Annual Report
2015–16

NIMR

ICMR-National Institute of Malaria Research
(Indian Council of Medical Research)
Sector 8, Dwarka, New Delhi–110 077

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Preface

The ICMR-National Institute of Malaria Research (NIMR) has been constantly involved in the development of practical solutions to the evolving challenges in the field of malaria, and supporting the changing research practices and needs related to prevention and control of malaria, and other vector-borne diseases. The activities and major achievements of the Institute during the year 2015–16 are compiled and presented in this Annual Report.

Through, the network of its 10 field units set-up in different eco-epidemiological settings across the country, the Institute undertook the diverse research activities in various aspects of malaria and other vector borne diseases, in the form of studies on vector bionomics and disease epidemiology, insecticide/drug resistance in malaria vectors/parasites, molecular and proteomic studies on vector-parasite interactions, malaria transmission dynamics, field testing of new vector control tools, efficacy of new antimalarials, studies on population genetic diversity of malaria parasites, etc.

Studies on vector biology and epidemiology were centered in different malaria endemic districts for mapping the distribution and biological attributes of malaria vectors, generation of malaria risk maps in the context of climatic change, molecular characterization of novel haemocyte transcript, proteogenomic analysis of salivary microbiome, molecular population genetics, phase-III studies for evaluation of NetProtect LLIN, and health impact assessment of various developmental projects.

Research on parasite biology was focused on development of new antimalarials which included molecular characterisation of *IspE* kinase gene from *Plasmodium vivax*, *PfPMT* gene from *P. falciparum*, immunomodulatory role of mesenchymal stem cells in the pathogenesis of malaria infection, next generation sequencing to study antimalarial drug resistance in the Indian *P. falciparum* population, etc.

Clinical studies were focused on projects with operational and translational approach. The Institute has been accredited as a WHO-recognized laboratory for quality assurance of malaria rapid diagnostic tests (RDTs) and to link with other lot-testing laboratories, so as to provide credibility to the results of monitoring quality of malaria RDTs through external quality assurance scheme. Phase-III of a project on quality assurance of malaria RDTs was initiated in the reporting year to assess the quality of RDTs procured and supplied by NVBDCP. Open label trial to assess the safety, tolerability and efficacy of DHA/piperaquine in paediatric patients with uncomplicated malaria has been initiated at two sites. Additionally, projects on monitoring therapeutic efficacy of other antimalarial medicines, studies on effective and safe interventions for prevention of malaria in pregnancy and its burden were continued. The malaria clinic at the Institute, one of the sentinel sites for diagnosis of malaria and dengue provided diagnosis and treatment to ~3700 cases.

For enhanced surveillance and development of vector control methods, several collaborative projects were undertaken with the support and grants from national and international agencies like National Vector Borne Disease Control Programme; Department of Biotechnology; All India Institute of Medical Sciences, National Centre for Biological Sciences; WHO Global Malaria Programme; Global Fund for AIDS, TB and Malaria; New York University; Pennsylvania State University (National Institute of Health); London School of Hygiene and Tropical Medicine; University of Oxford, etc.

The outcome of the various projects and researches against malaria and other vector borne-diseases was reflected in the form of a good numbers of high impact scientific publications, research papers (>60) and books. Several information, education and communication (IEC) activities were undertaken for mass awareness about malaria/ dengue intervention measures for disease control.

Towards development of human resource and manpower, different educational and academic activities were undertaken by the Institute in the form of trainings to young entomologists, district programme officers, VBD consultants, laboratory technicians; and classroom/laboratory programmes to research scholars, Ph.D. and M.Sc. students. The basic support for research and development was supplemented from the in-house facilities- like malaria parasite bank, central instrumentation facility, insectary and animal house at the Institute. The research scientists and scholars attended various national and international conferences, workshops, delivered lectures and received awards/ fellowships for their valuable contribution in the areas of their expertise.

Along with the *Journal of Vector Borne Diseases,* the Institute's other scientific publications—*Malaria Patrik*a and *Plasmodium* Newsletter were published on regular basis for wider dissemination of biomedical information and recent findings about vector borne diseases. Other activities included celebration of *Hindi Pakhwad*a to encourage the progressive use of Hindi in official work, cleanliness and hygiene-related activities under *Swachh Bharat Abhiyan* and observance of National days.

I, sincerely acknowledge the guidance and direction of the Secretary, Department of Health Research, Government of India and Director General of the Indian Council of Medical Research and trust her continuous support ahead. I also wish to acknowledge the sincere efforts of all the scientists and staff for their contribution towards the betterment of the Institute.

Neena Valecha
Director

Executive Summary

Vector Biology & Control

- Entomological surveys were carried out in three districts of Jharkhand, namely Ranchi, Giridih and Latehar to study the bionomics of malaria vectors and their sibling species in order to establish their role in malaria transmission in Jharkhand. Villages falling in different ecological settings like foothills, plain, urban, forested, riverine, stream, dam, hill top etc. were selected for the study. During 2015, entomological surveys were carried out in pre-monsoon (February-March 2015) and monsoon season (June-July 2015), whereas post-monsoon survey in all the three districts is in progress.
- Entomological, parasitological and KAP investigations were carried out in malaria epidemic prone Nuh CHC of District Mewat, Haryana.
- Proteomic analysis of midgut and salivary gland of *Anopheles culicifacies* was carried out using high through put mass spectrometry in order to understand the importance of functional proteins in vector behaviour following blood meal. Differential protein expression between salivary glands of sugar-fed (SF) vs blood-fed (BF) mosquitoes was analyzed using 2-D E-gel electrophoresis combined with MALDI-TOF mass spectrometry. A total of 45 spots in SF and 71 spots in BF between a pI range of 4–10 and MW 6–80 kDa were detected. Among these total spots, 29 spots were found to be differentially expressed between SF and BF mosquitoes. Further among 29 annotated spots, only 11 spots were further processed for MALDI as remaining spots did not match the criteria for MALDI-TOF/TOF mass spectrometry.
- Molecular analysis of mosquito olfactory system in *An. culicifacies* uncovered that adult female may encode at least 68 putative odorant proteins, facilitating key function of host seeking behavioural modulation.
- Horizontal gene transfer (HGT), an important evolutionary drive to acquire and retain beneficial genes, have been well-documented in prokaryotes, for their survival in diverse ecologies. However, in eukaryotes, functional role of HGTs remains questionable. In a study RNAseq analysis of *An. culicifacies* revealed horizontal gene transfer of plant-like transcript (PLTs), encoding functional proteins. A comprehensive analysis of tissue associated microbiome and PLTs, predicts that mosquito may have feeding and survival benefits in diverse ecologies.
- A study was carried out to find out the role of rearing temperature and container material in larval development of *Aedes aegypti* mosquitoes. Larval susceptibility studies with larvae reared at experimental temperatures against temephos are in progress.
- An integrated vector management approach was undertaken in order to reduce dengue transmission in Najafgarh zone of Delhi. KAP, socio-economic and entomological data were also collected.
- Phase-III evaluation of NetProtect LLIN (impregnated with deltamethrin) against malaria vectors in the states of Haryana, Uttar Pradesh and Jharkhand revealed that NetProtect LLIN failed to fulfil WHO criteria for long-lasting insecticidal nets. However, the positive aspect of the trial was high compliance rate of the net usage and collateral benefits experienced by the community.
- To evaluate the efficacy of PermaNet®3.0 long-lasting insecticidal nets in areas with

