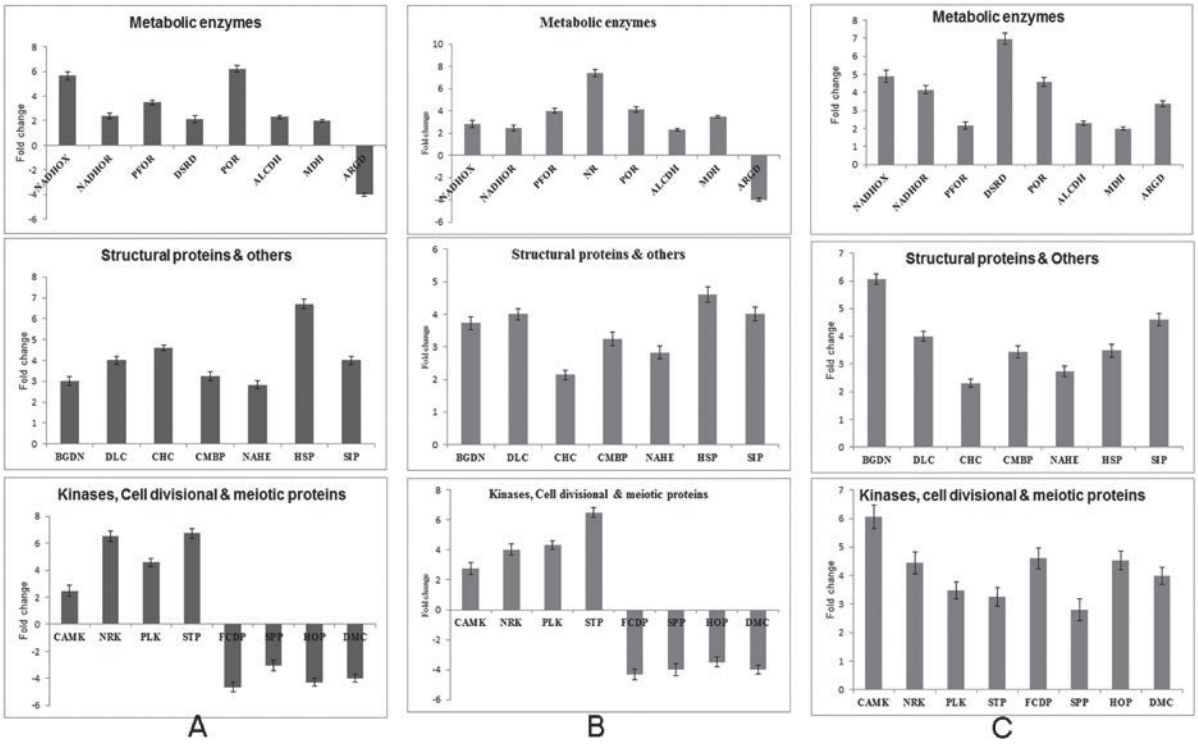


Fig 26 Real time PCR graphs showing differential gene expressions under different modes of oxidative stresses. A. Gene expression (fold change) under H_2O_2 stress. **B.** Gene expression under metronidazole treatment. **C.** Gene expression during modified medium treatment. (Gene abbreviation used: **Metabolic enzymes:** NADHOX – NADH oxidase, NADHOR – NADH oxidoreductase, PFOR – Pyruvate-ferredoxin oxidoreductase, DSRD – Disulfide reductase, NR – Nitroreductase, POR – Peroxiredoxin, ALCDH – Alcohol dehydrogenase, MALDH – Malate dehydrogenase, ARGD – Arginine dihydrolase. **Structural proteins & others:** BGDN – β giardin, DLC – Dynein light chain, CHC – Clathrin light chain, CMBP – Cysteine rich membrane binding protein, NAHE – Na-H exchanger, HSP – Heat shock protein, SIP – Stress induced phosphoprotein. **Kinases, cell divisional & meiotic proteins:** CAMK – CAM kinase, PLK – Polo-like kinase, NRK – NIMA related kinase, STP – Serine threonine phosphatase, FCDP – Fts-J cell divisional protein, SPP – Spindle pole protein, HOP – Homologous pairing, DMC – Disrupted meiotic protein.

Mode of cell death

It was hypothesized that the origin of eukaryotic programmed cell death is a consequence of aerobic metabolism (Frade *et al*, 1997). In the previous paragraph, a hypothesis by Blackstone *et al*, 1999 was discussed partly. According to them, in metazoans, the mechanism of apoptosis involving Cytochrome c may be a vestige of the process where programmed cell death is triggered instead of sexual reproduction. In another study, we reported a protease independent programmed cell death in *Giardia* under oxidative stress (Ghosh *et al*, 2009). When oxidative stress exceeds the limit of the parasite's tolerance level, the cells commit suicide. In the dying cells, a protein named Programmed cell death protein has been found to be up-regulated. A time-kinetics has been done where the increased expression of this protein has been observed. This protein is a type of phosphatase. Another protein named cathepsin B required for cell viability is inversely regulated with the previous one i.e. when the cells undergo death phase, expression level of this protein gradually decreases. Cathepsin B precursor gene has also been observed to be regulated under cell death during oxidative stress. Cysteine Protease has been found to be down-regulated in the initial phase whereas, Signal Recognition Particle 64 (SRP 64) gets up-regulated in the primary stage but gets down-regulated during the commencement of cell death (Fig. 27). The total cell death regulation under oxidative stress has been found to be a unique feature of this parasite. In our previous study, we obtained some results on the changes in parasitic cell cycle, cell morphology and cell death under oxidative stress. Transcriptomic data from the Real-time PCR study has further elaborated that concept indicating the name of some significant genes regulating cell death of this parasite during oxidative stress.

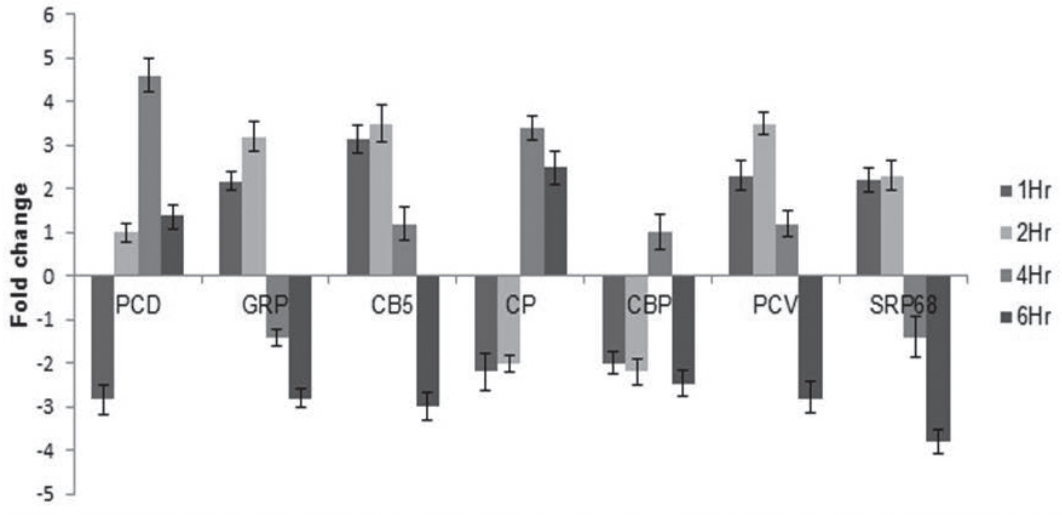


Fig 27 Real time PCR graph of cell death regulating proteins

X axis: Name of the gene; Y axis: Fold change

PCD: Programmed cell death protein like protein, GRP: Glucose regulated protein 94KDa (Anti apoptotic death regulator), CB5: Cytochrome B5, CP: Cysteine protease, CBP: Cathepsin B precursor, PCV: Protein for cell viability, SRP68: Signal recognition particle 68

Modulation of different metabolic genes under differential pathogenic regulation

To better understand the effect of different oxidative stress in transcriptional regulation of gene expression in *Giardia lamblia*, we performed time course analysis of gene expression of pyruvate metabolism pathway upon H_2O_2 (0.10 µM) and cysteine-ascorbate deprived medium stress using a quantitative RT-PCR. We have chosen six genes, related to the pyruvate metabolism of *Giardia lamblia* modulated by at least 2 fold at one or more time points in response to H_2O_2 and cysteine-ascorbate deprivation. The metabolism of *Giardia* species is essentially fermentative, with glucose and amino acids being used as energy sources (Jaroll *et al*., 1995). Arginine is metabolized by Arginine dihydrolase pathway (Knodler *et al*., 1995) and aspartate can be metabolized to pyruvate via a variety of enzymes (Mendis *et al*., 1992). In our study, we

have shown that arginine deiminase (ARGD)-encoding gene was up-regulated in *Giardia* trophozoites during cysteine-ascorbate deprived medium stress but remains down-regulated under hydrogen peroxide stress (Fig. 28B, 28A). Glucose is metabolized by a glycolytic pathway that is modified such that ATP is replaced by pyrophosphate at several key points (Mertens, 1993). This makes the pathway reversible and produces an increase in net ATP synthesis. In *Giardia lamblia*, pyruvate can be produced by three different pathways. Phosphoenolpyruvate carboxyphosphotransferase together with malate dehydrogenase and malate dehydrogenase (decarboxylating), serves as a pathway to convert phosphoenol pyruvate (PEP) into pyruvate (Lindmark, 1980). Malate dehydrogenase (MDH) gene was up regulated at one or more time points upon cysteine-ascorbate deprivation (Fig. 28B). The gene showed a maximum induction at 6 h of cysteine-ascorbate deprivation. In contrast to the induction in response to hydrogen peroxide stress, the gene was down-regulated after (4-8 h) time points (Fig. 28A). However, pyruvate phosphate dikinase (PDK) (Bruderer *et al*., 1996; Park and Sinskey, 1997) and pyruvate kinase (PK) (Ellis *et al*., 1993) can also convert phosphoenolpyruvate into pyruvate in *Giardia*. Pyruvate phosphate dikinase, an enzyme that catalyzes the irreversible conversion of phosphoenolpyruvate (PEP) to pyruvate, the energy generating step of pyruvate biosynthetic pathway, was up-regulated till 8 h under both H2O2 stress and cysteine-ascorbate deprivation (Fig. 28A, 28B). Other genes that were slightly modulated by cysteine-ascorbate deprivation and H_2O_2 stress included pyruvate kinase, which was induced at early (4-6 h) time points under H_2O_2 stress (Fig. 28A) but it was getting down-regulated after (8 h) time points. In response to cysteine-ascorbate deprivation pyruvate kinase remained down-regulated after 6 h time points but up-regulated after 8 h time points (Fig. 28B). This has suggested that the three-enzyme pathway may have an alternative function, such as transferring equivalents from NADH to NADPH (Lindmark, 1980; Ellis *et al*., 1993). Pyruvate ferredoxin oxidoreductase (PFOR) (Townson *et al*., 1996), an enzyme system involved in the conversion of pyruvate to acetyl-coA and finally from acetyl-CoA to acetate is formed by the enzyme acetyl-CoA synthase (ACS). Giardia displays a significant sensitivity to O_2 (Lloyd *et al*. 2000) that are attributed to the expression of O_2-labile key metabolic enzymes such as PFOR (Townson *et al*., 1996). In our study, PFOR-encoding gene was up-regulated during first couple of hours under cysteine-ascorbate deprived medium stress (Fig. 28B) but it was getting down-regulated after (6-8 h) time points upon H_2O_2 stress (Fig. 28A). The enzyme acetyl-CoA synthase transcript was up-regulated during both stresses. However, both hydrogen peroxide and cysteine-ascorbate deprivation has been shown to significantly modulate the metabolic flux across pyruvate metabolism in *Giardia lamblia*.

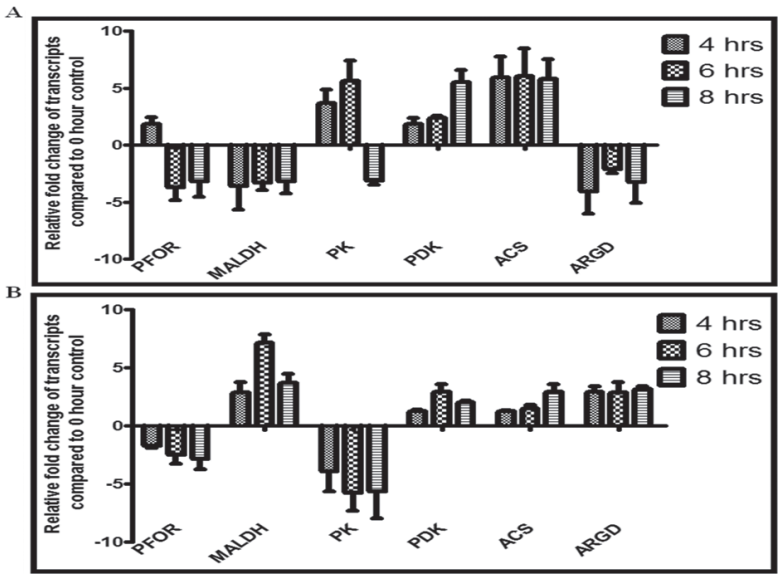


Fig 28 Effect of H_2O_2 and cysteine-ascorbate deprived medium on the expression of genes involved in pyruvate metabolism. Modulation of transcripts encoding enzymes involved in pyruvate metabolism. A. Gene expression (fold change) under H_2O_2 stress. **B**. Gene expression during modified medium treatment. Data are shown as fold change in relative expression compared with Actin on the basis of Comparative Ct ($2^{-\Delta\Delta Ct}$) method. Values are shown as mean ± SEM of three independent experiments, each performed in triplicate. (Gene abbreviation used: **Metabolic enzymes:** PFOR: Pyruvate-ferredoxin oxidoreductase, MALDH: Malate dehydrogenase, ARGD: Arginine deiminase, PK: Pyruvate kinase, PDK: Pyruvate dikinase, ACS: Acetyl coA synthase)

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.
- Recipient of Mother Teresa Gold Medal Award for outstanding achievement and service in the field of health research in 2015.

Institutional committees as member and these are:

- Head, Training and extension division
- Nodal Officer, AADHAR Enabled Biometric Attendance System
- Member, Technical Committee
- Member of Website committee
- Member of Electronic communication committee
- Chairman, Canteen Committee

Conferences/Seminars/Workshops/Trainings Attended/Organised

S. Ganguly

- Organized Institutional Ethical Committee meeting on 13th May 2015 from Division of Training and Extension.
- Organized a conference on Swachh Bharat Campaign on 9thOct 2015 from Division of Training and Extension.
- Organized the one day conference on Pharmacovigilance on 22ndFeb 2016
- Dr Ganguly has been invited to "Japan India Bilateral Meeting on the collaborative research projects" and the International Seminar on Infectious Diseases at the National Institute of Infectious Diseases (NIID), Tokyo during Jan 25th-27th 2015. Dr. Ganguly was invited to present progress of his work on epidemiology of Giardia and other parasitic diarrhoeal etiologies in Kolkata, India. 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 KatoKatz technique", at National Institute of Cholera and Enteric Diseases During July 2-11th, 2015.
- Organized the one day workshop cum training course for MSc students from Vidyasagar University, West Bengal on 3rd May 2016 from Division of Training and Extension.

PATHOPHYSIOLOGY

This division is involved in several projects related to the role of microbial proteases in pathogenesis and tumor regression. Several proteases have been purified, cloned and expressed from *V. cholerae* and *Escherichia coli*. In our earlier studies we had showed the role of hemagglutinin protease (HAP) in pathogenesis in ctx negative *V. cholerae* non-O1, non-O139 strains (Infect and Immun 2006). We also reported for the first time the role in pathogenicity of VesC a 59 kDa serine protease from *V. cholerae* and showed its role in hemorrhagic fluid response in rabbit ileal loop (PloS ONE, 2010). We were among the first to report the transport of proteases in *V. cholerae* through outer membrane vesicles (Infect and Immun 2016). Recently we have identified, cloned, sequenced, characterized and shown the pro-inflammatory response on intestinal cells by YghJ a secreted metalloprotease from neonatal septecemic *E. coli* (IJMM. 2016).

Hemagglutinin protease from *V. cholerae* showed apoptotic response on several cancer cells like EAC (mice 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 EAC cells on which HAP can activate and induce apoptosis (Apoptosis, 2016). Further work is in progress to design and synthesize pro-apoptotic peptides to specifically target cancer cells.

Our laboratory is also working on transport proteins involved in diarrhoea. A novel potassium channel inhibitor TRAM34 has been shown to have anti-secretory effect on cholera toxin induced diarrhoea (JBC, 2013). The role of tight junction protein in inflammatory diarrhoea has also been explored (FASEB, 2015). Further work is in progress to study the mechanism of action of accessory cholerae enterotoxin (Ace) of *V. cholerae*.