variable deltamethrin-resistant malaria vectors in India, 21 districts in five states have been selected. Further studies are in progress.

- In a comparative genomics study of insecticide resistance gene families in mosquito vector, identification and classification of detoxification enzyme families *CYP 450, GST* and *COE* genes in *An. culicifacies* and *An. stephensi* were completed. The outcome of this investigation will give a broad view of the organization and evolution of the insecticide resistance conferring gene families in the different mosquito species.
- A multidisciplinary approach was undertaken to study the impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in India. Studies on impact of interventions on malaria transmission are in progress.
- The bottle assay method developed by NIMR was validated at VCRC, Puducherry and the NIMR Field Units at Raipur, Bengaluru and Chennai are using laboratory/field collected mosquitoes.
- In a laboratory simulation study by differential exposures in *An. stephensi* to DDT, it was found that variable differential exposure has resulted in precipitation of increased resistance while complete exposures resulted lower levels of resistance.

Parasite Biology

- Molecular characterisation of 4-diphosphocytidyl-2C-methyl-d-erythritol (*IspE*) kinase gene from *Plasmodium vivax* – ligand recognition in a template for antimalarial drug discovery was done. 3-D model of *Pf*IspE protein was generated with I-TASSER and depicted active sites were molecularly docked with 73 inhibitors at Patchdock server and further narrowed down to four lead compounds. These 4 compounds showed a very good drugability score and observed to be non-toxic. Further optimization and testing of these compounds are in process to develop novel antimalarial.
- Molecular characterization of a novel antimalarial drug target *Pfpmt* gene (*P. falciparum phosphoethanolamine methyltransferase*) was carried out. Partial gene amplification of *Pfpmt* gene of *P. falciparum* culture from parasite laboratory, NIMR and multiple alignment of PMT protein showed that PMT protein is functionally as well as structurally conserved among all *Plasmodium* strains. Further modelling and docking studies are in process.
- Allelic typing of region-3 in gametocyte gene *Pfg377* was compared between field isolates and culture adapted samples. Three allelic types of *Pfg377* as A, B, and C, as distinguished by size polymorphism of PCR amplified fragments were detected in the field isolates and culture adapted samples. The cryopreserved and adapted samples of the same isolates showed difference in their genotypes on allele typing indicating the presence of multiple clones in the same sample on sequence comparisons. Further functional analysis of *Pfg377* gene is in process to understand its role in transmission.
- Infusion of mesenchymal stem cells (MSCs) derived from malaria-infected animals to naïve animals protected against *P. berghei* infection. Adoptive transfer of these Sca-1$^+$ CD44$^+$CD29$^+$CD34$^-$ cells showed decrease in number of Sca-1$^+$CD29$^+$ cells on 7th day of post-infection compared to infected mice, which was further confirmed by immunohistochemistry (IHC). Results indicated that these mesenchymal stem cells might be differentiated to other lineage, *viz.* CD34$^+$ haemopoetic cells and / lymphopoietic cells. A significant increase in the number of CD34$^+$ in spleen as well in lymph node and thymus were observed. The number of lymphocytes (both CD4$^+$ and CD8$^+$ cells) increased equal to that of uninfected control wild type mice indicates that mesenchymal stem cells may be helping in rescuing the lymphocytes.
- Ion torrent PGM platform was used for genotyping a panel of candidates for antimalarial resistance. An amplicon sequencing protocol has been established to examine genetic diversity in five genes (*pfk-13, pfcrt ,pfdhfr, pfdhps* and *pfmdr1*) implemented in resistance against various effective antimalarial drugs in field isolates. Three sites exhibiting different malaria epidemiology were selected for the study, *i.e.* Rourkela, Nadiad and Chennai. The

sequencing of 124 isolates identified 58 mutations (synonymous = 12 and non-synonymous = 46) in above five discussed genes. Highest number of mutations were observed in *pfmdr-1* > *pfcrt* > *pfdhfr* > *pfdhps* > *pfk-13* gene. The field sites also showed marked disparity in the distribution and frequency of mutations; highest in isolates from Rourkela > Nadiad > Chennai.