Scientists

Dr. M.K. Chakrabarti, ICMR Emeritus Scientist

Dr. A. Pal, Scientist 'F

Staff:

Mr. B. Roy, Technician 'B'

DBT Ramalingaswami Fellow:

Dr K.M. Hoque

Post-doctoral fellow:

Dr T. Ray ICMR Postdoctoral fellow

Predoctoral Fellows:

Ms. Paramita Sarkar (DST Inspire)

Mr. Joydeep Ayoun (SRF ICMR)

Ms. Tultul Saha (SRF DBT)

D. Kar (CSIR)

A. Mondal, ICMR

R. Bhowmick (submitted thesis, CSIR)

Irshad Ali Sh

Title: Role in proinflammatory response of YghJ, a secreted metalloprotease from neonatal septicemic *Escherichia coli*

Investigator : A. Pal

Neonatal sepsis is the invasion of microbial pathogens into the blood stream. Sepsis is the result of an infection associated with a systemic inflammatory response with production and release of a wide range of inflammatory mediators. The increased serum levels of cytokines were found to correlate with the severity and mortality in the course of sepsis. There have been no reports on the role of microbial proteases in stimulation of proinflammatory response in neonatal sepsis. We have identified YghJ a secreted metalloprotease from EB260 a neonatal septicemic *Escherichia coli* strain. The protein was partially purified from culture supernatant, concentrated and run successively in anion and gel filtration chromatography. The MS/MS peptide sequencing of the protease showed homology with YghJ. YghJ was cloned, expressed and purified in pBAD vector. YghJ was found to be proteolytically active against Methoxysuccinyl Ala-Ala-Pro-Met-p-nitroanilide oligopeptide substrate, but not against casein and gelatin. YghJ showed optimal activity at pH 7-8 and at temperatures 37-40°C. YghJ showed clear changes in cellular morphologies of Int407, HT-29 and HEK293 cell lines. YghJ stimulated the secretion of cytokines such as IL-1α, IL-1β and TNF-α in murine macrophages (RAW 264.7) and IL-8 secretion on human intestinal epithelial cell line (HT-29). YghJ can also down-regulate the production of anti-inflammatory cytokines such as IL-10. Ygh is present in both septicemic (78%) and fecal *E. coli* isolates (54%). However, expression of YghJ is significantly higher among the septicemic (80%) than the fecal isolates (33%). This is the first study to show the role of a microbial protease YghJ in triggering proinflammatory response in NSEC.

Title: PAR1 mediated apoptosis and tumor regression of breast cancer cells by *V. cholerae* hemagglutinin protease

Investigator : A. Pal

Conventional anticancer therapies are effective but have side effects, so alternative targets are being developed. Bacterial toxins have emerged as promising agents in cancer treatment strategy. Bacterial toxins that can kill cells or alter the cellular processes like proliferation, apoptosis and differentiation have been reported for cancer treatment. *V.cholerae* hemagglutinin protease (HAP) induces apoptosis in breast cancer cells and regresses tumor growth in mice model. One µg of HAP showed potent antitumor activity when injected into Ehrlich Ascites Carcinoma (EAC) tumors in Swiss albino mice. Weekly administration of this dose is able to significantly diminish a large tumor volume within three weeks and increases the survival rates of cancerous mice. The success of novel cancer therapies depends on their selectivity for cancer cells with limited toxicity for normal tissues. HAP causes PAR-1 activation in breast cancer cells (EAC). Increased expression of Protease Activated Receptor-1 (PAR-1) has been reported in different malignant cells compared to normal cells. HAP mediated activation of PAR-1 caused nuclear translocation of p50-p65 and the phosphorylation of p38 which triggered the activation of NFKB and MAP kinase signaling pathways. These signaling pathways enhanced the cellular ROS level. The level of ROS in malignant cells is reported to be higher than the normal healthy cells; so malignant cells cross the threshold level of ROS faster than the normal healthy cells and induce the intrinsic pathway of cell apoptosis. PAR-1 mediated apoptosis by HAP of malignant cells without effecting normal healthy cells in the same environment makes it a good therapeutic agent for treatment of cancer (Fig 29).

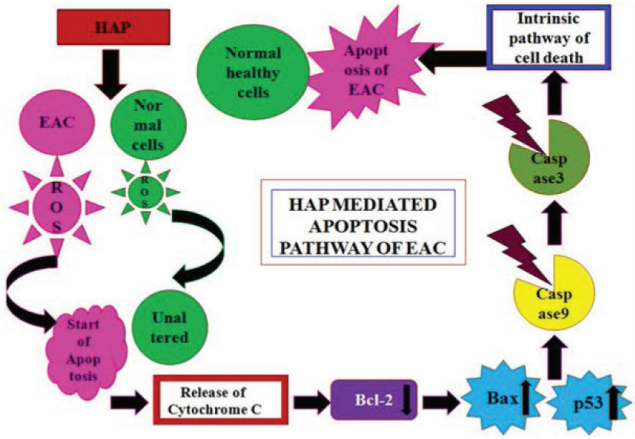


Fig 29 HAP mediated apoptotic pathway

HAP activates the cellular ROS level in malignant cells which allows the threshold level of cellular ROS to reach earlier than the normal healthy cells. ROS activates the intrinsic pathway of apoptosis in malignant cells.

Title: Zinc stimulates the activity of NHE3 and rescue barrier function altered due to *Shigella flexneri 2a* infection in T84 cell monolayers of human colon

Principal Investigator : M. H. Kazi

Previously we established that Zn acutely stimulates NHE3 [Sodium-hydrogen Exchanger isoform 3] in polarized human intestinal cells, Caco-2 by increasing surface NHE3 amount. In this reported period we translated our *in vitro* findings into *in vivo* model to demonstrate that Zn activated NHE3 activity in mouse ileum. Slices from jejunum and ileum were glued onto coverslip and loaded with 95% O_2, 5% CO_2 gassing. The dye loaded in tissue was excited at 780nm with emission ratio imaging at 580 and 640 nm using a 60x/1.00 water immersion objective (Nikon). The images of the jejunal and ileal villus cells were visualized and stored after which fluorescence intensity was calculated using Meta Morph 5.0 software. To determine NHE3 activity, tissues were acidified perfusing with 60mM NH_4Cl for 30 min followed by N methyl-D glutamine (NMDA) buffer for 20-25 min. To monitor NHE3 activity (Na^+/H^+ exchange activity) as the initial rate of pH recovery, the NMDA buffer was switched to Na+ buffer. NHE3 activity was defined as the initial rate of $\Delta pH/\Delta time$ during the Na^+-dependent alkalinization, when Na^+ solution with or without Zn perfused the ileal tissue as shown in Fig 30. The effect of Zn (100µM) added to the luminal surface on NHE3 activity was presented from six independent experiments. We have found that Zn application caused NHE3 stimulation by 112% [Control 0.08; Zn 0.17]. Our study in intact mouse ileum confirmed and extended in vitro results from HANHE3 expressing Caco-2 cells. That is, the stimulation of NHE3 activity by Zn truly happened in native tissue.

Examine whether altered tight junction function due to bacteria and their toxin is recovered by Zn application, we studied the barrier function in human colonic cell line T84 by infecting with invasive bacteria, *Shigella flexneri* 2a in the presence or absence of Zn. We hypothesized that upon infection, Shigella target and disrupt barrier function and Zn could repair this disruption. Polarized T84 monolayers grown onto the permeable filter supports were challenged with *S. flexneri* 2a [Strain # NY 269/92] either apically or basolaterally. At 6 hours post infection with *S. flexneri* 2a, TER decreased significantly over the time period by 75 ± 5% and 25 ± 2% respectively due to basolateral and apical infection, (Fig 31). The inocula of *Shigella flexneri 2a* evaluated in this study do correspond to the MOI of 200. At 12 hours post infection with *Shigella flexneri 2a* we recorded almost total disruption of the monolayer TER value comparable to those of initial cell seeding when very little tight-junction are still formed between cells [3600 ± 27 $\Omega.cm^2$ vs 800 ± 23 $\Omega.cm^2$, Fig 31a]. Together all these data suggests that basolateral infection of shigella greatly affected monolayer barrier integrity. We further studied the alteration of barrier function following interaction with basolateral *Shigella flexneri* -2a by measuring paracellular flux of fluorescently labeled dextran of 4kDa and 72kDa

rhodamine. As shown in Fig 31c, T84 cells exposed to *S. flexneri 2a* showed a significantly increased [2.1 ± 0.6 vs 0.8 ± 0.2 µg/3.5 hr/cm^2] transport of FITC-Dextran (4KDa) with respect to uninfected control. However, permeability to rhodamin-dextran (72kDa) was not altered when *Shigella flexneri 2a* was applied to T84 monolayer. These data indicated *S. flexneri 2a* affect epithelial barrier integrity by increasing T84 monolayer paracellular permeability along with TER. EGTA was used as maximum permeability control for its ability to completely open tight junction. It remains to be clarified whether the effects of Zn may be beneficial in the modification of specific tight junction (TJ) proteins which was altered in T84 cells by *S. flexneri 2a* infection. We measured TER and paracellular flux of dextrans (4kDa and 72 kDa) in T84 cells infected with *S. flexneri 2a* in the presence or absence of either apical or basolateral Zn. As shown in Fig 31a,b, apical administration of 200uM Zn upon basolateral infected T84 cell showed a recovery of TER by 72 ± 2%. Similarly, in the presence of Zn, the flux of 4kDa FITC-dextran was reversed by 57%. This recovery effect was not due to cytotoxic effect of Zn on Shigella flexneri 2a because no inhibitory effect on the growth of Shigella flexneri 2a was seen at this concentration (data not shown). However a 50% inhibitory effect was evident beyond this concentration of Zn.

Because both the cellular and paracellular pathway determine net transport, we hypothesized that the paracellular pathway might be affected in parallel with the cellular pathway due to shigella infection, we measured dilution potential in mV under open-circuit conditions and used them to calculate passive transport of Na^+ vs, Cl^-. This was done by reducing the apical bathing solution NaCl concentration from 120mM to 60mM (1:2 dilutions). If the junction is selectively altered due to infection and rescued by Zn application, an electrical potential does generate accordingly and the size of the potential (mV) represents a measurement of the degree of junctional charge selectivity. Under baseline condition, the paracellular pathway was cation-selective (Fig 32a), which is consistent with earlier reports [Proc. Natl. Acad Sci(USA), 2009, 106(9): 3591-3596. As shown in Fig 32, we further observed that the dilution potential was decreased due to shigella infection which was rescued by the application of apical Zn. We also considered that if Shigella infection simply disrupted tight junction integrity, then PNa^+/PCl^- would fall. Indeed this was the case that paracellular permeability of Na^+ was decreased in shigella infected cells than the uninfected cells (Fig 32b). In conclusion, several observations from our study argue that Zn is beneficial for the infection induced alterations in tight junctions. First, we used several methods [TER, Transepithelial Electrical Resistance; Paracellular flux of non-charged particle of different size; dilution potential and paracellular permeability of Na^+ by electrophysiological study in Ussing chamber] and all suggested that Zn rescued the altered tight junction function caused by shigella infection in T84 cells. Second, the tight junction is the epithelial structure that determines paracellular ion selectivity, and thus altered ion selectivity implicates change in tight junction functions which exactly happened in our study that paracellular permeability to Na^+ was decreased in shigella infected cells which lead to dehydrate the mucus layer, and provide the nidus for bacterial colonization to induct inflammation. Third, the decreased dilution potential due to shigella infection was significantly reversed back by the application of Zn suggests that Zn has potential for the inflammatory diarrhea. Taken together, the data described above suggests that Zinc rescued the affected epithelial barrier integrity induced by Shigella infection.

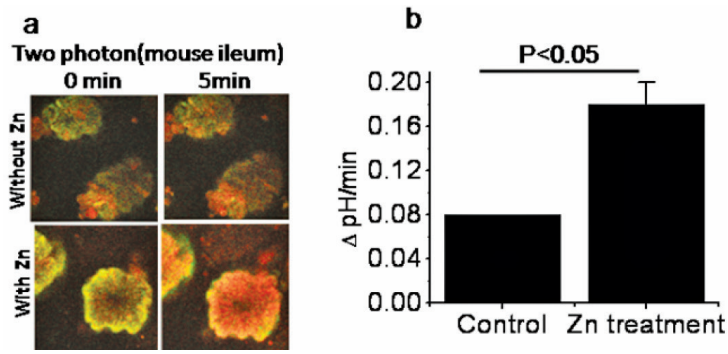


Fig 30 Measurement of NHE3 (Na^+ - H^+ exchange) activity in mouse ileum (a) representative images of the measurement of SNARF-4F fluorescence intensity at 580 and 640nm in optical section, 20µM from the villus tip. (b) summary of the mean effect of Zn on NHE3 activity in mouse intestinal tissue. N = 6 independent experiments

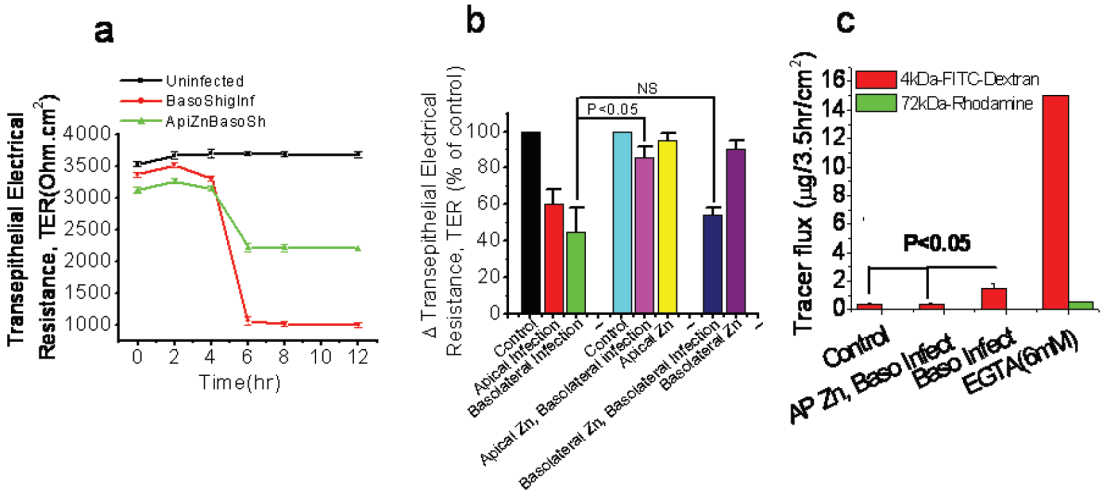


Fig 31 Luminal Zn application rescued severely affected paracellular transport function primarily mediated by tight junction complex in T84 cell monolayer of human colon. Time course regulation of TER by shigella infection either in presence or absence of Zn. (a) Quantification of data derived from Fig a to demonstrate that shigella had severe effect in the reduction of TER which was rescued by luminal Zn. (b) Shigella infection increased paracellular flux of 4kDa FITC dextran while luminal Zn was able to reverse this effect. (c) Data are expressed as mean ± SEM for triplicate samples fpr at least three independent experiments.