Epidemiology

- Epidemiological studies were carried out during low transmission season in low endemic study area of Mewat, Haryana during April-May 2015 and in high endemic study area of Jharkhand during June-July 2015 for establishing immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions in India. Further studies on the malaria transmission dynamics are in progress
- Comprehensive case management programme (CCMP), an operational research study under programmatic conditions is being carried out jointly by Government of Odisha, NIMR and MMV with aims to assess the impact of early diagnosis and treatment, supported by a strong surveillance system, on the incidence of malaria in different transmission settings in the state of Odisha. CCMP has led to a significant increase in access to diagnosis and treatment in all the intervention areas.
- Studies were undertaken in Odisha to: (i) quantify the role of environmental conditions in determining malaria transmission intensity (risk) in different eco-epidemiological contexts, including the future effects of climate change; and (ii) evaluate the evolutionary responses of key mosquito vectors to the increasing adoption of insecticide-based interventions, quantifying the implications for malaria transmission in different eco-epidemiological contexts.
- Health Impact Assessment was initially started in 2004 in three major dam areas in Madhya Pradesh, which was extended further for 5 years in 2010 to cover entire Narmada Basin. Three study centres each working at Narmada Nagar, Bhopal and Jabalpur carried out entomological, parasitological and microbiological surveys in the affected areas of Narmada Basin.
- Epidemiological study on dengue prevalence was carried out in Delhi which includes active human with virus and vector surveillance. NS1 and IgM sero-prevalence with or without IgG evidenced high proportion of asymptomatic dengue infection (63%).
- A prospective field study was conducted to detect dengue virus with the help of immuno-fluorescence assay in adult *Ae. aegypti* mosquitoes collected from 18 localties in Delhi. Out of 2408 female *Ae. aegypti*, 14 were found positive with MIR of 5.8. LIG localities showed highest mosquito infectivity (9.8) followed by MIG localities (6.2) and least in HIG localities (1.3) which clearly showed that the risk of acquiring dengue infection is higher in residents of LIG in comparison to MIG and HIG.
- Based on case mean ratio and properties of sine curve, a mathematical model for determination of outbreak and early warning of malaria has been developed. The rainfall cut-off for different regions indicated that in states like Assam, 100 mm rainfall in the month of March while in Maharashtra state, >200 mm cumulative rainfall in the month of July-September could result into outbreak in the month of December.
- District level scenario of projection of windows of malaria in view of climate change has been generated using PRECIS model with fuzzy logic technique. Climate suitability maps for malaria transmission based on climatic parameters (temperature and relative humidity), malaria endemicity and other geographical features have been generated. The reasons of low malaria endemicity in Kerala and southern India were identified.
- Based on the Oceanic Nino Index (ONI), the predicted SST anomaly values for November–December 2015, January–February 2016 and annual malaria cases in different states of India, a map was generated to show the impact of El Nino on malaria. As per available projections, the ONI values suggest strong El Nino conditions in first half of 2016.

Clinical Research

- The NIMR has been established as a WHO-recognized Laboratory for Quality Assurance of Malaria RDTs.

- In an active pharmacovigilance study conducted for primaquine radical cure for the treatment of *P. vivax*, no hemolytic symptoms were observed among 39 enrolled patients. Average hemoglobin fall was 1.1 g%; hemoglobin level recovered by Day 28.
- Phase-III of the project 'Quality Assurance of Malaria Rapid Diagnostic Tests' was initiated in the year 2015 to assess the quality of RDTs procured and supplied by NVBDCP. As a part of pre-dispatch QA, 3248 RDTs were tested in the year 2015 and 1134 RDTs in 2016. Post-dispatch testing was carried out on 489 RDTs in the year 2015 and on 816 RDTs till March 2016. The panel detection score of these RDTs was 100%. There were 8 invalid results but no false positives.
- A Phase-IIIb, open label trial, to assess the safety, tolerability and efficacy of DHA/piperaquine in paediatric patients with uncomplicated *P. falciparum* malaria has been initiated on children under 18 years at Ranchi and Mangalore.
- A multicentre, open-label randomised trial to assess the efficacy, safety and tolerability of triple ACTs compared to ACTs in uncomplicated falciparum malaria was initiated.
- In malaria clinic at Delhi a total of 75 malaria cases (70 *P. vivax*, 4 *P. falciparum* and 1 mixed infection) were reported during the year 2015. A total of 3534 samples were tested for dengue, of which 935 were confirmed dengue cases. About 70% of the total dengue cases reported in the month of September. Serotyping performed in 18; serotype 2 was detected in 17; while as one sample each had serotypes 2 and 3. During follow up, of the 580 cases contacted, 42 required hospitalization.
- The joint NIMR-NVBDCP nation-wide sentinel surveillance system have guided the national drug policy for malaria in the country and based on observed high treatment failure of recommended ACT (AS+SP), the national drug policy on malaria for northeast states has been revised to Artemether+Lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria since 30 April 2013. The studies conducted till 2015, showed >95% PCR-corrected efficacy at 48 sites after 28 days follow up and between 90–95% at two sites. Only three sites in NE region showed efficacy <90% during 2012. The efficacy of AL remains >95% at all the six sites, so far. The efficacy of chloroquine in *P. vivax* malaria has been observed as 100% at nine sites.
- NIMR in collaboration with the London School of Hygiene and Tropical Medicine (LSHTM) has conducted a cluster randomized trial to assess the burden of malaria in pregnancy and to evaluate the benefits and risks of intermittent screening and treatment (IST) for malaria in pregnancy. The preliminary findings suggest that compared to the passive case detection (PCD) arm, the detection rate of malaria in pregnancy in the IST arm is approximately 10-fold higher at the first visit (enrolment and ANC1) and 5 fold higher at subsequent ANC visits. Also, the screening for malaria using RDT can be done alongwith the HB testing during routine ANC.

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Vector Biology and Control

1

1.1 Vector Biology

1.1.1 Bionomics of malaria vectors and their sibling species to establish their role in malaria transmission in Jharkhand, India

Study initiated for determining bionomics of malaria vectors in three districts of Jharkhand, namely Ranchi, Giridih and Latehar was continued in the year 2015. Entomological surveys were carried out in different ecological settings like foothill, plain, urban, forested, riverine, stream, dam, hill top etc. in pre-monsoon (February-March 2015) and monsoon season (June-July 2015), whereas post-monsoon survey in all the three districts is in progress.

During pre-monsoon season 22 villages (9 foothills, 6 forested and 7 plains), 21 villages (8 foothills, 6 forested and 7 plains), 22 villages (6 foothills, 7 forested and 9 plains) were surveyed in Latehar, Ranchi and Giridih districts, respectively, whereas during monsoon season 21 villages (3 foothills, 12 forested, 4 plains and 2 hill tops), 22 villages (14 foothills, 3 forested, 4 plains and 1 hill top), 21 villages (3 foothills, 3 forested, 13 plains and 2 hill tops) were surveyed in Latehar, Ranchi and Giridih districts, respectively.

Both adult and immature mosquitoes were collected using hand catch, total catch, light trap, evening collection, landing collection, larval collection from indoor/outdoor, human dwellings/ cattlesheds by standard WHO techniques (Fig. 1). District-wise results are as follows:

Fig. 1: Mosquito collection from different breeding sites.

Giridih: In pre-monsoon season, two vector species viz. *Anopheles fluviatilis* and *An. culicifacies* were recorded from all collection methods. The landing time observed was 2000-2400 hrs for *An. culicifacies*, whereas for *An. fluviatilis* it was 2100-2400 hrs. In monsoon season three vector species, *viz. An. fluviatilis*, *An. culicifacies* and *An. stephensi* were recorded from all collection methods. Extended landing time was observed, *i.e.* 2000-0300 hrs for *An. culicifacies*.

Ranchi: In pre-monsoon season two vector species viz. *An. fluviatilis* and *An. culicifacies* were recorded from all collection methods. The landing time observed was 2000-2400 hrs for *An. culicifacies*, whereas for *An. fluviatilis* it was 2100-2300 hrs. In monsoon season two vector species, *viz. An. culicifacies* and *An. fluviatilis* were recorded from all collection methods. Extended landing time observed was 2000-0200 hrs for *An. culicifacies*.

Latehar: In pre-monsoon season two vector species viz. *An. fluviatilis* and *An. culicifacies* were recorded from all collection methods. In Latehar, the landing time in pre-monsoon season for *An. culicifacies* was extended, *i.e.* 1900-0100 hrs, whereas for *An. fluviatilis* it was shortened, *i.e.* 2200-2300 hrs. In monsoon season three vector species, *viz. An. fluviatilis, An. culicifacies* and *An. stephensi* were recorded from all collection methods. The landing time observed was 2000-0200 hrs for *An. culicifacies*.

Results of susceptibility test carried out in different villages of three districts on *An. culicifacies* using 4% DDT showed mortality in the range of 6 to 26% in pre-monsoon season whereas it was 17 to 33% during monsoon season. Using 0.05% deltamethrin, mortality was in the range of 90 to 98% in pre-monsoon season, whereas it was 46 to 58% during monsoon season.

1.1.2 Entomological and parasitological investigations in malaria epidemic prone Nuh CHC of District Mewat, Haryana

Entomological, parasitological and KAP studies were carried out pertaining to malaria transmission dynamics in malarious villages of epidemic prone Nuh CHC of District Mewat. Seasonal abundance of *An. culicifacies* and *An. stephensi* showed variation, being lowest in winter and peak densities were observed during monsoon months (July-September). A build up of *An. stephensi* densities was also observed during March-April probably because of water storage practices and favourable environmental conditions (Fig. 2). Blood meal source identification of malaria vectors revealed *An. stephensi* to be almost zoophagic, whereas human blood index (HBI) was comparatively high in *An. culicifacies* (Table 1). An upsurge in the feeding on humans was observed in case of *An. culicifacies* during monsoon which coincided with peak prevalence period of this species. *Anopheles culicifacies* was also incriminated during monsoon period for *Plasmodium vivax* CS antigen by ELISA test. These observations suggest that this species might be playing a major role in malaria transmission. Analysis of sibling species composition of *An. culicifacies* population using cytotaxonomy showed that it almost comprised of species A (>99%) which was found polymorphic i^1 inversion (Fig. 3). Assessment of insecticide

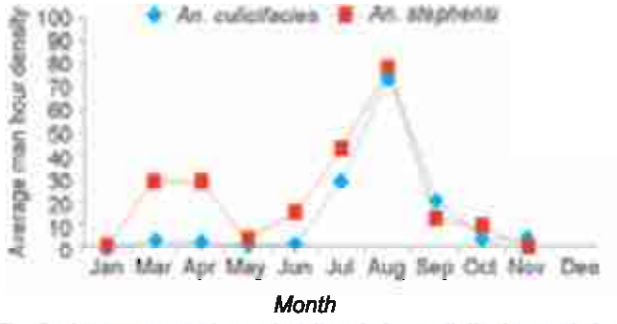

Fig. 2: Average man hour density of *An. culicifacies* and *An. stephensi* in study villages of PHC Ujina, CHC Nuh.

Table 1. Host preference of malaria vectors in study villages in PHC Ujina, CHC Nuh*

Vector species	Total tested	Human (+)ve	Mix (+)ve (H+B)	Bovine (+)ve	Negative for H & B	HBI
An. culicifacies	401	25	53	305	18	0.195
An. stephensi	241	0	4	220	17	0.017

*Pooled data of all surveys in study villages; H= human; B = bovine; HBI = human blood index.

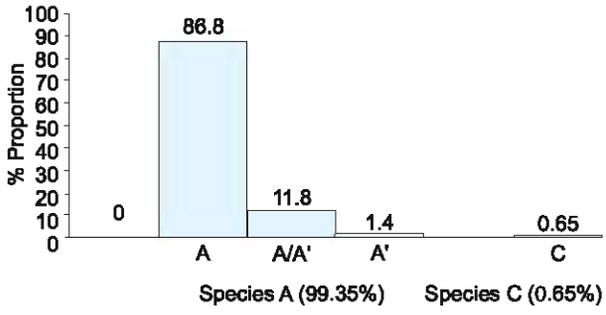

Fig. 3: Sibling species composition of *An. culicifacies* complex in study villages (n=153, Pooled data of all study villages).

susceptibility status of malaria vectors against 0.5% deltamethrin (presently being used for IRS in the district) revealed that *An. culicifacies* was comparatively more susceptible (95% mortality) than *An. stephensi* (85% mortality). Mass blood survey in selected malarious villages in June-July showed average slide positivity rate (SPR) of 1.3 with predominance of *P. vivax* cases, whereas in active surveillance of fever cases in these villages during August-September, the average SPR was 31.6 and an increase in the proportion of *P. falciparum* cases was observed. A questionnaire-based household survey is underway to generate information on the economic status, awareness about malaria, personal protection practices, health seeking behaviour *etc.* of the inhabitants of study villages in order to assess the possible influence of above mention factors on malaria transmission. The study is likely to delineate the risk factors contributing to malaria incidence and would be helpful in planning effective malaria control strategy.