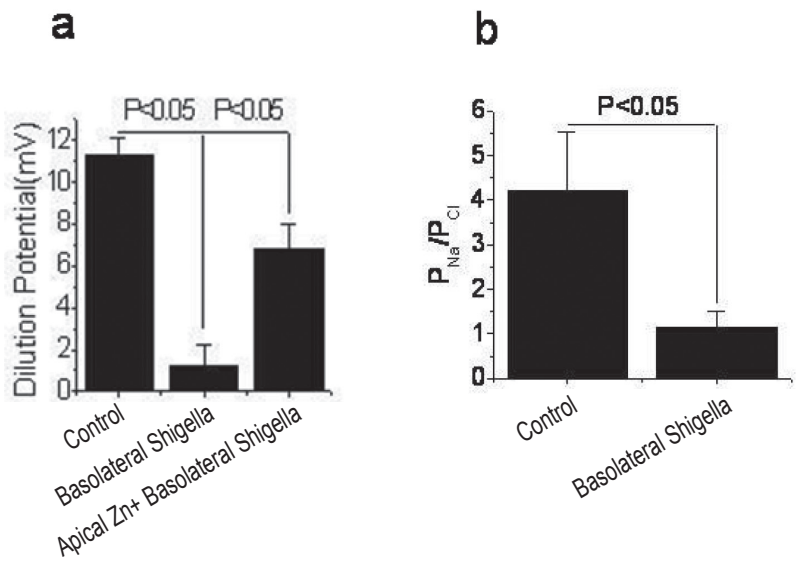


Fig 32 Shigella infection caused reduction of dilution potential (a) and paracellular permeability of Na^+ (b). Dilution potentials were measured on T84 cell monolayers grown on transwell inserts by diluting NaCl concentration from 120mM to 60mM at the apical side. PNa/PCl were calculated from the dilution potential using the Goldman-Hodgkin-Katz equation. Data are mean ± SEM. N = 5-6 independent experiments

Title: Knockdown of Epac1 alters barrier function in Human colonic cell line (T84): implication in intestinal infection

Principal Investigator : M.H. Kazi

Here we have explored a better understanding of the intestinal epithelial barrier function which is regulated by Epac to foster new ideas for the development of therapies for diarrhea in patients with IBD. We have demonstrated earlier that depletion of Epac1 caused reduction of Transepithelial Electrical Resistance (TER), and increment of paracellular flux of non-charged particle. We tentatively interpreted such decreased TER and increased paracellular permeability as subtle forms of junctional barrier disruption. The junctional

barrier disruption due to Epac1 depletion was further confirmed by the effect of Epac agonist 8-pCPT-2'-O-Me-cAMP on the ability of monolayers to reforms tight junction, an incubation period between the opening phase and the recovery phase that is required. The sensitivity of TER to 8-pCPT-2'-O-Me-cAMP in wild type T84 cells (T84WT) and Epac1 depleted cells (T84Epac1KD) is shown in Fig 33a,b. When T84WT cells were incubated with 8-pCPT-2'-O-Me-cAMP, the transmonolayer resistance fell rapidly over time. Whereas in T84Epac1KD cells, the basal TER remained low, confirming that the low TER due to Epac1 depletion correlated with a increase in paracellular permeability through the monolayer. Another important question that requires clarification is how Epac1 may change the anion vs. cation selectivity because the identity and quantitative contribution of the ion(s) carrying the paracellular conductance and the electrophysiological basis for the differences (if any) may be altered due to Epac1 depletion. To test the hypothesis of whether T84Epac1KD cells have an altered paracellular charge selectivity, we measure the transmonolayer dilution potential with an basal-to-apical chemical gradient (60mM NaCl at the apical to 120mM at the basal side. In Epac1KD cells, we found that a -6.6 ± 0.25 mV dilution potential had developed across the monolayer which was lower than the T84WT cells [-3.75 ± 0.18mV, $P<0.05$], indicating that the junctional pores of T84Epac1KD cells were less permeable to anions than cations. The experiments were also performed with a basal-to-apical chemical gradient and we found the direction of gradient had no effect on our measurements of dilution potential. The Goldman-Hodgkin-Katz equation calculated the ratio of permeability of Na^+ over Cl^- at 1.5 ± 0.02 in control cells compared to 1.17 ± 0.01 ($P<0.05$) in Epac1 depleted cells (Fig. 33c). This suggested that depletion of Epac1 altered the ion selectivity of tight junctions to reduce cation permeation between T84Epac1KD cells. Taken together these data further illustrate that paracellular barrier formation and tight sealing along with ion selectivity requires Epac1 protein and perhaps it's associated signaling. Since occluding is a member protein it might be involved in the sealing of tight junction. Therefore, we next examined the cellular distribution of occludin in T84WT cell vs. T84Epac1KD cell monolayers grown onto transwell inserts by confocal microscopy. To precisely compare the distribution of occluding, optical sections were taken in the xy and xz plane to test whether Epac1 depletion can alter the characteristic tight junction staining of occludin. The images shown in Fig. 34a illustrate section at the horizontal plane revealed that occludin transported to tight junction level and formed a continuous junctional ring in T84WT cells. The staining of occludin never revealed any discontinuities in Epac1 depleted cells (Fig. 34b). Z-sections generated with confocal microscope demonstrated that occludin localized in the most apical part of the lateral plasma membrane of both T84WT and Epac1KDT84 cells suggesting that Epac1 depletion did not have any effect onto the mislocalization of occludin in Epac1KDT84 cells. We conclude that occludin may not be involved in the Epac1 mediated regulation of tight junction function. We need to explore our understanding of the molecular mechanism of Epac1 regulation of barrier function which may have clinical relevance in intestinal inflammation. Further work is in progress.

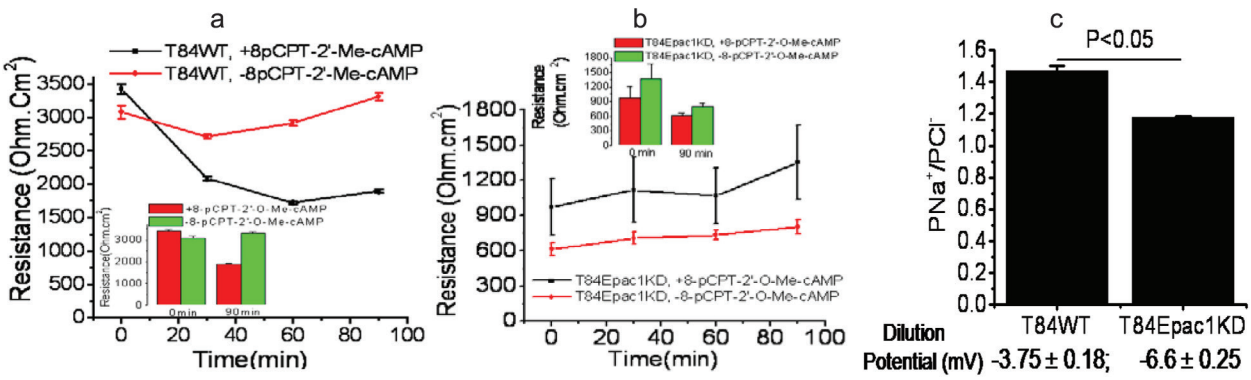


Fig 33 Comparison of 8-pCPT-2'-O-Me-cAMP effects of tight junction perturbation on Transepithelial Electrical Resistance (TER) across T84WT (a) and Epac1 depleted cells Epac1KDT84. (b) TER measurements were performed in triplicate, and the results are the average of three separate experiments. Dilution potentials were compared between wild type T84 cells and Epac1 knock down cells. Depletion of Epac1 decreased dilution potential from -3.7mV to -6.6 mV. Permeability of Cl^- relative to Na^+, as determined by dilution potential measurements. (c) Each data point represents of the mean ± SEM of six monolayers

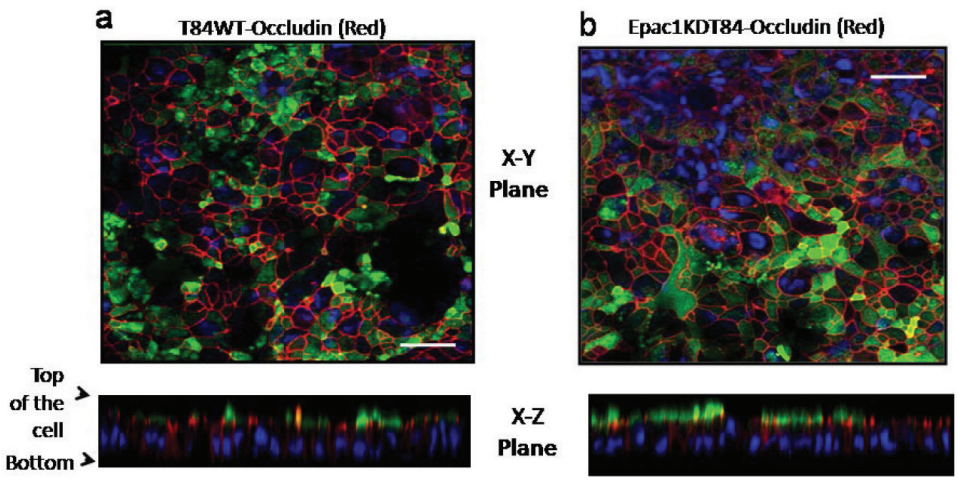


Fig 34 Immunofluorescence staining of tight junction protein occludin in T84WT (a) and Epac1KDT84 (b) cells cultured on transwell inserts were fixed with methanol and stained with occludin antibody labeled with alexa fluor 568 and apical plasma membrane marker Wheat Germ Agglutinin (WGA). Occludin (red) and WGA (green) are localized to the apical tight junction domain as shown in xy plane (top) and xz plane (bottom) sections

Title: Luminal TRAM-34 inhibits second messengers stimulated chloride secretion –Ex vivo evidence for better therapeutic intervention in secretory diarrhea

Principal Investigator : M H. Kazi

Potassium channel inhibiting drug therapy has got attention with respect to diarrheal disease therapy because effective chloride ion (Cl^-) secretion only happens if potassium channels are activated. In intestinal epithelial cells, potassium channel recycles potassium ions (K^+) across the membrane to maintain necessary driving force for luminal exit of Cl^-. It is usually assumed that only a serosal potassium conductance produced from second messenger stimulation facilitate the efficiency of the secretion mechanism. However, there is considerable experimental evidence to suggest that the calcium-activated potassium channel of the intestinal mucosal membrane has a significant role in Cl^- secretion.

To directly test the hypothesis that apical K^+ channels play a role in providing the driving force for the cAMP-stimulated Cl^- secretion, we first investigated the pharmacological profile of various K^+ channel blockers added on the apical side after the adenylate cyclase activator forskolin(FSK) stimulated Isc, a measure of Cl^- secretion. Fig 35a shows the inhibitory effect in Isc responses of FSK to the effective concentrations of various K^+ channel blockers. We found that clotrimazole (91 ± 4% of control), which is often used as a probe of calcium -activated K^+ channels, and TRAM-34 (97 ± 3% of control), a specific calcium-activated KCNN4 channel blocker, almost completely inhibited Cl^- secretion stimulated by FSK. However, addition of apamin, a specific SK channel blocker; iberiotoxin, a specific BK channel blocker; tetraethylammonium, a nonspecific potassium channel blocker; and $ZnCl_2$, a KCNQ1/KCNE3 channel blocker, had no effect on FSK-stimulated Isc. $BaCl_2$, a nonspecific potassium channel blocker, only required a 5 mM concentration to significantly (21 ± 4 versus 32 ± 2 A/cm^2 (34%), $p< 0.05$) reduce FSK-stimulated Isc. The complete inhibition of FSK-stimulated Isc by clotrimazole and TRAM-34 compared with the lack of effect of apamin, iberiotoxin, or Zinc suggests a possible role for apical KCNN4 channels but not BK, SK, or KCNQ1/KCNE3 channels in cAMP-stimulated Cl- secretion. Previously we have investigated that serosal addition of zinc significantly inhibited cAMP stimulated chloride secretion in rat intestinal tissue (Am J Physiol Gastrointest Liver Physiol. 2005; 288(5):G956-63). However apical addition of zinc did not have any effect on the cAMP stimulated Cl- secretion. This was further confirmed in human colonic cell lines, T84 as well as in an *in vivo* mouse ileal loop model of diarrhea. Serosal addition of 10 µM FSK resulted in an immediate and sustained increase in Isc (ΔI_{sc}: 30 ± 1.1 µA/cm^2) that reached a peak in 5 min following the addition of FSK and remained near maximal levels throughout the experimental period. The subsequent addition of 150µM $ZnCl_2$ to the

serosal solution resulted in a prompt decrease in I_{sc}. In contrast, mucosal addition of zinc had no effect on FSK-stimulated I_{sc} (Fig. 35b). To assess whether zinc or TRAM-34 modified cholera toxin induced diarrhea, mouse ileal loop experiments were performed. A series of closed loops of small intestine were created, and the lumen of alternate loops was injected with small volume of saline or saline containing cholera toxin or cholera toxin with zinc or TRAM-34. Data from series of experiments were summarized in Fig 35c. Mucosal addition of TRAM-34 dose dependently reduced diarrhea, whereas zinc had minimal effect. The electrophysiological data together with *in vivo* mouse loop experimental data suggested that zinc required serosal access for efficacy. Along this line of thought, Bzik *et al*. (J Pharma and Pharmacol. 64: 644-653) provide novel data that pharmacological opening of epithelial tight junctions with the established modulator, cytochalasin D, enabled increased zinc permeability to the serosal side and conferred anti-secretory action for apically-added concentrations of zinc. These observations raised question on zinc's effectiveness from mucosal (oral) side and may limit widespread support for its use as adjunct therapy in diarrhea. Heat-stable enterotoxin (STa) and NSP4 of rotavirus enterotoxin stimulates intestinal Cl^- secretion by increasing cGMP and intracellular calcium respectively. We next explored if TRAM-34 sensitive luminal potassium channel KCNN4 remains operative in cGMP and calcium stimulation of Cl- secretion in mouse tissue. We treated mouse tissue with 100nM STa for 15 min. STa caused an increase of Cl^- secretion which was completely inhibited by luminal application of TRAM-34. Similarly, the effect of calcium signaling specific analogue [mimics NSP4] carbachol on Cl^- transport was measured electrophysiologically in Ussing chamber. TRAM-34 strongly inhibits calcium stimulated Cl- secretion [Fig. 36a,b]. Taken together all these data provide a strong rationale for further trial testing of TRAM-34 (KCNN4c channel inhibitor) for its efficacy in specific clinical setting for diarrhea. The study is in progress.

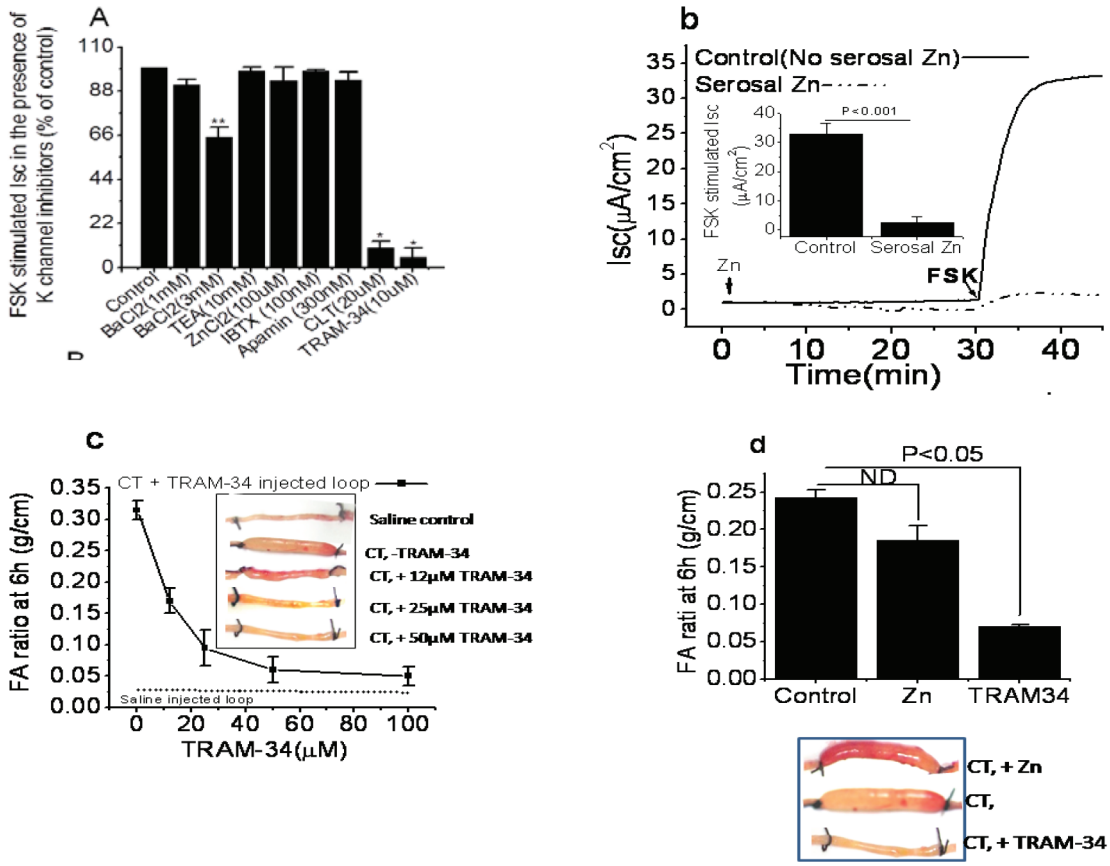


Fig 35 Effect of luminal potassium channel blockers (including zinc and TRAM-34) on forskolin stimulated currents of human colonic T84 cells monolayers. (a) Summary of calculated Isc in presence of various K+ channel blockers added on the luminal side. (b) Luminal zinc application had no inhibitory effect (data not shown) while serosal zinc promptly decreased in forskolin stimulated Isc. (c) Data from series of mucosal ileal loop experiments demonstrate that luminal TRAM-34 dose dependently reduced cholera toxin stimulated intestinal fluid accumulation (d)while zinc had minimal effect. Data are mean ± SEM. N = 10 mice per group

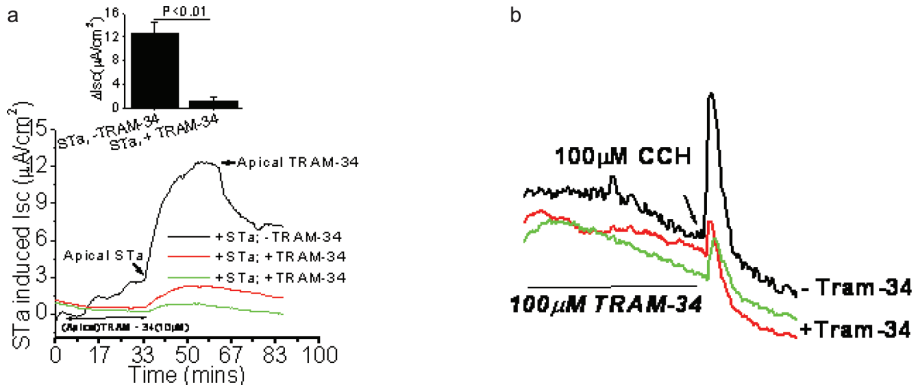


Fig 36 TRAM-34 inhibits STa (a) and carbachol (b) (CCH) stimulated Cl- secretion in mouse intestinal tissue in Ussing chamber. STa was added on luminal side and CCH was added on the serosal side. TRAM was used only on the luminal side either before or after STa or CCH stimulation as indicated. Data are mean ± SEM

Awards/Honours received

M.H. Kazi

- The UK Physiological Society's Travel Grant Award for attending the Experimental Biology Meeting, held at San Diego, CA, USA
- Invited as an editorial board member of the journal 'The Physiological Report' – a sister journal of American Physiological Society and UK Physiological Society from 2015 for a three year term.
- Invited speaker to the International conference on Gastroenterology and Hepatology (ICGEH 2016) held from December 1-3, 2016 Sanya, China.