1.1.3 Proteomic analysis of midgut and salivary gland of *Anopheles culicifacies* using high throughput mass spectrometry

The salivary glands are the site for the maturation of sporozoites where a set of sporozoite-vector interactions takes place and these interactions determines the competence of parasite and success of malaria transmission. Salivary glands of blood sucking insects have evolved to complement their blood feeding behaviour and produces large array of biochemically active molecules which deactivates host's hemostatic response triggered by biting and helps in food ingestion and digestion.

In order to understand the importance of functional proteins in the vector behavior following blood meal, a proteomic dataset is essential for

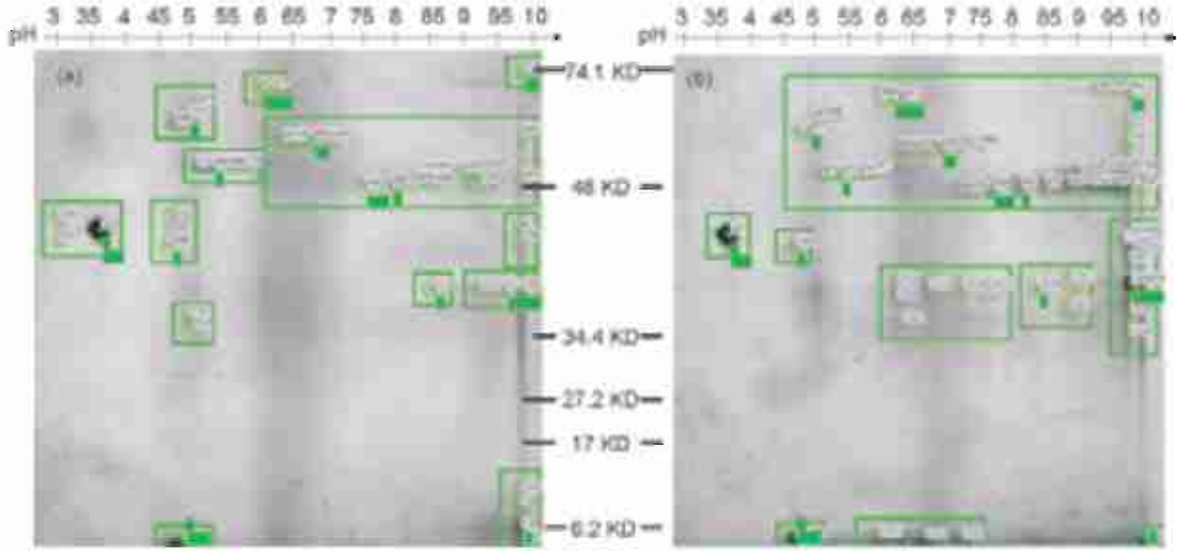

Fig. 4: Representation of 2D gel picture of *An. culicifacies* salivary gland proteins: (a) Gel picture of sugar-fed species showed total spot ID number (473-535, black coloured); (b) Gel picture of blood-fed species showed total spot ID number (940-1058,black coloured).

providing insights into the physiology of blood feeding. In the present study differential protein expression between salivary glands of sugar-fed (SF) *vs* blood fed (BF) mosquitoes was analyzed using 2-DE gel electrophoresis combined with MALDI-TOF mass spectrometry. Bioinformatics analysis were carried out using Blast P and SMART algorithm (*http://smart.embl-heidelberg.de*) in order to find out functions and conserved domains. Gene ontology (GO) search engines (*http://www.geneontology.org/*) were used for further identification of biological process and molecular function of all identified annotated putative functional proteins.

A total of 45 spots in SF and 71 spots in BF between a pI range of 4–10 and MW 6–80 kDa were detected using ImageMaster 2D Platinum software (Fig. 4 a & b). Among these total spots, 29 spots were found to be differential expressed between SF and BF mosquitoes. Further among 29 annotated spots, only 11 spots were further processed for MALDI as remaining spots did not match the criteria for MALDI-TOF/TOF mass spectrometry.

Among 11 differentially expressed spots three spots were found to be over expressed (match ID 5, 9, 11) and 8 spots were found to be under expressed (match ID 0, 2, 3, 12, 14, 25, 26, 27) in BF as compared to SF profile (Fig. 5 a & b).

Analysis of raw data files of all the 11 annotated

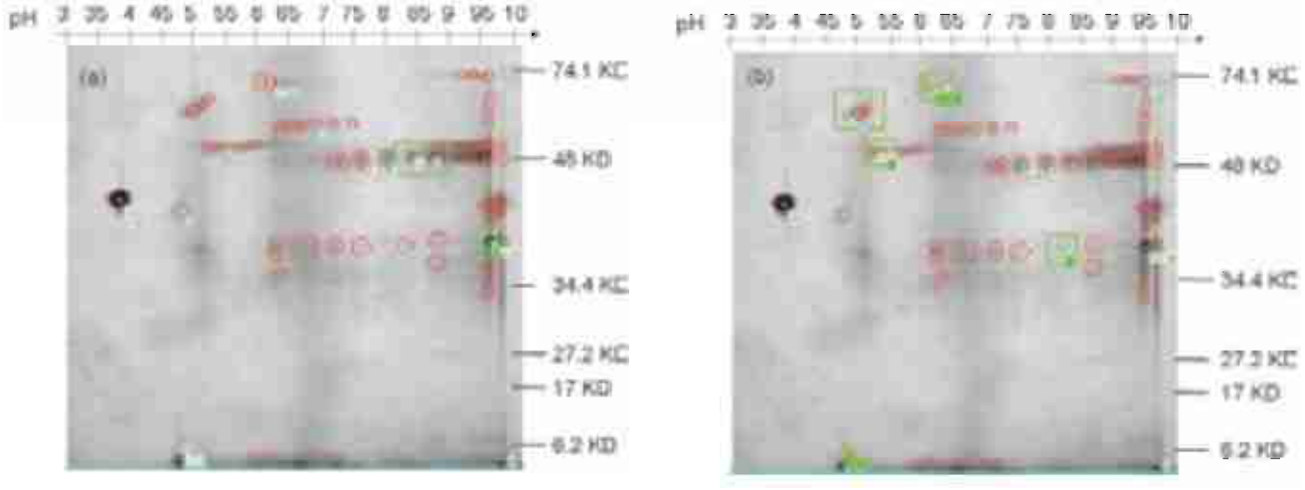

Fig. 5: Representation of 2-DE gel picture of salivary gland extracts of *An. culicifacies*: (a) Depiction of over-expressed spots (5, 9 11) in blood-fed (green squared and numbered in red); and (b) Depiction of under-expressed spots (0, 2, 3, 12, 14, 25, 26, 27) in blood-fed (green squared and numbered in red). 2D expression profile of sugar-fed mosquitoes was taken as control.

spots carried out using peptide mass finger-printing (PMF) that leads to identification of total 29 up-regulated proteins and 9 down-regulated proteins and their description with fold change is given in Tables 2 and 3, respectively. All these proteins were further analysed using Gene ontology and other bioinformatics algorithms for the identification of molecular function.

The alterations in the differential expression of total 38 proteins was observed out of which 29 proteins like beclin-1, phosphorylating proteins, haeme oxygenase 1, ferritin, apoptotic proteins,

Table 2. Annotated up-regulated salivary proteins in *An. culicifacies* upon blood feeding

Match ID/ Fold increase	Accession	Protein	Organism, % similarity	Peptide matches	Function/ Signal peptide (if any)
5/1.94	A0A023EV62	Putative ceramide kinase	*Ae. albopictus*, 15	13	Phosphorylation
	A0A023EU04	Serine/threonine kinase	*Ae. albopictus*, 15	9	Phosphorylation
	B0XFC4	Phosphatidylinositol transfer protein SEC14	*Cx. quinquefasciatus*, 14	6	Signal transduction
	B0WIF0	Serine protease inhibitor	*Cx. quinquefasciatus*, 15	9	Proteolytic
	C4N137	30 kDa salivary antigen family	*An. darling*, 16	6	Antigen / Signal P: 1-29
	A0A023EDZ3	Putative secreted protein	*Ae. albopictus*, 35	7	Signal P: 1-26
9/1.79	A0A084W2T3	Ribokinase	*An. sinensis*, 61	8	Phosphorylation
	W5JB49	Calcium/calmodulin-dependent protein kinase 1	*An. darlingi* 30	15	Phosphorylation
	A0A023EV08	Putative creatine kinase	*Ae. albopictus*, 28	12	Phosphorylation
	B0X4Y5	Polo kinase	*Cx. quinquefasciatus*, 19	26	Phosphorylation
	A0A023EIY7	Tyrosine phosphatase iva1	*Ae. albopictus*, 32	6	Phosphatase
	A0A084W4J8	MRAS2, putative	*An. sinensis*, 37	14	Signal transduction
	Q7QGU9	Oxysterol-binding protein	*An. gambiae*, 23	11	Signal transduction
	Q0IEK7	Lipoyltransferase 2, Mt	*Ae. aegypti*, 32	11	Protein modification
	B0X918	Ferritin subunit	*Cx. quinquefasciatus*, 37	7	Iron homeostasis
	D3KAG7	CLIPB14	*An. gambiae*, 32	9	Proteolytic
	A0A084VN17	AGAP004754-PA	*An. sinensis*, 63	10	Caspase like domain
	W5J7X6	Glucose dehydrogenase	*An. darling*, 24	9	Oxidoreductase
	J9E8U1	AAEL017136-PA	*Ae. aegypti*, 32	13	Cyt P450
	A0A084WNZ9	Putative antennal carrier protein TOL-2	*An. sinensis*, 73	4	Chemosensory
	W5J836	Takeout	*An. darling*, 17	3	Chemosensory
11/1.5	B8RJ80	5'-AMP-activated protein kinase, alpha-2	*Cx. tarsalis*, 30	5	Phosphorylation
	B0WVM0	Beclin-1	*Culex quinquefasciatus*,12	6	Autophagy
	A0A023EM51	Putative heme oxygenase 1	*Ae. albopictus*, 20	8	Heme oxidation
	W5JD00	26S proteasome regulatory subunitS3	*An. darling*, 15	9	Proteolytic
	Q7QKL3	AGAP003249-PA	*An. gambiae*, 11	8	Serine protease
	A0A023EFV7	Putative galactose-specific c-type lectin	*Ae. albopictus*, 34	4	Immune
	B0WQX3	Aldehyde dehydrogenase	*Cx. quinquefasciatus*,10	5	Oxidation reduction
	Q8I8P8	OBP39	*An. gambiae*, 14	5	Chemosensory

Table 3. Annotated down-regulated salivary proteins in *An. culicifacies* upon blood feeding

Match ID/ Fold decrease	Accession	Protein	Organism, % similarity	Peptide matches	Function/ Signal peptide (if any)
0/0.17	B0WIV8	Putative uncharacterized protein	*Cx. quinquefasciatus*, 22	7	Caspase recruitment domain
	A0A084VT63	Isocitrate dehydrogenase	*An. sinensis*, 29	12	Oxidation reduction
3/0.38	A0A023EUU0	Putative juvenile hormone epoxide hydrolase ii	*Ae. albopictus*, 22	8	Physiological change Signal P:1-19
	Q6TRY1	Putative salivary OBP 2	*Cx. quinquefasciatus*, 27	2	Signal P: 1-20
	A0A023EVV5	Putative pftaire-interacting factor 1a	*Ae. albopictus*, 23	14	Unknown
12/0.37	B0W7K8	Chemosensory protein 1	*Cx. quinquefasciatus*, 71	8	OBP 10
14/0.42	A0A023EFB5	Putative 11 kDa salivary protein	*Ae. albopictus*, 60	4	Magnesium transport Signal P: 1-19
27/0.34	W5JJH0	Sphingosine phosphate lyase	*An. darling*, 13	8	Apoptosis regulation
	O17491	Iron regulatory protein	*An. gambiae*, 17	5	Iron homeostasis

coagulation and immunity like, serine proteases, serpins, c-type lectin and protein in regulation of blood feeding behavior were found to be up regulated while 9 proteins related to blood feeding, juvenile hormone epoxide hydrolase ii, odorant binding proteins and energy metabolic enzymes were found to be down regulated. To our knowledge, this identification of differential proteins serves as a basis for future work concerning the possible role of these proteins in the physiological processes and the vector behavior and sporozoite transmission during the process of blood feeding.