Conferences/Seminars/Workshops/Trainings Attended/Organised

A. Pal

- Attended the 103rd Indian Science Congress held at Mysore University, Mysore from 3rd to 7th January, 2016 and delivered a lecture on "Hemagglutinin protease secreted by
- *V. cholerae* induced apoptosis in breast cancer cells by ROS mediated intrinsic pathway and regresses tumor growth in mice model"
- Nominated for the DST approved GMP for Scientists held at Administrative Staff College of India, Hyderabad from July 06 to 17, 2015.

M. H Kazi:

- Invited speaker to the US-Japan Medical Science Program joint panels on cholera & other bacterial enteric infection at the 49th US-Japan conference on 14th-18th Jan 2015 held at University of Florida, Gainesville, USA. Title of talk: Luminal TRAM 34 is superior than zinc in the cholera toxin stimulated fluid secretion- the in vivo evidence for a better therapeutic intervention for cholera.
- Mirajul H. Kazi, Saha T., Woodward W., Guggino WB. Identification of Ano1 (Tmem16A) activator purified from Cucumis sativas: 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 , I.A. Sheikh, T. Saha, J. Aoun, M.H. Kazi. Zinc restores altered intestinal ion-transport, barrier functions and counteract inflammatory mediators induced by Shigella infection in T84 cells at 17th International Conference on Infectious diseases (ICID) , March 3-5, Hyderabad, India.
- I.A. Sheikh, J. Aoun, P. Sarkar, T. Saha , M.H. Kazi. Recombinant accessory cholera enterotoxin of vibrio cholera activate ANO6 via RhoA- ROCK-PIP2 signaling to induce secretary diarrhea at 17th International Conference on Infectious diseases (ICID), March 3-5, Hyderabad, India.
- Two day workshop on the role of the Microbiota in infectious diseases sponsored by NIAID (NIH) on September 1-2, 2015 at 5601 Fisher Lane, North Bethesda, MD, USA.

VIROLOGY

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

The scientists and staff of Division of Virology are actively associated with the surveillance studies undertaken by the National Institute of Cholera and Enteric Diseases to understand the etiological role and disease burden of different diarrhoeagenic viruses in and around Kolkata. Molecular characterization studies are undertaken to gather knowledge on the genetic diversity and to determine the circulation of new variants and emerging viruses; phylogenetic nature of the circulating enteric viruses with focus on Rotaviruses, Caliciviruses viz. Norovirus and Sapovirus, Astroviruses, Picobirnaviruses, emerging Picotrirnavirus-like agent and Adenoviruses.

Studies towards understanding dynamics of Rotavirus-Host cell interaction

The basic research activities cater towards understanding functional aspects of host pathogen interaction through analysis of the signaling mechanisms during Rotavirus- host cell interactions with special reference to study of host cellular proteins required for viral replication and pathogenesis.

Surveillance of Influenza and other respiratory viruses

Strain surveillance for respiratory viruses using molecular methods in collaboration with NIV Pune and Centers for Disease Control and Prevention, Atlanta, USA is ongoing to monitor seasonality, emergence of new subtypes and antiviral resistance among circulating strains. The laboratory staff actively conduct investigations during sudden outbreaks to diagnose the influenza virus subtypes.

HIV surveillance in North-East India

The Division of Virology of NICED has been actively engaged in HIV/AIDS research and National AIDS Control program since mid-1980s. HIV transmission in North eastern states is routinely monitored for better understanding of the molecular aspects of HIV strains among infected individuals and their partners. Current focus includes (i) HIV Surveillance (Sentinel as well as Integrated Biological and Behavioural Surveillance) for HIV estimation in different risk groups (ii) Molecular studies for HIV detection among babies born to HIV infected mothers, HIV viral load assay and HIV drug resistance mutations for understanding the molecular diversity of HIV in eastern India and (iii) Quality Assurance in HIV testing.

Human resource development and collaborations

Training is imparted to graduate and doctoral students and staff so as to improve the human resources capable of studying viral diseases of national importance across the country. The research programs include intramural projects and extramural projects with national and international funding and collaborating scientists. The current programs are associated with DBT, ICMR, CDC Atlanta and Okayama University, Japan.

Scientists:

Dr. T. Krishnan, Scientist F

Dr. M. K. Saha, Scientist F

Dr. M. Chawla-Sarkar, Scientist E

Staff:

Dr S. Bhunia Technical Officer A

Mr. S. Omesh Technical Officer A

Dr S. K. Sadhukhan Technical Officer A

Ms. M. Mullick Technical Officer A
Mr. K. Sen Technical Assistant
Ms. P. De Technician B
Md. M. Hossain Technician B
Ms. C Das MTS

DST Ramanujan Fellow:

Dr Anupam Mukherjee

Project Scientists:

Dr Mukti Kant Nayak
Dr. Subrata Biswas. Project Coordinator
Ms. Srijita Nandi- Research Officer
Dr. Mallika Ghosh- Research Officer

Post-Doctoral Fellow:

Dr. Nalok Dutta

Pre-Doctoral Fellows:

Ms. Shampa Chanda UGC-SRF
Ms. Paulami Mandal CSIR-SRF
Ms. Arpita Mukherjee UGC-SRF
Mr. Upayan Patra ICMR- JRF
Ms. Anindita Banerjee DST-Women Scientist
Mr Edwin Anthony JRF Maulana Azad National Fellowship for Minority Students
Ms. Urbi Mukhopadhyay UGC – JRF
Mr. Rakesh Sarkar UGC-JRF
Ms. Piyali Ghosh. Project Assistant

PhD Awarded:

Dr. Rahul Bhowmick received Ph D. from University of Calcutta

Title of the Thesis: A study on Rotavirus mediated modulation of Cell survival and Apoptotic Machinery during Infection.

Dr. Satabdi Nandi received Ph D. from University of Calcutta

Title of the Thesis: Studies on viral and cellular determinants of rotaviral infection.

Dr. Satarupa Mullick received Ph D. from University of Calcutta

Title of the Thesis: Genomic characterization of circulating Group A Rotavirus strains in an urban slum community in Kolkata.

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

Principal Investigator : T. Krishnan

Co-Investigators : M.K. Bhattacharya, P. Indwar, NICED; Principal, B.C Roy Post Graduate Institute of Paediatric Sciences (BCRPGIPS); Superintendent, Infectious Diseases and Beliaghata General Hospital.

The diarrhoeal disease burden is a major concern and the surveillance of enteric viruses with special reference to non-Rotaviral diarrhoea is being carried out in the Division of Virology of National Institute of Cholera and Enteric Diseases in Kolkata, India.Screening for emerging viruses was carried out from faecal specimens of hospitalized diarrhoea cases, admitted for treatment in Infectious Diseases and Beliaghata General Hospital, Kolkata, India. The genomic RNA was extracted and electrophoresed in agarose gel and stained with ethidium bromide to screen for other viral etiological agents. Some of the non- rotaviral pathogens associated till date with diarrhea cases were Group B rotavirus, Group C rotavirus, Norovirus, Sapovirus, Astrovirus, Picobirnavirus and Adenovirus.

Faecal specimens of approx. 2363 diarrhoea cases of all age groups, enrolled in the ongoing surveillance program were screened for detection of non-Group A rotaviruses by agarose gel electrophoresis, followed by ethidium bromide staining. An etiological agent showing trisegmented genomic RNA profile was detected in diarrhoeic faecal specimens of 160 diarrhoea cases where 80 positives were males and another 80 positives were females. The age group of the diarrhoea cases where the trisegmented profile was detected was found to be children below 5 years in 41 cases, children between 5 to 12 years in 7 cases, older children aged above 12 yrs and below 18 years in 14 cases and adults in 98 cases respectively. [Fig 37]

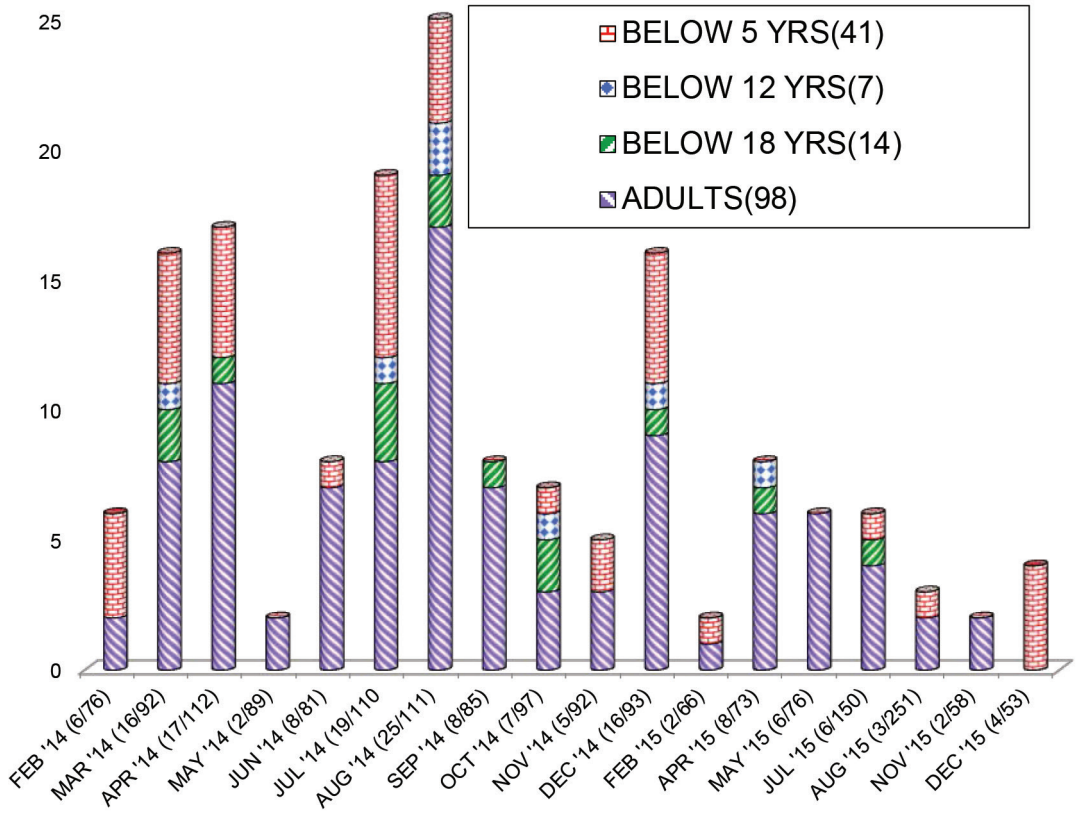


Fig 37 Detection of an emerging etiological agent with three segmented genome profile among different age groups of hospitaliseddiarrhoea cases in Kolkata, India

The clinical features of the trisegmented genomic RNA profile positive diarrhoea patients showed that nature of the stool was watery in 131 cases, loose stool in 23 cases and bloody mucoid stool in 6 cases. The positive cases presented with vomiting in 129 cases; fever in 41 cases and abdominal pain in 92 cases. Some dehydration was observed in 145 cases and severe dehydration was recorded in 8 cases and no dehydration was found in 7 cases. [Table 8]

The picotrirnavirus-like agent was the only detectable agent in 94/160 positive cases; coinfection with another diarrhoeagenic virus was seen in 13 cases, coinfection with bacteria was seen in 36 cases, coinfection with parasites was seen in 3 cases while 14 cases showed mixed infection with viral/bacterial/parasitic enteric pathogens. [Table 9]

Table 8 Clinical findings associated with the detection of an emerging etiological agent showing three segmented genomic profile among hospitaliseddiarrhoea cases in Kolkata, India

Study period (PTV positives)	Stool_ watery	Stool_ loose	Stool_ bloody &mucoid	Vomiting	Fever	Abdominal pain
FEB '14(6)	4	2	0	4	0	3
MAR '14(16)	14	1 (sole)	1 (sole)	14	3	7
APR '14(17)	12	3	2	11	2	11
MAY '14(2)	2	0	0	1	0	1
JUN '14(8)	5	2	1	5	1	6
JUL '14(19)	17	2	0	16	1	7
AUG '14(25)	22	2	1	22	2	14
SEP '14(8)	7	1	0	8	8	7
OCT '14(7)	6	1	0	6	3	5
NOV '14(5)	5	0	0	4	4	3
DEC '14(16)	15	0	1	13	11	11
JAN '15(0)	0	0	0	0	0	0
FEB '15(2)	2	0	0	2	1	1
MAR '15(0)	0	0	0	0	0	0
APR '15(8)	6	2	0	5	3	4
MAY '15(6)	4	2	0	4	4	5
JUN '15(0)	0	0	0	0	0	0
JUL '15 (6)	4	2	0	6	2	1
AUG '15(3)	3	0	0	3	1	2
SEP '15(0)	0	0	0	0	0	0
OCT '15(0)	0	0	0	0	0	0
NOV '15(2)	1	1	0	2	1	1
DEC '15(4)	2	2	0	3	1	3
JAN '16(0)	0	0	0	0	0	0
FEB '16(0)	0	0	0	0	0	0
MAR '16(0)	0	0	0	0	0	0
	131	23	6	129	41	92

Table 9 Detection of emerging etiological agent showing three segmented genome profile as sole or mixed infection among male and female patients and the dehydration status among hospitalised diarrhoea cases in Kolkata, India

Study Period	PTV Pos	Only PTV	Coinfection Virus	Coinfection Bacteria	Coinfection Parasite	Coinfection Mixed	Male	Female	Some Dehydration	Severe Dehydration	No Dehydration
Feb '14	6	3	2	0	0	1	4	2	6	0	0
Mar '14	16	11	3	1	1	0	7	9	13	3	0
Apr '14	17	11	2	1	1	2	11	6	15	2	0
May '14	2	2	0	0	0	0	1	1	2	0	0
Jun '14	8	7	0	1	0	0	3	5	7	1	0
Jul '14	19	9	2	6	1	1	9	10	19	0	0
Aug '14	25	10	0	11	0	4	13	12	25	0	0
Sep '14	8	2	0	5	0	1	3	5	8	0	0
Oct '14	7	3	0	3	0	1	4	3	7	0	0
Nov '14	5	3	1	0	0	1	2	3	4	1	0
Dec '14	16	10	2	4	0	0	11	5	15	1	0
Feb '15	2	2	0	0	0	0	1	1	2	0	0
Apr '15	8	8	0	0	0	0	3	5	7	0	1
May '15	6	6	0	0	0	0	0	6	6	0	0
Jul '15	6	3	0	2	0	1	3	3	2	0	4
Aug '15	3	2	0	1	0	0	1	2	1	0	2
Nov '15	2	2	0	0	0	0	1	1	2	0	0
Dec '15	4	0	1	1	0	2	3	1	4	0	0
	160	94	13	36	3	14	80	80	145	8	7

Title: National Integrated Biological and Behavioral Surveillance (IBBS) for HIV

Principal Investigator : M K Saha

Eight leading Government Public Health Institutes (AIIMS, NARI, NICED, NIE, NIHFW, NIMS, PGIMER, and RIMS) were involved in implementing IBBS (largest study of its kind in the world), a community based cross-sectional study using probability based sampling to generate evidence on risk behaviors among FSW, MSM, IDU, Transgender, Migrant and Currently Married Woman with the aim of monitoring trends, level and burden of HIV among different HRG population to facilitate delivery of effective response to control the epidemic.

Among FSW, national HIV prevalence was 2.2% (95% CI: 1.8-2.6), Nagaland (with Manipur & Mizoram) recorded a high 5.9% (95% CI: 4.0 – 8.6) and WB, Assam, & Meghalaya recorded < 2 % prevalence. Nearly half (45% to 52%) of the FSWs were separated, widowed or divorced in WB (grouped with Manipur, Mizoram, Kerala, & Puducherry), which is higher than all other states/UTs. In N-E states, higher levels of consistent condom use with occasional clients were reported by FSWs (Nagaland -80% and Assam -75%), compared

to other states where it ranged between 37% and 55%. Alcohol consumption was higher among FSWs in Nagaland (88%) as well as West Bengal (62%), and Meghalaya (43%) compared to national average of 31%.