1.1.4 RNAseq reveals molecular signature of horizontal gene transfer of plant-like transcripts in *Anopheles culicifacies*

Horizontal gene transfer (HGT) is an important evolutionary drive to acquire and retain beneficial genes, have been well documented in prokaryotes, for their survival in diverse ecologies. However, in eukaryotes, functional role of HGTs remains questionable. Here, in our recent comparative salivary RNAseq analysis not only demonstrated that adult female mosquito salivary glands are evolved with unique ability to manage meal specific responses, but also unraveled the presence of several 'Plant Like Transcripts', associated with sugar fed library, absent in the blood fed salivary transcriptome database (Fig. 6a). The surprising discovery of these transcripts raised several puzzling, but arguable questions that prompted us to clarify: (i) whether the nature of the plant like transcripts is mosquito origin; (ii) do they express in the mosquito tissues and/or other developmental stages; (iii) if express in mosquito, what is possible evolutionary and functional correlation of these transcripts in feeding; (iv) do these transcripts have any molecular relationship to plant-mosquito-microbe interactions/symbiotic association. To uncover the molecular nature and possible functions of the putative PLTs, we performed following systematic and comprehensive analysis of PLTs, revealing a unique case of the massive transfer of HGTs from plant-to-mosquito.

PLTs are of mosquito origin: For technical validation of the PLTs origin, we conducted a series of experiments: (i) in two independent experiments, first we examined and verified the RT-PCR-based expression of few selected PLTs, in the salivary glands of adult male and female mosquitoes; (ii) interestingly, we also observed that PLTs expression is not only restricted to the mosquito tissues, but also express during the aquatic developmental stages, *viz*. egg, larva, and pupa of the laboratory reared mosquitoes (Fig. 6b). Our relative gene expression analysis revealed that PLTs dominantly express in the egg, pupa and adult than larvae stages (Fig. 6 c,d & e); (iii) we also observed positive amplification of selected PLTs through genomic DNA PCR (Fig. 6f); (iv) we further carried out the functional validation of one of the plant homolog PLT encoding dehydrin protein, by Immunoblot analysis as well as immunofluorescence assay (Fig. 7); and (v) finally to test whether PLTs expression are associated with feeding machinery components, we monitored the relative expression of PLTs in four tissues that included salivary glands, midgut, olfactory tissue and haemocytes, collected from 3-4 days-old Naïve adult female mosquitoes, by real-time PCR. Additionally, we also examined the expression of PLTs in response to blood meal in the salivary glands (see also Figs. 11 a & b). Interestingly, we not only observed that PLTs dominantly expresses in the tissues associated with mosquito feeding machinery (olfactory tissue, salivary gland and midgut), but also noticed a significant down regulation in response to blood meal in the salivary glands (Figs. 6a & b), evidencing that the mosquito genome may code plant like proteins.

Mosquito encoded plant-homolog dehydrin: A functional validation: Dehydrin are group2 members of late embryogenesis abundant (LEA) proteins, originally identified from land plants, are known to be associated with desiccation (water stress) tolerance. Dehydrins are characterized by lysine rich conserved K-segment comprising consensus amino acid sequence EKKGIMDKIKEK-LPG, towards the C-terminus and may be repeated one to many times to encode 9-200 kDa protein. This unique feature renders these proteins cationic, providing cryoprotective activity towards freezing sensitive enzymes. To the contrary of the antifreeze proteins, ice-nucleator proteins, or stress-response proteins previously described in insects, the biochemical characterization of a novel cryoprotective protein in freeze-tolerant *Eurosta solidaginis* larvae, shows dehyrin like activities, but a true homolog of dehydrin is yet to be verified.

Mosquito dehydrin have not been reported so far, though a putative transcript AGAP000328 has