Among MSM, countrywide HIV prevalence was 4.3% (95% CI: 3.7 - 5.1). In WB 6.7% (95% CI: 3.7-12.0) and in Assam & Nagaland 1.8% (95% CI: 1.1 – 3.0). The proportion of MSM who reported taking no action for the last STI episode was 6% at the national level and higher in WB (25%), and Nagaland (29%) compared to all other states. In East and N-E states, consistent condom use with casual female partner among MSM populations was 30% or less, with the exception of WB (40%) and Nagaland (48%). The proportion of MSM visiting government facilities was < 40% in WB and Nagaland. (Fig 38).

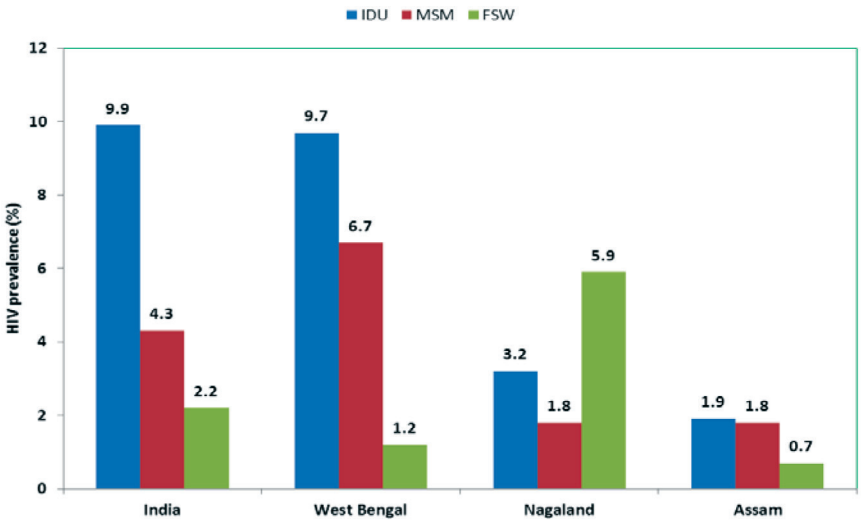


Fig 38 HIV sero-positivity among HRG population from East & North-East India

HIV prevalence among IDU in the country was 9.9% (95% CI: 9.0-10.9) and ranging from 9.7% (95% CI: 6.2-14.8) in WB, 3.2% (95% CI: 2.2-4.7) in Nagaland and 1.9% (95% CI: 1.1-3.1) in Assam, Meghalaya, and Sikkim. A higher proportion of the IDUs were younger in many of the N-E states (Assam, Meghalaya, Nagaland, and Sikkim). Heroin and Spasmoproxyvon were used predominantly and in majority of the N-E states common location/ place where IDUs had injected in last three months was home, ranging between 28% in Assam and 86% in Nagaland.

Title: HIV drug resistance mutations among patients on anti-retroviral therapy

Principal Investigator : M.K. Saha

HIV drug resistance (HIV DR) compromises antiretroviral therapy (ART) outcome. Patients on 2nd line ART have shown high HIV viral load (virologic failure, >1000 copies of HIV/ml) and HIV DR mutations. HIV-1 genotyping employing Viroseq (Abbott Diagnostics) for virologic failure samples detected HIV DR mutations. For (i) Nucleoside reverse transcriptase inhibitor (NRTI) drugs M184V and M41L were predominant mutations, for Non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs A98G and Y181C were predominant and for Protease inhibitor (PI), l54V, A71V, V82A and M46L were predominant.(Fig 39)

Interestingly, no HIV DR mutation was detected among 45% patients on 2nd line ART and having virologic failure. This significant observation warrants further in-depth studies on virologic failure in the absence of HIV DR mutations for better understanding of HIV DR dynamics as well as planning evidence based clinical management for HIV infection.

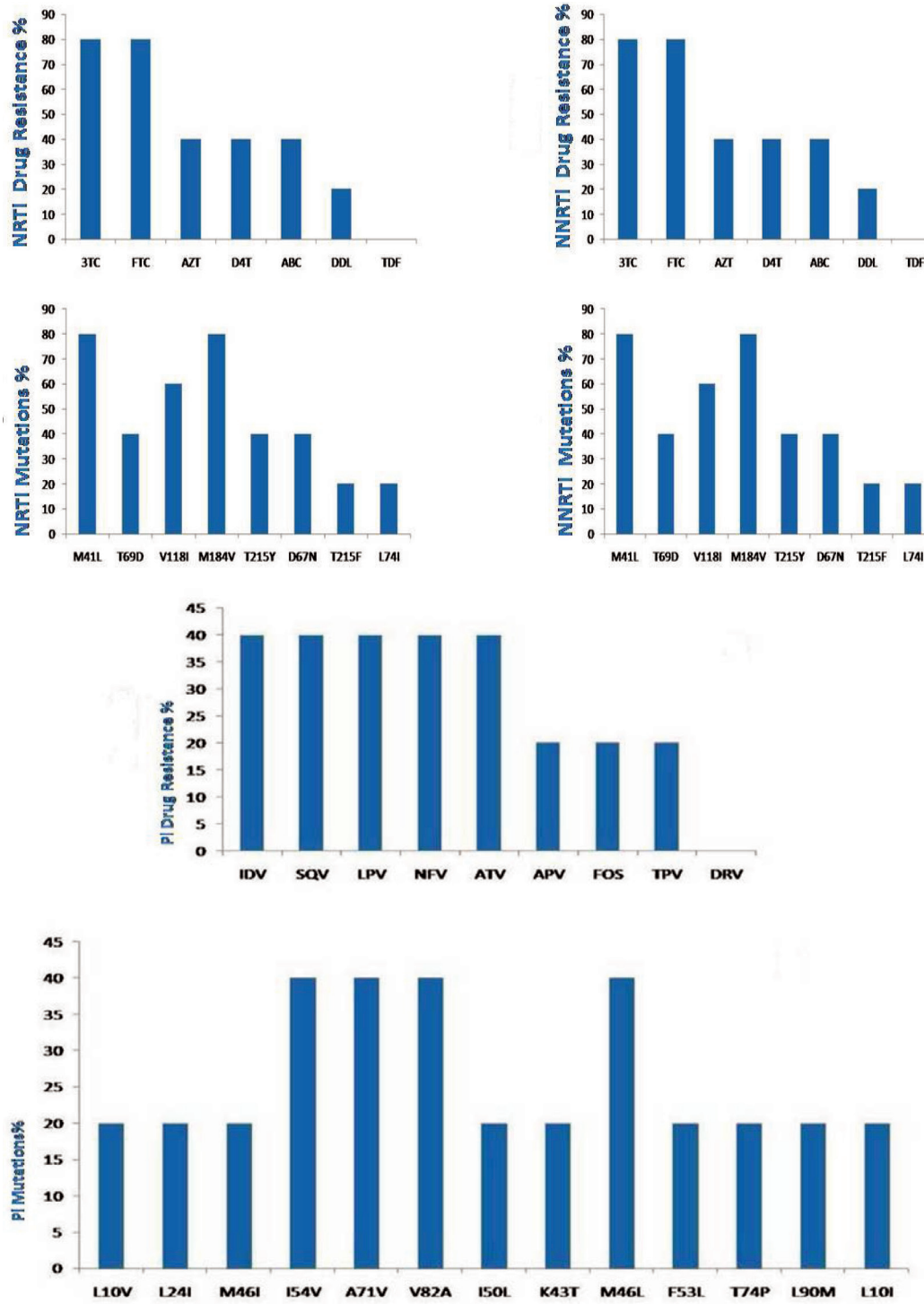


Fig 39 HIV Drug resistance pattern among patients on 2nd line ART

Title: HIV risk among long distance truckers in West Bengal

Principal Investigator : M. K. Saha.

Long Distance Truckers (LDT) are at higher risk of sexually transmitted infections including HIV but study on socio-behavioral risk factors of HIV among LDT is limited in India. A cross-sectional study in WB among 998 LDTs recruited through consecutive sampling, interviewed and tested for HIV anonymously in

HSS found 3.71 % (2.53 - 4.88) HIV sero-positivity. Regression analyses revealed that 72.62% and 17.03% LDTs had sex with female-casual-partner and male-partner, respectively, in last 6 months. Participants who were unmarried/widower/separated, educated up to higher-secondary level and visited HSS sites for medical care/testing and for recreational purposes were less likely to have paid for sex with a casual female-partner. Odds of having sex with men were lower among subjects with better education, but higher in rural areas. Educated LDTs and those who stayed longer at home were less likely to be HIV positive.

Title: Immobilization of Cellulase onto single-walled carbon nano-tube for activity enhancement and thermo-stability

Principal Investigator : M. K. Saha

Cellulase is being used extensive in food and agriculture industry. Primarily due to its potential use in the fermentation of biomass into biofuels its demand is in rise. But, instability, high water solubility, low catalytic efficiency and high cost of enzyme have become the main obstacles for the development of large scale operations and applications. Cellulase was immobilized on carbon nano-tubes to enhance its stability and activity (Fig 40).

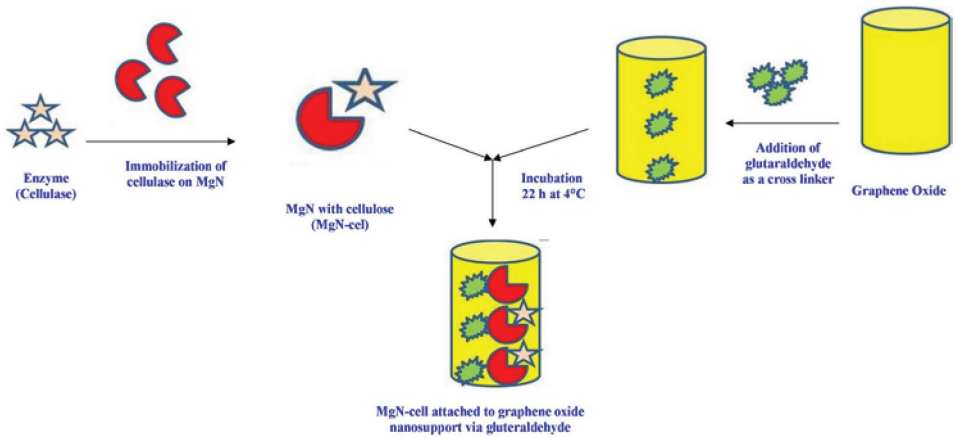


Fig 40 Immobilization of Cellulase on Graphene Oxide nanosupport

Cellulase enzyme purified from natural source showed optimum activity at 15°C and pH 8.0. Whereas, magnesium oxide nanoparticle supplemented enzyme when immobilized on graphene oxide nanosupport via glutaraldehyde as cross linker showed 2.98 fold increase in enzymatic activity at 8°C and more than 3.5 fold activity increment at 90°C. Activity of modified enzyme was retained even after 12 repeated uses and showed storage stability at 4°C for more than 120 days. This nanoparticle assisted enzyme immobilization technique might be an effective solution for bioprocessing industries which require functioning at extreme temperature ranges.

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

Principal Investigator : M. Chawla-Sarkar

As part of 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 1918 samples were tested during 2015-16, of which 790 (41.2%) were positive for rotavirus. Genotyping revealed circulation of G1 P[8]/P[6], G2P[4]/P[8] and G9P[8]/P[4]. Uncommon strains of zoonotic origin such as G10 P[14] and G12 P[11] were also identified (Fig.41).

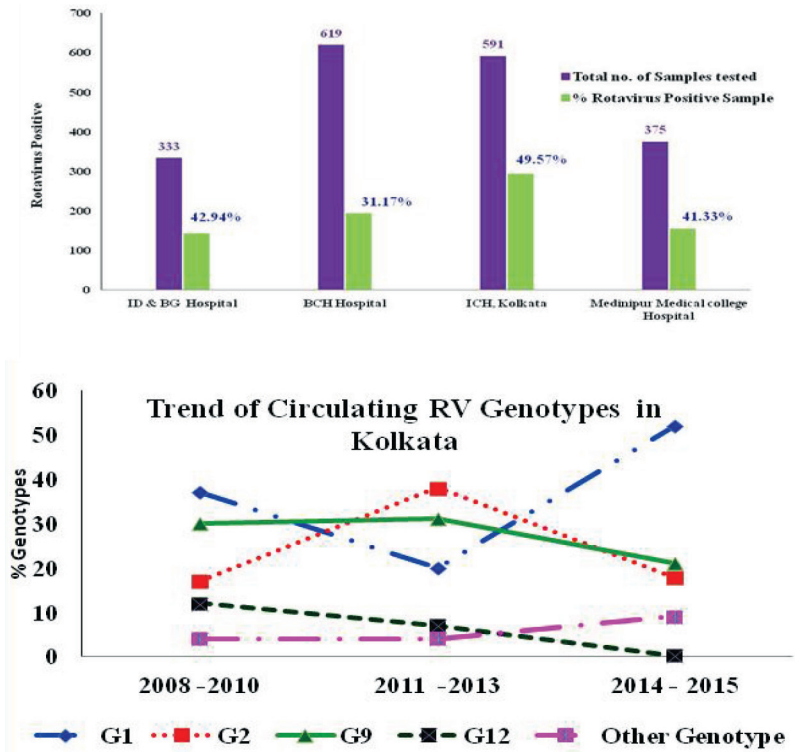


Fig 41 Surveillance and Genotyping of Circulating Rotavirus Strains among children (<5yrs) seeking health care facility for treatment of acute gastroenteritis

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

Global high-throughput studies like whole cell proteomics and tyrosine phosphoproteomics were undertaken to identify the novel host determinants as targets for developing anti-rotaviral drugs. Calmodulin a cellular Ca regulator and Cordycepin, a derivative of nucleoside adenosine, are being evaluated as anti-viral therapeutics both *in vitro* and in mouse model. W-7 (CAM inhibitor) and Cordeycepin (adenosine analog) reduced RV titers/ shedding both *in vitro* and in an illeal loop model. Protection was obtained up to 24hr, after virus infection at both low and high multiplicity of infection. In Cordycepin treated cells, increased induction of IFN was observed suggesting antiviral effects of Cordycepin are due to activated innate immune responses. Incubation of cell with Cordycepin leads to RIG-MAVS interaction which through IRF3 activation resulted in induction of IFNs and downstream antiviral proteins (Fig. 42). By using PatchDock, FireDock and GOLD v5.2 programs probable docking sites and orientation of Cordycepin and RIG-I was predicted. Cordycepin was found to have relatively higher binding probability for ATP binding domain than other domains. Overall results highlight anti-rotavirus activity of Cordecypin through modulation of host innate immune responses (Chanda S *et al.*, 2015). As an effective alternative or in combination with vaccine mediated prevention, anti rotaviral therapeutic drugs have potential to reduce the morbidity and hospitalization rates of the disease.

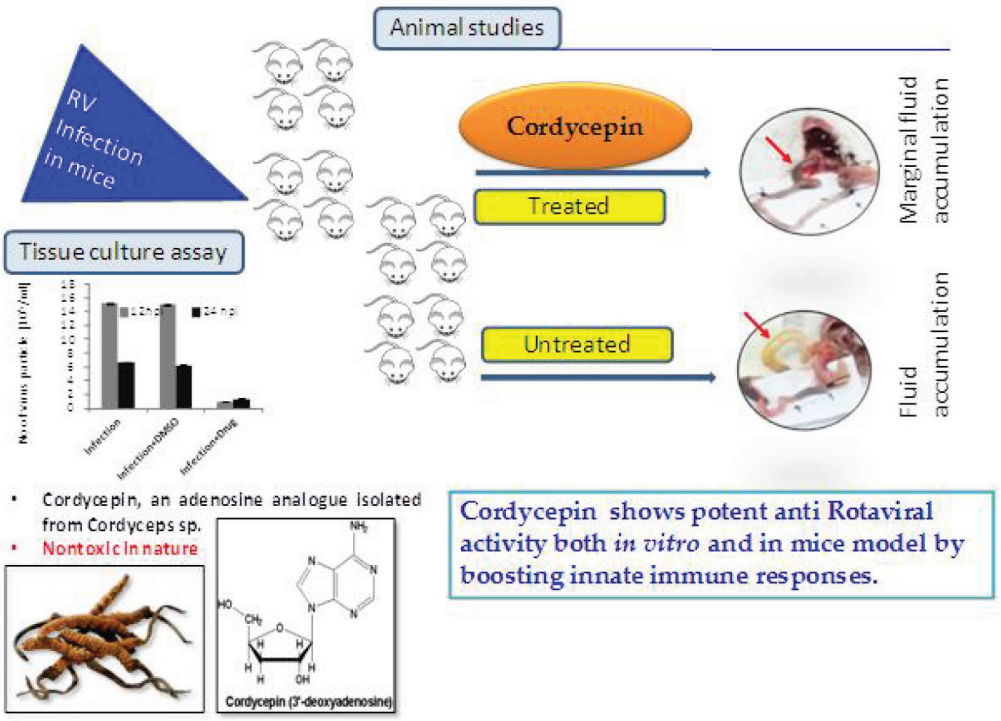


Fig 42 Assessment of small molecule such as Cordecypin as potential anti-viral candidate

Awards/Honours received

T. Krishnan

- Recognised by International Biographical Centre as a member of the IBC Top 100 Professionals 2015, Ely England Great Britain in April 2015
- Update was invited in April 2015 for current biography to be included in Who's Who in the World 2016 33rd Edition
- Evaluation of research project 14103316-M as external reviewer for Research Grants Council (RGC) of Hong Kong in April 2015
- Invited to deliver a lecture titled 'Emerging variants of human astroviruses associated with severe acute watery diarrhoea among children in Kolkata' on 16th April 2015 in the Scientific Session II Viral Genetics and Evolution at the 4TH Molecular Virology Meeting held between 16 - 17, April 2015 at Rajiv Gandhi Centre for Biotechnology Thiruvananthapuram, Kerala.
- Recognised by International Biographical Centre as a member of the IBC Leading Scientists of the World 2015, Ely England Great Britain in May 2015
- Selected as the Da Vinci Laureate for The Da Vinci Diamond for inspirational achievement by International Biographical Centre, Ely England Great Britain in June 2015.
- Member of the Academic Council/Research Review Committee (RRC) of National Institute of Cholera and Enteric Diseases since July 2015.
- Invited to chair the session and deliver a lecture titled 'The emerging etiology of viral gastroenteritis agents in Kolkata, India from acute watery diarrhoea cases' at the Indo Global Health Summit in the section Health Issues Part 1 on 23 July 2015.
- Biography was included in the 2000 Outstanding intellectuals of the 21st Century 2015 Edited by Sara Rains from International Biographical centre, Ely, England, Great Britain in July 2015.
- Invited to deliver a lecture on 'Viral diarrhoea' to celebrate Intensified Diarrhoea Control Fortnight on 7th August 2015 in Department of Microbiology, Lady Brabourne College, Kolkata.
- Highlighted as an outstanding scientist and worthy recipient of THE CAMBRIDGE CERTIFICATE for outstanding scientific achievement by International Biographical Centre, Ely England Great Britain in September 2015.
- Invited to celebrate Science Day and deliver the lecture titled 'Understanding genomic diversity of emerging

viruses' during the One Day National Seminar on Genomic Perspectives of Host Pathogen Interaction sponsored by the West Bengal Department of Science and Technology on 3rd December 2015 held in the Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata.

- Invited to join the Editorial Board of the World Journal of Clinical Infectious Diseases for a term period from 2016 to 2019 in December 2015.
- Chairperson of Complaints Committee on Sexual Harassment of Working Women in National Institute of Cholera and Enteric Diseases since December 2015
- Evaluation of PhD Thesis submitted to National Institute of Technology, Rourkela, Odisha as external examiner in January 2016
- Invited to deliver a lecture titled 'Gastroenteritis Viruses of Public Health Importance' during the seminar on Modern Trends in Environmental Microbiology on 26 February 2016 held in Department of Microbiology, Scottish Church College, Kolkata.
- Compiled and edited the Annual Report of National Institute of Cholera and Enteric Diseases during 2015-2016 with the team, for the period April 2014 to March 2015.
- Selected as a peer reviewer of Dove Medical Press for the journals Advances in Genomics and Genetics, Clinical Epidemiology and Research and Reports in Biology.
- Selected by the Science Publishing Group journal American Journal of Life Sciences as a peer reviewer.
- Recognised by Publons as one of the top reviewers for British Journal of Pharmaceutical Research of SCIENCEDOMAIN international.
- Recognised by Publons as one of the top reviewers for Archives of Virology, official journal of the virology division of the International Union of Microbiological Societies.
- Recognised by Publons as one of the top reviewers for British Biotechnology Journal of SCIENCEDOMAIN international.
- Recognised by Publons as one of the top reviewers for Amer J Trop Med and Hygiene official journal of American Society of Tropical Medicine and Hygiene.
- Recognised by Publons as one of the top reviewers for Archives of Medical Science from Poland.
- Recognised by Publons as one of the top reviewers for British Microbiology Research Journal of Science of SCIENCEDOMAIN international.
- Recognised by Publons as one of the top reviewers for Infection Genetics Evolution Journal of Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases.
- Recognised by Publons as one of the top reviewers for Journal of Disease and Global Health (ISSN: 2454-1842) [NLM ID: 101664146] aims to publish high quality papers in all areas of Disease and Health Research.
- Recognised by Publons as one of the top reviewers for Journal of Clinical Virology The Official journal of the Pan American Society for Clinical Virology and The European Society for Clinical Virology.
- Recognised by Publons as one of the Editors and top reviewer for BMC Infectious Diseases for Viral Diseases of BioMed Central The Open Access Publisher.
- Recognised by Publons as one of the Editors and top reviewer for International Journal of Tropical Disease and Health that was formerly known as American Journal of TROPICAL MEDICINE & Public Health.
- Recognised by Publons as one of the Editors for W J Clinical Infectious Diseases of Baishideng Publishing Group of open access journals.

Dr. M.K. Saha

- Has been actively contributing as an Expert for the Development of National Resources by providing scientific & technical inputs for leading National Institutions/Organization:
- Member, Sectional Committee Immuno-Biological Diagnostic Kits, Bureau of Indian Standards. New Delhi, Govt of India. Since 2010.
- Member, Expert Committee, Strengthening of Quality Control Testing Procedure of Immuno Diagnostic Kit Laboratory (IKDL), National Institute of Biologicals, New Delhi, Govt of India, Since 2014.
- Member, Technical Resource Group, Lab Services, NACO, Govt of India.
- Technical Expert for HIV Rapid Test Kit. West Bengal SACS.

Development and strengthening of National Human Resources

- Vice-Chancellor's nominee, Committee for Promotion under Career Advancement Scheme for Asst & 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.
- External Examiner, West Bengal University of Animal and Fisheries Sciences.
- Ph D thesis Examiner, West Bengal University of Health Sciences.

Development of Laboratory Quality System as per International Standards for achieving Excellence and Accreditation.

- Untiring efforts of Dr. M.K. Saha with support from Institute management NICED lab achieved the ISO-15189: 2012 standard and through rigorous process of assessment by the Assessors, the Lab was accredited by NABL for the QUALITY & COMPETENCE.
- NICED laboratory has been in the process of expanding scope of NABL accreditation including Microbiology and Virology for Biochemical, Serological and Molecular Testing which are being conducted for different National Program and Clinical Research activities.
- On the journey of Excellence in Performance, the HIV Molecular lab has been receiving appreciation from different National and International Organizations. Division of Global HIV/AIDS, CDC, USA, recognized NICED lab for outstanding performance for the Qualitative HIV 1 DNA Testing Using Dry Blood Sample (DBS) Consecutively for 4 years (2012, 2013, 2014 & 2015).
- NICED being in the forefront of National HIV Control Program has been implementing External Quality Assurance for the State Reference Labs of Andaman & Nicobar, Orissa, Jharkhand, Assam, Meghalaya and Mizoram.
- All the NICED labs involved in National AIDS Control Program received 100% scoring in proficiency testing conducted by outside agencies.

Conferences/Seminars/Workshops/Trainings Attended/Organised

T. Krishnan

- Oral presentation titled 'Emerging variants of human astroviruses associated with severe acute watery diarrhoea among children in Kolkata' on 16th April 2015 in the Scientific Session II Viral Genetics and Evolution at the 4TH Molecular Virology Meeting held between 16-17th April 2015 at Rajiv Gandhi Centre for Biotechnology Thiruvananthapuram, Kerala.
- Oral presentation titled 'The emerging etiology of viral gastroenteritis agents in Kolkata, India from acute watery diarrhoea cases' on 23 July 2015 at the Indo Global Health Summit in the section Health Issues Pat 1 on 23 July 2015 in Hyderabad, India.
- Oral presentation titled 'Viral diarrhoea' to celebrate Intensified Diarrhoea Control Fortnight on 7th August 2015 in Department of Microbiology, Lady Brabourne College, Kolkata.
- Oral presentation titled 'Understanding genomic diversity of emerging viruses' during the One Day National Seminar on Genomic Perspectives of Host Pathogen Interaction sponsored by the West Bengal Department of Science and Technology on 3rd December 2015 held in the Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata. and celebration of Science Day.
- Oral presentation titled 'Gastroenteritis Viruses of Public Health Importance' during the seminar on Modern Trends in Environmental Microbiology on 26 February 2016 held in Department of Microbiology, Scottish Church College, Kolkata.

M.K. Saha

- Sensitization and Continued Medical Education (CME) on HIV World AIDS day. Kothari Medical Centre. Kolkata. 1st December, 2015. Invited Speaker (Topic: HIV Sentinel Surveillance).
- Workshop on "HIV/AIDS: Socio-behavioral Perspective at Sociology Department, Kalyani University. 21st March 2016. Invited Speaker (Topic: HIV/AIDS: Socio-behavioral Perspective).

M. Chawla Sarkar

- 17th International Congress on Infectious Diseases-2016, Hyderabad. 2-5 March, 2016. Poster Presentation. Surveillance and molecular characterization of Rotavirus strains in hospitalized children with gastroenteritis in West Bengal. M. K. Nayak, P. Mandal, N. Ganguly, P. Niyogi, C. Ghosh, S. Panda, M. Chawla-Sarkar.
- 17th International Congress on Infectious Diseases-2016, 2-5 March, 2016, Hyderabad. Poster Presentation.

Molecular characterization of human enteric adenovirus circulating among children below five years of age in Kolkata, India. A. Banerjee, M. Chawla-Sarkar.

- 17th International Congress on Infectious Diseases-2016, Hyderabad. 2-5 March, 2016. Poster Presentation. Detection and molecular characterization of unusual rotavirus group A genotypes G12P[11] and G10P[14] in hospitalized children with acute gastroenteritis in Kolkata, India. P. Mandal, S. Mullick, M. Chawla-Sarkar.
- 12th International dsRNA Virus Symposium 2015, Goa. 6-10th October 2015. Oral presentation. Modulation of both cell survival and apoptotic pathways during virus infection by rotavirus encoded non-structural proteins. M. Chawla Sarkar.
- 12th International dsRNA Virus Symposium 2015, Goa. 6-10th October 2015. Poster Presentation. miR-142-5p Augments Rotavirus Infection by Targeting Non Canonical TGFβ Signaling and Apoptosis. S. Chanda, S. Nandi, M. Chawla-Sarkar.
- 12th International dsRNA Virus Symposium 2015, Goa. 6-10th October 2015. Poster presentation. Rotavirus Disrupts Cytoplasmic Processing Bodies during Infection. U Patra, R Bhowmick, A Mukherjee, M. Chawla Sarkar.
- "Hands on training on Molecular diagnosis of InfA/H1N1 viruses" on 13th January 2016 at Virology Division, NICED. Organizer Dr. M Chawla Sarkar, Scientist E; Trainees: WB State Health Virologists and Technicians from Tropical School of Medicine Kolkata. (Pic)

Training workshop for WB State Health Department Virologists for 'Molecular Diagnostics of INF A/ H1N1/2009'

S. Dutta

Confirmed the identification and serotyping of *Salmonella & Shigella* isolates received at NICED from various Medical colleges and other academic institutions of India. Timely feedbacks were sent to the concerned organizations.

M.K. Bhattacharya

1. Associated with the Diarrhoeal Treatment & Training Unit (DTU) at the Infectious Diseases Hospital, Kolkata and Diarrhoea treatment unit in the outpatient department (OPD) at Dr. B.C Roy PGIPS. The objectives of the DTU are as follows:
   a) To treat the children and infants suffering from some dehydrating diarrhea with ORS.
   b) To educate mothers on how to treat a diarrhoeic child with ORS or other home available fluid to prevent dehydration and also give necessary health care.
   c) To educate the community about the use of ORS in diarrhoea and impact of health care through mothers who are getting education at the DTU.
   d) To increase the awareness for using ORS in the community through mothers who are getting education at the DTU.
2. First time in West Bengal on behalf of NICED, We started surveillance programme for Diarrhoeal Diseases at the Infectious Diseases Hospital, Kolkata, with the following objectives:
   a) To monitor changes in disease patterns including drug sensitivity (particularly for cholera and shigellosis).
   b) To create database on diarrhoeal diseases and to generate preliminary information for researchers to design new research protocols.
   c) To develop an early warning system for forecasting an epidemic.
   d) To furnish information to be applied for improvement in patient care and better preventive measures.
3. We are also helping the Health Department, Govt. of India and Govt. of West Bengal supplying the report as suggested by the institutional head
4. We are actively involved in routine teaching of internees from different medical colleges of Kolkata at the DTU ward of I.D. Hospital.
5. We are responsible for investigation of any outbreak/epidemic that occurs due to diarrhoeal illness or any other illness.

B.L. Sarkar

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

K. Sarkar

Staff of Epidemiology Division actively participated in 'Swachh Bharat' Mission and educated school children of various schools in Kolkata.

S. Panda

Scientists of NICED participated in the Sundarban Kristimela (fair) and Loko Sanskriti Utsab, Kultali, Basanti during December 20-29, 2015. Participation of NICED as an ICMR Institute in this event was part of the Council's effort to highlight activities of public health importance at the grass-root level and enhance

awareness about common health problems among the local mass. Everyday a team from NICED constituted by various scientists and other staff were present at the Kristimela throughout the aforementioned period. On one of these days, Dr. V.K. Srivastava, Scientist G and Head, Division of Publication and Information, ICMR and Dr. Shanta Dutta, Director-in-Charge, NICED visited Krishtimela and addressed the attendees on ways to contain diarrhea. Posters with messages on different health issues were displayed at the stall of NICED in this fair. Activities at this stall revolved around answering queries from visitors, explaining the contents of the posters to the audience and distributing brochures on 'what to do at home during diarrhea' and 'steps of hand washing' (photo of the NICED stall attached).

NICED team at Sundarban Kristimela

T. Krishnan

Short term training was accorded to post graduate students from various institutions to prepare a dissertation based on the research work, as part of their curriculum.

M.K. Saha

A. Services provided by NICED

1 Quality Assurance for HIV Testing:

a) **External Quality Assurance** for HIV testing for the State Reference Labs (SRLs) of Andaman & Nicobar, Assam, Jharkhand, Meghalaya, Mizoram and Orissa.

- Conducting Proficiency Testing for 12 SRLs and approx. 432 ICTCs of these statesQuality Assurance for HSS Lab result (Retesting of all positive and 5% negative).
- Quality Assurance for HCV lab results generated by 12 SRLs (Retesting of all positive and 2% negative)
- Testing laboratory for HIV Sentinel Surveillance (ANC) 2015.
- Referral service for confirmation of HIV testing results of the samples received from different SRLs and other organizations.
- Training for Medical Officers, Lab/Program Supervisors and Medical Lab Technologists for HIV testing as and when requested by different organizations.
- Testing of HIV and HCV Dry Blood Spot samples for Integrated Biological and Behavioral Surveillance (IBBS). (Table 1) and (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 Medicine	04	04	00

Table 2 HIV Sentinel Surveillance 2015 (ANC): Quality Assurance for SRLs under NACO NRL, NICED, Kolkata and other Testing Center (sample received from April 2015 to March 2016)

Sl. No	Name of SRL/Testing Centre	Samples sent by SRL		Samples rejected by NRL	Confirmed Result at NRL		Discordant
		HIV_{-ve}	HIV^{+ve}		HIV_{-ve}	HIV^{+ve}	
1.	Assam Medical College Dibrugarh, Assam	140	03	00	140	03	Nil
2.	Guwahati Medical College Guwahati, Assam	320	09	160	161	08	01
3.	Silchar Medical College Silchar, Assam	80	07	00	80	07	Nil
4.	Pataliputra Medical College, Dhanbad, Jharkhand	24	00	00	24	00	Nil
5.	MGM Medical College, Jamshedpur, Jharkhand	11	00	00	11	00	Nil
6.	SCB Medical College, Cuttack, Orissa	144	11	00	144	11	Nil
7.	VSS Medical College, Burla, Orissa	85	02	00	85	02	Nil
8.	MKCG Medical College, Beharampur, Orissa	162	10	04	158	10	Nil
9.	GB Pant Hospital, Port Blair, A & N	84	01	02	82	01	Nil
10.	Pasteur Institute, Shillong, Meghalaya	81	05	00	81	05	Nil
11.	Tura Civil Hospital, Meghalaya	75	00	00	75	00	Nil
12.	Regional Institute of Medical Sciences, Imphal	134	25	01	134	24	Nil
13	School of Tropical Medicine, Kolkata, WBl	99	04	00	99	04	Nil

b) 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) and (Fig 1)

Table 3 Referral Service done for the institutions at NACO NRL, NICED, Kolkata

Sl. No.	Source of Samples	No. of sample Tested	No. of sample Positive
1.	Command Hospital, Kolkata	40	34

Table 4 HCV Testing 2015 (ANC samples): Testing Center data, NACO-NRL, NICED, Kolkata

District	Site Name	Sample received	Sample rejected	Sample tested
Kolkata	Abinash Dutta Maternity Home	400	00	400
Kolkata	Vidyasagar State General Hospital	400	00	400
Nadia	Nabadwip State General Hospital	400	00	400
Nadia	Aranghata BPHC	202	02	200
Nadia	Ranaghat Sub-Divisional Hospital	201	01	200
24 Parganas(North)	Madhyamgram Rural Hospital	206	06	200
24 Parganas(North)	Barasat District Hospital	205	05	200
TOTAL SAMPLE TESTED = 2400				

Table 5 HCV Testing using Dried Blood Spot Samples for IBBS 2014-2015

State	No. of Sample received	No. of Sample rejected	No. of Sample tested
Assam	2831	00	2831
Chhattisgarh	5155	20	5135
Nagaland	2009	03	2006
TOTAL SAMPLE TESTED = 9972			

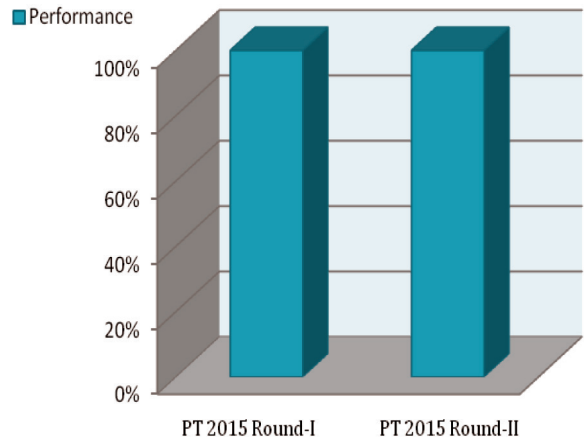


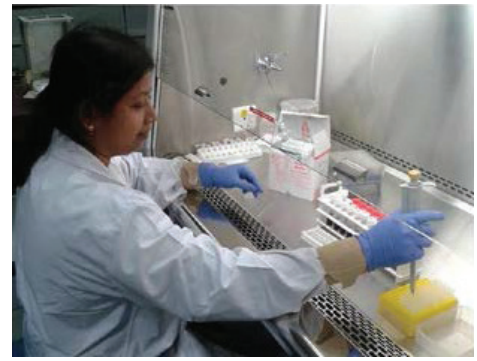
Fig 1 Performance of NRL, NICED in Proficiency

2. Diagnostic Kit Evaluation by Consortium of NRLs at NICED

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)

Table 6 Diagnostic Kits Evaluated by NICED Consortium Lab Testing Center data, NACO-NRL, NICED, Kolkata

Type of Kit	No of Batch/ Lot evaluated
HIV ELISA	06
HIV Rapid	11
HBsAg ELISA	09
HBsAg Rapid	01
HCV ELISA	04
HCV Rapid	01
Total	32



Testing at ICTC Lab

Integrated Counselling & Testing Centre (ICTC)

- Providing information on modes of HIV transmission.
- Promoting behavioural change to reduce vulnerability.
- Conducting HIV diagnostic tests.
- Providing psychological support.
- Link people with HIV prevention, care & treatment services. (Table 7) and (Table 8)

Table 7 HIV testing details at ICTC (April'15-March'16)

Total Tested	Positive	Positivity	Pre ART Registration	Referred from RNTCP	HIV-TB Co-infection
1340	33	2.46%	30	154	6

Table 8 HRG Client details

HRG	Number tested	Positive	Positivity
Female Sex Worker	26	0	0
Men who have Sex with Men	413	6	1.45%
Transgender/Transsexual	218	3	1.38%

3 Early Infant Diagnosis

Molecular diagnosis of HIV among babies (up to 18 months) born to HIV infected mothers employing Dried Blood Spot (DBS) samples in massive scale covering all the Eastern and N-E states (Andaman & Nicobar, Arunachal, Assam, Bihar, Jharkhand, Manipur, Mizoram, Meghalaya, Nagaland, Sikkim, Tripura, and WB) of India is being done at NICED Regional Reference Lab (RRL) to address the monumental challenge of implementing the nationwide program to ensure early initiation of ART for the infected babies and also to monitor effectiveness of existing practice of PPTCT (Prevention of Parent To Child Transmission).

Evidence generated rationalizes national ART regimen for preventing mother to child HIV transmission (aiming zero transmission) as well as use of only DBS sample (removing whole blood sample test for confirmation) for molecular diagnosis of HIV.

Automated Sample Processor for Molecular Assay.Abbott m2000sp

Realtime Thermal Cycler Customized for HIV, HBC & HCV Molecular Assay. Abbott

Molecular Testing (HIV-1 PCR)

Presently, 1200 ICTCs are involved in collection of DBS samples in 14 states under NICED-RRL for DBS HIV-1 TNA PCR. All the DBS HIV-1 PCR reactive/detected specimens are confirmed by a 2nd PCR assay using another DBS sample of the baby.

A total of 1317 DBS samples was received. At NICED-RRL and among those 1312 DBS samples were tested for the period of and their status is depicted below. (Table 9) and (Fig 3)

Table 9 Status of EID DBS samples received and tested

Name of States	No. of total DBS samples received	No. of total DBS samples tested	HIV-1 DNA detected
West Bengal	349	348	22
Orissa	141	140	14
Chhattisgarh	187	187	14
Bihar	150	150	12
Jharkhand	100	100	10
Mizoram	132	131	4
Assam	82	81	3
Manipur	69	69	8
Nagaland	90	89	9
Meghalaya	14	14	1
Arunachal Pradesh	1	1	0
Sikkim	0	0	0
Tripura	0	0	0
A & N Islands	2	2	0
TOTAL	1317	1312	97

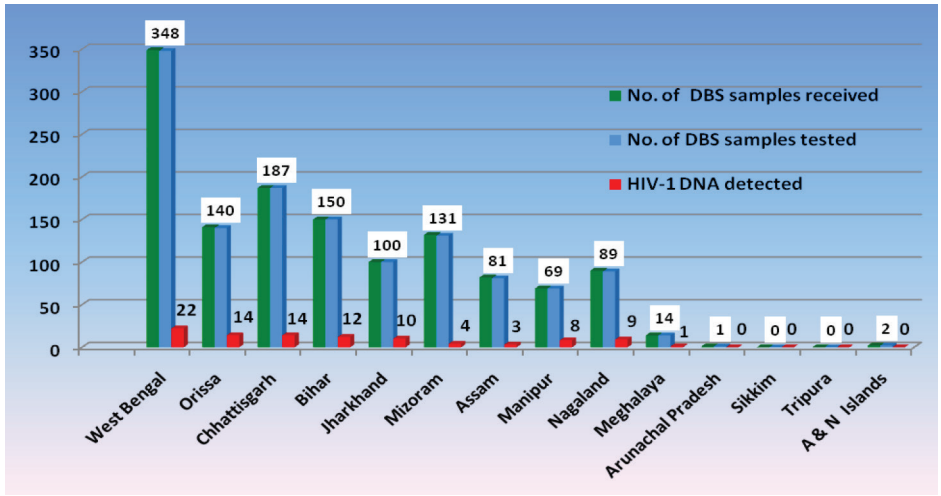


Fig 3 Status of EID DBS samples received and tested (with HIV-1 positivity) at NICED

4 Regional Institute (East) for HIV Surveillance

NICED is involved in implementation of HIV Sentinel Surveillance (HSS) and Integrated Biological & Behavioral Surveillance (IBBS) for the East and North Eastern states with the aim to monitor the (i) trends in prevalence of HIV infection, (ii) distribution and spread of HIV prevalence in different population subgroups and in different geographical areas and (iii) to identify emerging pockets of HIV epidemic in the country. RI (E) also has an important role in data entry and data management of HSS and IBBS. (Table 10)

Table 10 No. samples allotted for HSS-ANC round

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

- Technical support & guidance to State AIDS Control Society (SACS) in overall planning & implementation of HSS in allocated Indian states, facilitating smooth implementation of surveillance activities by liaising with concerned state authorities addressing specific problems at sentinel sites/ testing labs.
- Technical validation & approval of new site reviewing relevant data & site visits.
- Regional Pre and Post Surveillance co-ordination & planning meetings, Regional Trainings and Workshops for HSS & IBBS.
- Technical & Supervisory support for state level training of site & lab personnel.
- Monitoring & Supervision during HSS & IBBS 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 specific proposal from SACS related to HSS & IBBS.
- Submission of report of activities undertaken during surveillance and analysis of the surveillance findings in the allocated states. (Fig 4)

State level training for HSS, ANC Round

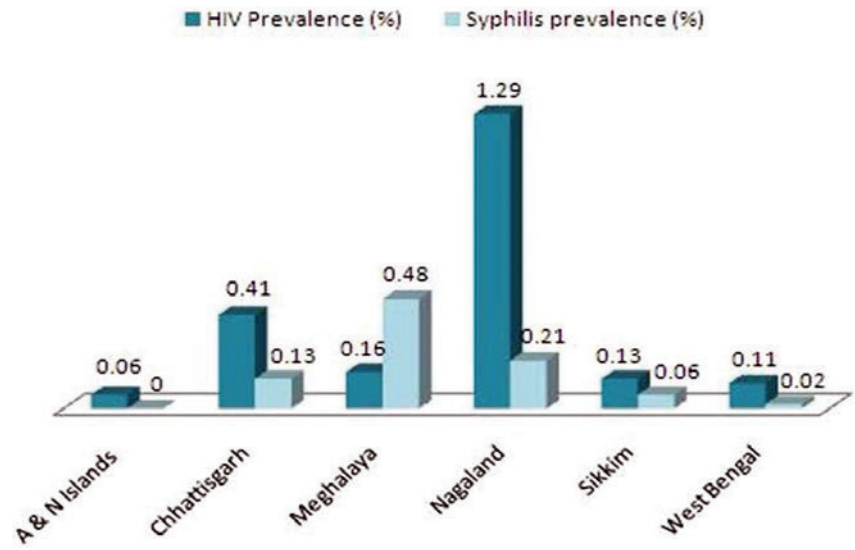


Fig 4 HIV & Syphilis Prevalence among Ante Natal Clinic Attendees

5 HIV Viral Load Assay

- HIV Viral load assay for East & N-E for ensuring efficacy of ART and evidence based decision for continuation or initiation of further treatment.
- NICED Molecular HIV lab restarted HIV viral load assay for the patients under ART for monitoring effectiveness of ongoing treatment as per national guidelines and also to assist in HIV drug resistance mutation assay.
- During 2015-16, a total of 551 viral samples were received at NICED for HIV viral load estimation and assay was performed for all the samples. (Table 11) and (Fig 5)
- In most of the cases HIV 1 viral load was below detectable level (<37 copy/ml).
- A significant proportion of patients under ART showed Virologic failure (HIV -1 Copy no > 1000 /ml).
- Continuous support for HIV viral load estimation is essential for effective treatment options

Table 10 No. samples allotted for HSS-ANC round

Total Sample	Sample Having <37 HIV copy /ml	Sample Having Between 37 to 1000 HIV copy/ml	Sample Having >1000 HIV copy /ml
551	270	85	196

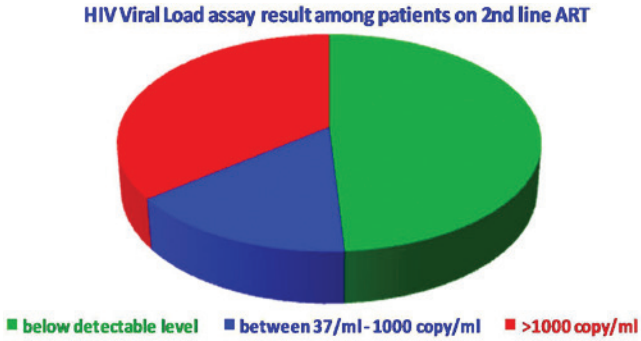


Fig 5 Distribution of HIV Copy/ml

S.S. Das

The outpost of the OPD of B.C. Roy PGIPS 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.

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.

S. Ganguly

- Training and support on parasite detection and isolation.
- Field studies have been performed during last fiscal year from this division, in Chakdah, Nadia, West Bengal for investigation of presence of different enteric parasites by improper hand wash and in Sikkim, Tripura, Uttar Pradesh, Chattisgarh and Telengana for identification of different soil transmitted helminths among school children.
- Quality Control and Quality Assessment support facility in eastern India for parasitic detection.

M. Chawla-Sarkar

NICED virology lab was identified as a single point testing facility for H1N1/2009 outbreak during January 2015. A total of 1923 samples (hospitalized cases under category bii and c) were tested during the year (Jan-Dec 2015), where 448 cases were identified as InfA/ H1N1/2009 and 12 were subtyped as InfA/H3N2.

S. Kanungo

As a part of the endeavor towards making our environment clean and healthy, several activities were taken up in the community, involving school children and local clubs in the urban slums of Kolkata. These include public lectures, seminars and sit and draw competition among young children in urban slums. Participants were mostly people living in urban slums, school children, parents and teachers. The students were encouraged to disseminate the information in their field activity at present and future by remembering if they can educate a mother / woman of a family about cleanliness as it will improve the society.

Interacting with students on the importance of personal hygiene and clean environmen

Students engaged in sit and draw competition on the theme ‘Clean Environment Leading To Healthy Life’

Encouraging hand washing among school children

OUTBREAK INVESTIGATION

NICED participation in National Task Force (NTF) programme on Laboratory Containment of Wild Polio Viruses

This institute primarily works on bacterial, viral and parasitic pathogens isolated from stool specimens collected from diarrheal patients. Institute conducts vaccine trials linking epidemiological findings with laboratory based analysis in the area of bacteriology, virology, parasitology, immunology, molecular biology and clinical medicine. To maintain aforementioned activities, a large number of stool specimens and/ or water samples from diarrheal outbreak affected areas are being collected and stored in the freezer(s) before being analyzed at NICED laboratories. In addition, this Institute works on Influenza virus infected specimens as and when requested by the Ministry of Health & Family Welfare, State or Central Government. Aforementioned all specimens as collected for research/ diagnostic activities have been categorized under potentially Polio risk materials by the National Task Force (NTF) from the point of view of Laboratory Containment of Wild Polio Viruses.

NICED actively participated in the national programme on Laboratory Containment of Wild Polio Viruses in India since its inception in 2012. Considering potential risk from the standpoint of laboratory containment of wild polio viruses special attention has been attributed for non-storage and destruction of clinical specimens like stools, water samples, and nasopharyngeal swabs and these are to comply with Phase II activity under the NTF guidelines and Global Action Plan (GAP) III protocol (2014). Institutional 'Risk Assessment and Risk Management Committee for Containment of Wild Polio P2 Virus' was formed on August 3, 2015. Since then this Institute periodically submitted containment action reports to National Coordinator, NTF. All these activities are to comply with guidelines of 'Global Polio Eradication and the Endgame Strategic Plan (PEESP) (2013-2018)'. In addition, NICED jointly with NTF, hosted a meeting on August 14, 2015 for sensitization on the importance of PEESP and this meeting was attended by several senior scientists/ head of divisions from Institutes/ Universities who are working with potentially Polio infected materials. Finally, a certification stating that NICED is not storing any potentially Polio infected materials in the freezers have been issued in April 2016.

A. Palit

Outbreak investigations (2015-16) by the Environmental Microbiology Laboratory

During the epidemic outbreaks (2015-16) of diarrhea spreading across different southern districts of West Bengal, microbial analysis and examination of samples of potable water sources, from different parts of West Bengal and reporting of results to the Govt. agencies, has been a routine activity of the environmental laboratory of our 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 reported to the respective agencies with a copy of the same to State Health Secretariat, Govt. of West Bengal. During the period under report, 74 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 12).

Table 12 District-wise distribution of the Outbreak Water Samples, their respective sources and identification of *V. cholera*

Sl. No.	District	No. of samples received	Source					Culture Positive	PCR positive
			Tap	Tube well	Drinking water	Pond	Others		
1.	North 24 Parganas	23	15	8	-	-	-	18	6
2.	Nadia	3	-	2	-	-	1	3	2
3.	Hooghly	12	9	1	2	-	-	10	4
4	Howrah	14	10		2	2	-	10	7
5	East Midnapore	10	4	2	-	4	-	6	6
6	Kolkata	12	10	-	2	-	-	4	2
	Total	74	48	13	6	6	1	51	27

K. Sarkar

A proposal on 'Mosquito-borne surveillance to forecast any Dengue outbreak & its control at New Town City' was submitted to the New Town Development Authority for necessary funding. The proposal is accepted by the New Town Development Authority in principle and possibility of funding is being reviewed.

Alok Deb

Investigated an outbreak along with Dr Shanta Dutta of foodborne diarrheal illness that occurred in Sashan block of 24-Parganas (North) following consumption of offerings devoted to the idol in a local temple during March 1-5, 2016. More than 1000 people of different age groups, who were mostly from a particular village and consumed the offerings made to the God in a local temple, fell sick with diarrhea and vomiting within a few hours of its consumption. Some of them also presented with mild fever and headache. Around 300 people were admitted in the I.D. & B.G. Hospital, Beliaghata from whom relevant information and stool specimens were collected from 30 patients. A sample of the food distributed among the affected devotees was also received and tested.

TRAINING AND EXTENSION:

Other than research, Human Resource Development (HRD) is an important mandate of National Institute of Cholera and Enteric Diseases (ICMR-NICED). On request, ICMR-NICED regularly takes part in training the scientists, research fellows, graduate and post graduate students, physicians, laboratory personnel and paramedical staff visiting this institute. Programs like National and International seminars, symposium, workshops and conferences are organized by the scientists with the help of support staff of Training & Extension Division, utilizing well-equipped modern facilities of ICMR-NICED.

In 1980, WHO recognized this Institute as a WHO Collaborating Centre for Research and Training in Diarrheal Diseases. Among the events contributed by the Training & Extension Division during the period between 1984 and 1989, most important ones were holding around ninety National Seminars on "Oral Rehydration Therapy" in joint collaboration with WHO-ICMR-DGHS for promotion of ORT usage among diarrhea patients by the attending physicians in different states of India. UNICEF also funded training programs on "Management of Diarrhea" in the states of West Bengal, Assam and Orissa organized by ICMR-NICED. Long term (2-3 months) residential Training programs for national and international WHO Fellows were organized by this Division.Holding fifty training programs on "Immunization Strengthening Project" funded by M/o Hlth & FW, Govt. of India for mid-level managers of the districts of 11 Eastern States, A&N Island, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Sikkim, Tripura, West Bengal are the example of other activities of this Division.

ICMR-NICED also conducted eight JICA-NICED Domestic and Third country Training Programs on "Molecular Epidemiology of Diarrheal Diseases with special reference to cholera" in collaboration with

Japan International Cooperation Agency with the help of this division.Training programs including hospital and field visit for the Medical Students of Japan were also been conducted by this Division.This Division took part actively in organizing "Training Program for German Doctors on Tropical and Travel Medicine" jointly with Jadavpur University for consecutive six years. This Division was also involved in conducting "Indo US Workshop on Opportunistic Enteric Parasites" and "Workshop on diarrhea and enteric pathogens"

ICMR conducted several programs like "Workshop on Research Ethics", "Role of ICMR in disease surveillance", "Development of an Atlas of Cancer in North East India", "ICMR Annual Day Celebration", "Integrated Disease Surveillance Program", "Drinking Water quality monitoring" and many more in close association with ICMR-NICED training and extension division.

ICMR-NICED conducts "Orientation course for Ph.D. fellows" on a regular basis as a mandatory event for registration of the candidate for Ph.D. under Calcutta University. Students of courses like M.Sc Biotechnology of Visva Bharati University, M.Sc Biotechnology of Kalyani University, M.Sc Life Science of North Eastern Hill University, Shillong were also benefitted by attending lecture and training classes specially arranged for them. Graduate and post graduate students of Calcutta National Medical College, Calcutta Medical College and National Homoeopathy Medical College are trained by the scientists belonging to different divisions of this Institute.

Training Conducted During April 2015-March 2016

- Epidemiology Training Program 'Multicentric Randomised double blind placebo controlled study to evaluate the efficacy and safety of live attenuated bovine human rotavirus reassortant pentavalent vaccine against severe rotavirus gastroenteritis in healthy Indian infants' by Dr Suman Kanungo on 23-24 April 2015
- Rotavirus Training Program by Dr Suman Kanungo, Dr B Manna & Mr Tapan at 12 pm on 28 May 2015
- STH training program in Parasitology Department organised by Dr Sandipan Ganguly between 2nd-15th July 2015.
- Bioinformatics Training organised by Dr S.S. Das on 6th July 2015.
- Epidemiology Training Program organised by Dr Suman Kanungo on 15th July 2015
- Epidemiology Training Program organised by Dr Suman Kanungo on 12th August 2015
- Dr. Sanjay Bhattacharya, Consultant Microbiologist, Tata Medical Center delivered a lecture on biosafety at the behest of Biosafety Training which was held on 22nd February 2016 (Monday) from 4:00 PM in NICED II Seminar Hall

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 CD8 Cells with Toll-like Receptor Expression and Responsiveness to Pathogen/Danger-associated Molecules
PI:	**Dr. T. Biswas**
Funding Agency	DBT
Duration	2014 to 2017

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

Title:	Assessment of malnutrition & anaemia status among primary & upper-primary school students of all districts of West Bengal
PI:	**Dr. K. Sarkar**
Funding Agency	Department of School Education, Govt. of West Bengal
Duration	2 year 3 month (1st April-14 to 30th June 2016)

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:	2015-2020

Title:	Study the molecular mechanism of anticancer and antitumor effect of bacterial protease
PI	**Dr. A. Pal**
Funding Agency	(ICMR)
Duration	2014-2016 (December)

Title:	External Quality Assurance for HIV testing
PI:	**Dr. M.K. Saha**
Funding Agency	National AIDS Control Organization
Duration	2002-2017

Title:	HIV Sentinel Surveillance
PI:	**Dr. M.K. Saha**
Funding Agency	National AIDS Control Organization
Duration	2008-2017

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	2012-2017
Title:	Counseling and Testing for HIV, Blood Borne Infections and STIs
PI:	**Dr. M. K. Saha**
Funding Agency	WBSAP&CS
Duration	2012-2017
Title:	Molecular assay for HIV-1 Plasma Viral Load
PI:	**Dr. M.K. Saha**
Funding Agency	National AIDS Control Organization
Duration	2015-2017
Title:	Molecular characterization of HIV for drug resistance mutations among infant using dried blood spot sample
PI:	**Dr. M.K. Saha**
Funding Agency	ICMR
Duration	2015-2018
Title:	Utility of prevention of mother-to- child HIV transmission program data for HIV surveillance
PI:	**Dr. M.K. Saha**
Funding Agency	National AIDS Control Organization
Duration	2013-2016
Title:	A prospective longitudinal study on changes of inflammatory cytokine levels among HIV infected subjects in an Eastern Indian hospital
PI:	**Dr. M.K. Saha**
Funding Agency	WB DST
Duration	2015-2017
Title:	Exploration of the biologic basis for underperformance of oral Polio and Rotavirus vaccine in India (PROVIDE)
PI:	**Dr. R.K. Nandy**
Funding Agency	International Vaccine Institute (IVI), South Korea
Duration	2012-2016
Title:	Gastro Intestinal Tract Pathogen Repository (GTPR)
PI:	**Dr. R.K. Nandy**
Funding Agency	Indian Council of Medical Research (ICMR)
Duration	2014-2016
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	2015-2017

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**
Funding Agency	AMED through Okayama University, Japan
Duration	2015-2020

Title:	Biomedical Informatics Center of ICMR, 2nd Phase of Task-force Project (IRIS ID: 2013-1551G).
PI:	**Dr. S.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.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.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.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:	Differential pathogenesis of Giardia: Role of Giardia Virus
PI:	**Dr. S. Ganguly**
Funding Agency	NIID, Japan
Duration	Three years (2014-2016)

Title	National Rotavirus Surveillance Network - Referral Lab Eastern India
PI	**Dr. M. Chawla Sarkar**
Funding Agency	ICMR
Duration	Four years/ 2013-2017

Title:	Screening of small molecules with antiviral activity as adjunct therapy for viral diarrhea
PI:	**Dr. M. Chawla Sarkar**
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 pathogens causing viral hemorrhagic fevers and respiratory infections.
PI:	**Dr. M. Chawla Sarka**r
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	Development and evaluation of a heat killed multi-serotype oral Shigella vaccine
PI	**Dr. H. Koley**
Funding Agency	Okayama University, Japan
Duration	Four years/ 2015 to 2019

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

Title	Phase III, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Live Attenuated Bovine-Human Rotavirus Reassortant Pentavalent Vaccine (BRV-PV) Against Severe Rotavirus Gastroenteritis in Healthy Indian Infants
PI	**Dr. S. Kanungo**
Funding Agency	PATH Vaccine Solutions, USA
Duration	Three Years/ 2014-2017

Title	Exploration of the Biologic Basis for underperformance of Oral Polio and Rotavirus Vaccine in India
PI	**Dr. S. Kanungo**
Funding Agency	International Vaccine Institute
Duration	Three and half years/ 2012-2015

PUBLICATIONS

Research Publications during April 2015-March 2016

1. Ali M, You YA, Sur D, Kanungo S, Kim DR, Deen J, Lopez AL, Wierzba TF, Bhattacharya SK, Clemens JD. Validity of the estimates of oral cholera vaccine effectiveness derived from the test-negative design. Vaccine. 2016 Jan; 34(4): 479-85.
2. Ali M, You YA, Kanungo S, Manna B, Deen JL, Lopez AL, Wierzba TF, Bhattacharya SK, Sur D, Clemens JD. Assessing different measures of population-level vaccine protection using a case-control study. Vaccine. 2015 Nov; 33(48): 6878-83.
3. Banerjee A, Ghosh MK, Karak K, Basu S, Mukhopadhyay BB, Mallik S, Saha B. Lower respiratory tract infection in Kolkata and multidrug resistant pathogen- with a focus on carbapenem resistant organism. Int J Contemporary Med Res.2016 Mar; 3(3): 873-880.
4. Banerjee R, Roy A, Das S, Basak S. Similarity of currently circulating H1N1 virus with the 2009 pandemic clone: viability of an imminent pandemic. Infect Genet Evol. 2015 Jun; 32:107-12.
5. Barman RK, Jana T, Das S, Saha S. Prediction of intra-species protein-protein interactions in enteropathogens facilitating systems biology study. PLoS One. 2015 Dec; 10(12): e0145648.
6. Bhattacharjya C, Patel SK, Panda S, Deb AK. Changing HIV epidemic in North-Eastern India and its relationship with development and programmatic indicators. World J AIDS 2015 Sept; 5: 265-74.
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8. Bhattacharya A, Moitra S, Sharma Sarkar B, Bhattacharya MK. Swine flu a clear and present danger that rocked India in 2014-15. J Int Academic Res Multidisciplinary, 2015 Dec; 3(11): 1-7.
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11. Bhowmick R, Mukherjee A, Patra U, Chawla-Sarkar M. Rotavirus disrupts cytoplasmic P bodies during infection. Virus Res. 2015 Dec, 210:344-54.
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13. Biswas S, Chattopadhyay M, Sen KK, Saha MK. Development and characterisation of alginate coated low molecular weight chitosan nanoparticles as new carriers for oral vaccine delivery in mice. Carbohydr Polym 2015 May 121, 403-410.
14. Biswas S, Saha MK.Uncertainty of measurement for ELISA in a serological testing laboratory. Immunochem. Immunopathol. 2015 Nov; 1(2):109.
15. Chatterjee T, Sheikh IA, Chakravarty D, Chakrabarti P, Sarkar P, Saha T, Chakrabarti MK, Hoque KM. Effects of small molecule calcium-activated chloride channel inhibitors on structure and function of Accessory Cholera Enterotoxin (Ace) of Vibrio cholerae. PLoS One. 2015 Nov 5; 10(11): e0141283.
16. Chowdhury R, Mandal RS, Ta A, Das S. An AIL family protein promotes type three secretion system-1-independent invasion and pathogenesis of Salmonella enterica serovar Typhi. Cell Microbiol. 2015 Apr; 17(4):486-503.
17. Das K, Chowdhury P, Ganguly S. Internal Transcribed Spacer 1 (ITS1) based sequence typing reveals phylogenetically distinct Ascaris population. Comput Struct Biotechnol J. 2015 Sep;13:478-83.
18. Das S, Thakur BK. Mucosal immune system of the respiratory tract: regulation of tolerance and immune response. The Pulmo-Face. 2015 Nov; XV(2): 61-70.
19. Dasgupta N, Kumar Thakur B, Ta A, Das S. Caveolin-1 is transcribed from a hypermethylated promoter to mediate colonocyte differentiation and apoptosis. Exp Cell Res. 2015 Jun; 334(2):323-36.

20. Datta R, Bansal T, Rana S, Datta K, Chattopadhyay S, Chawla-Sarkar M, Sarkar S. Hsp90/Cdc37 assembly modulates TGFβ receptor-II to act as a profibrotic regulator of TGFβ signaling during cardiac hypertrophy. Cell Signal. 2015 Dec;27(12):2410-24.
21. Datta S, Chatterjee S, Mitra S, Basu S. A reliable phenotypic assay for detection of ESBLs and AmpCs in MBL-producing gram-negative bacteria with the use of aminophenylboronic acid, dipicolinic acid and cloxacillin. J Microbiol Methods. 2015 Aug;115:100-3.
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23. Debnath A, Wajima T, Sabui S, Hamabata T, Ramamurthy T, Chatterjee NS. Two specific amino acid variations in colonization factor CS6 subtypes of enterotoxigenic Escherichia coli results in differential binding and pathogenicity. Microbiology. 2015 Apr;161(Pt 4):865-74.
24. Desai SN, Akalu Z, Teferi M, Manna B, Teshome S, Park JY, Yang JS, Kim DR, Kanungo S, Digilio L. Comparison of immune responses to a killed bivalent whole cell oral cholera vaccine between endemic and less endemic settings. Trop Med Int Health. 2016 Feb;21(2):194-201.
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29. Ghosh AK, Sinha D, Mukherjee S, Biswas R, Biswas T. IL-15 temporally reorients IL-10 biased B-1a cells toward IL-12 expression. Cell Mol Immunol. 2016 Mar;13(2): 229-39.
30. Ghosh AK, Sinha D, Mukherjee S, Biswas R, Biswas T. LPS stimulates and Hsp70 down-regulates TLR4 to orchestrate differential cytokine response of culture-differentiated innate memory CD8+ T cells. Cytokine. 2015 May;73(1): 44-52.
31. Ghosh M, Nandi S, Dutta S, Saha MK. Detection of hepatitis B virus infection: A systematic review. World J Hepatol. 2015 Oct 18;7(23):2482-91.
32. Ghosh P, Sarkar A, Ganguly M, Raghwan, Alam J, De R, Mukhopadhyay AK. Helicobacter pylori strains harboring babA2 from Indian sub population are associated with increased virulence in ex vivo study. Gut Pathog. 2016 Jan 12;8:1.
33. Jaiswal A, Sarkar S, Das P, Nandy S, Koley H, Sarkar B. Trends in the genomic epidemiology of Vibrio cholerae O1 isolated worldwide since 1961. Int J Antimicrob Agents. 2015 Oct;46(4):460-4.
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1. Dutta P and Das S. Antimicrobial peptides and infectious Diseases. In Mendez-Vilas A, The Battle against Microbial Pathogenesis: Basic Science and Educational Programmes, 5th series, 2015. Badazgoz Spain, Formatex Research Centre.
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Member Secretary	:	Dr. Hemanta Koley Scientist-C Division of Bacteriology, National Institute of Cholera and Enteric Diseases, Kolkata

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Member	:	Mr. Asit Ranjan Das, Caretaker

Vigilance Officer:

Dr. N.S. Chatterjee, Scientist-F

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Shri S. Parui, Technician-C (Eng. Support)
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Shri H. P. Das, Driver (Grade I)
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Shri S. Ghosh, Driver (Ordinary Grade)
Shri D. Dey, Driver (Ordinary Grade)

Employees transferred from / to NICED during 2015-16

Sl. No.	Name and Designation	Division	Date of Transfer/ Joining
1	Dr. K. Rajendran, Scientist C Transferred to TRC Chennai	Data Management	Transferred on 30.04.2015
2	Mr. D. R. Naik, Technical Assistant Transferred to National Institute of Nutrition, Hyderabad	Biochemistry	Transferred on 29.02.2016
3	Dr Nityananda Mandal, Scientist B Transferred from Desert Medicine Research Centre (ICMR), Jodhpur, Rajasthan	Parasitology	Joined on 23/09/2015
4	Mr. Tapu Barman, Technical Assistant Transferred from ROHC (South), Bangalore	Bacteriology	Joined on 16.03.2016

Retired Employees of the Institute during 2015-16

"Farewell dear Friends"

Sl. No.	Name and Designation	Division	Date of Retirement
1	Dr. Thandavarayan Ramamurthy, Scientist G	Bacteriology	31.05.2015
2.	Sk. Golam Mahboob, Technician C, (Engineering Support)	Vehicle Section	30.11.2015
3.	Mr. Subhash Chandra Saha, Technical Assistant	Bacteriology	30.11.2015
4.	Mr. Arun Sarkar, Technician C, (Engineering Support)	Maintenance, Instruments and Equipment Section	31.01.2016
5.	Mr. Shatrughna Parui, Technician C (Engineering Support)	Maintenance, Instruments and Equipment Section	29.02.2016

Obituary..our tribute and homage

"You will always be remembered ..Rest in eternal peace"

1. *Mr. R. N. Sarkar, Ex- Technical Officer, retired on 31.03.2003, passed away on 06.04.2015.*
2. *Mr. Sanat Kr. Ghosh, Ex-Section Officer voluntarily retired on 31.08.1999, passed away on 29.01.2016.*
3. *Mr. R. N. Mazumder, Ex-Technical Officer, retired on 31.07.2002, passed away on 22.02.2016.*

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