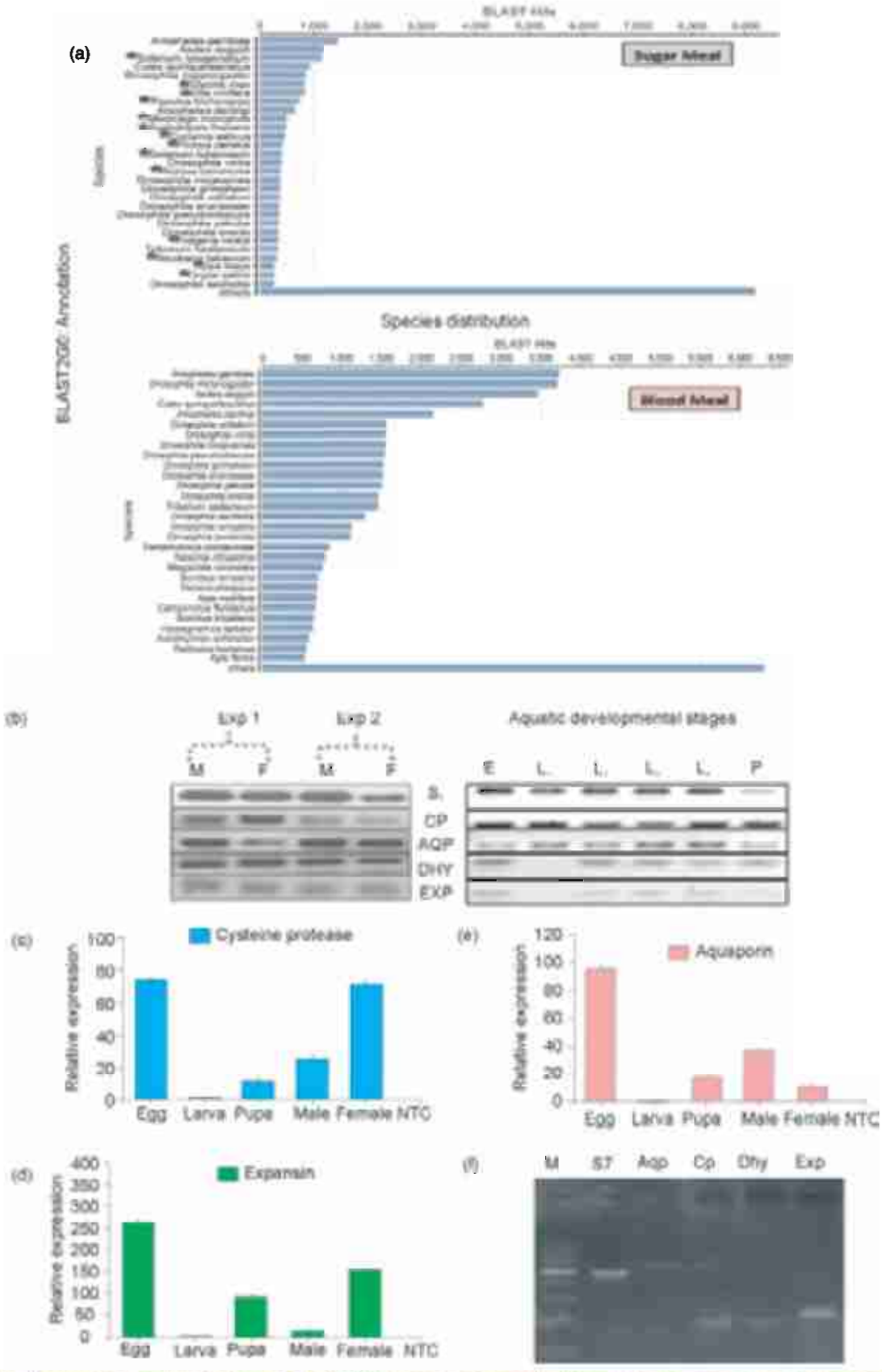

Fig. 6: Mosquito encodes plant-like proteins: **(a)** BLAST2GO based species distribution analysis of sugar and blood-fed mosquito salivary transcriptomic database. Green star mark indicates the name of plant species; **(b)** RT-PCR expression of PLTs during aquatic development of the mosquitoes; **(c-e)** Real-Time PCR-based developmental expression analysis of PLTs; **(f)** PCR-based genomic DNA amplification of PLTs; S7: Ribosomal protein S7; Aqp: Aquaporin; Cp: Cysteine protease; Dhyd/Dhy: Dehydrin; Exp: Expansin.

been predicted from mosquito *A. gambiae* genome, carrying (PF00257 domain) a signature of dehydrin like proteins. Finding of PLTs encoding proteins associated with dehydration stress, e.g. dehydrin, aquaporin, expansin etc. encouraged us to further examine their possible functions in the mosquito *An. culicifacies*. A comprehensive molecular analysis of the identified *AcDehydrin*, showed 100% identity to the plant dehydrin, including conserved lysine rich K-segment (Figs. 7 a,b,c & d). In our relative gene expression analysis, we observed a constitutive expression of *AcDehydrin*, throughout the aquatic developmental as well as adult stages of the mosquito, indicating that identified PLT *AcDehydrin* transcript may encode a putative functional protein (Fig. 7e). Our real-time PCR analysis repeatedly confirmed that dehydrins highly express in the egg and adult mosquitoes (Fig. 7a).

For functional validation of *AcDehydrin* protein, we examined the developmental expression of the dehydrin protein through immuno-blotting assay using antidehydrin antibody (kind gift from Dr Timothy Close). In these experiments we used wheat seedling protein sample as positive reference control. The anti-dehydrin antibody not only recognized the expected (28, 53 and 62 kDa) protein band in the wheat samples, but also identified at least two equivalent proteins (28 and 62kDa) abundantly expressing in different mosquito developmental stages, *viz*. egg, adult male and female mosquitoes (Fig. 7f). Additionally, we were also able to observe multiple isoforms ranging from (~10->70 kDa) expressing at low level in different developmental stages, an expected unique feature of dehydrin to form macromolecular structures. Finally, immunofluorescence assay not only corroborated with abundant expression in the egg, but also suggested that mosquito encoded *AcDehydrin* protein may play crucial role in the stress tolerance and survival of the embryo in the egg (Fig. 7g).

Like other late embryogenesis abundant proteins (LEA), dehydrins accumulate to high amounts in plant embryos, but remain undetectable in other vegetative tissues until their exposure to cellular dehydration stress. Taken together, we hypothesize that mosquito *A. culicifacies*, may have survival benefits of cold stress tolerance as well as developmental regulation, similar to plants. Future studies involving dsRNA mediated gene silencing approach may unravel molecular and functional relationship of the PLTs, controlling feeding and adaptation phenotypes in the mosquito.

Phylogenomic analysis of plant-like transcripts: Next, to understand the possible evolutionary relationship; we performed an extensive phylogenomic analysis of few selected transcripts. To do this, first we retrieved and analyzed all plant-homolog putative transcripts (537 PLTs), and performed an extensive BLASTX analysis against either NR database or Insect specific database. The above results prompted to follow up the associated evolutionary consensus, favouring plant-mosquito relationship: a parallelism setting where different species from unrelated taxa faces the common selective pressure. Initial multiple sequence alignment analysis revealed significant heterogeneity (substitution/deletion) of amino acid residues, but also indicated unique conservation of insect or plant specific residues within the mosquito *An. culicifacies*, result a clade formation with plant species (Fig. 8 a & b). Subsequently, we also tested whether the evolution of common traits from unrelated taxa owing to similar selection pressure favours adaptive significance.

A maximum likelihood (ML) estimation was applied to calculate and compare the site-wise likelihood (ΔSSLS) values between the two hypothesis, *i*.e. mosquito-mosquito species evolution (H_0) and mosquito-plant convergent adaptive evolution (H_1), for the selected PLTs. The site-wise log likelihood plot indicator, *i*.e. divergence towards negative (ΔSSLS) was compared with LRT (likelihood ratio test), using the parametric bootstrap at 1000 replicate analysis (cut off *p*-value 5%). Final data analysis and comparison stats favoured the convergent hypothesis, demonstrating that mosquito *An. culicifacies* PLTs followed convergent model favouring (H_1), an adaptive evolution for sugar feeding associated functional relationship with plants (Fig. 8c). Our analysis also supports the previous observations noted for the evolution of echolocating gene clusters among bats and bottlenose dolphins. Additionally, the predicted 3D structural analysis revealed fine conservation of the active functional domains in the mosquito and plant proteins, e.g. cysteine protease (Fig. 8d). From these studies, we concluded that mosquito feeding associated genes are not only evolving actively, but also acquiring new genes (e.g. dehydrin, expansin), to adapt successfully over plant host.

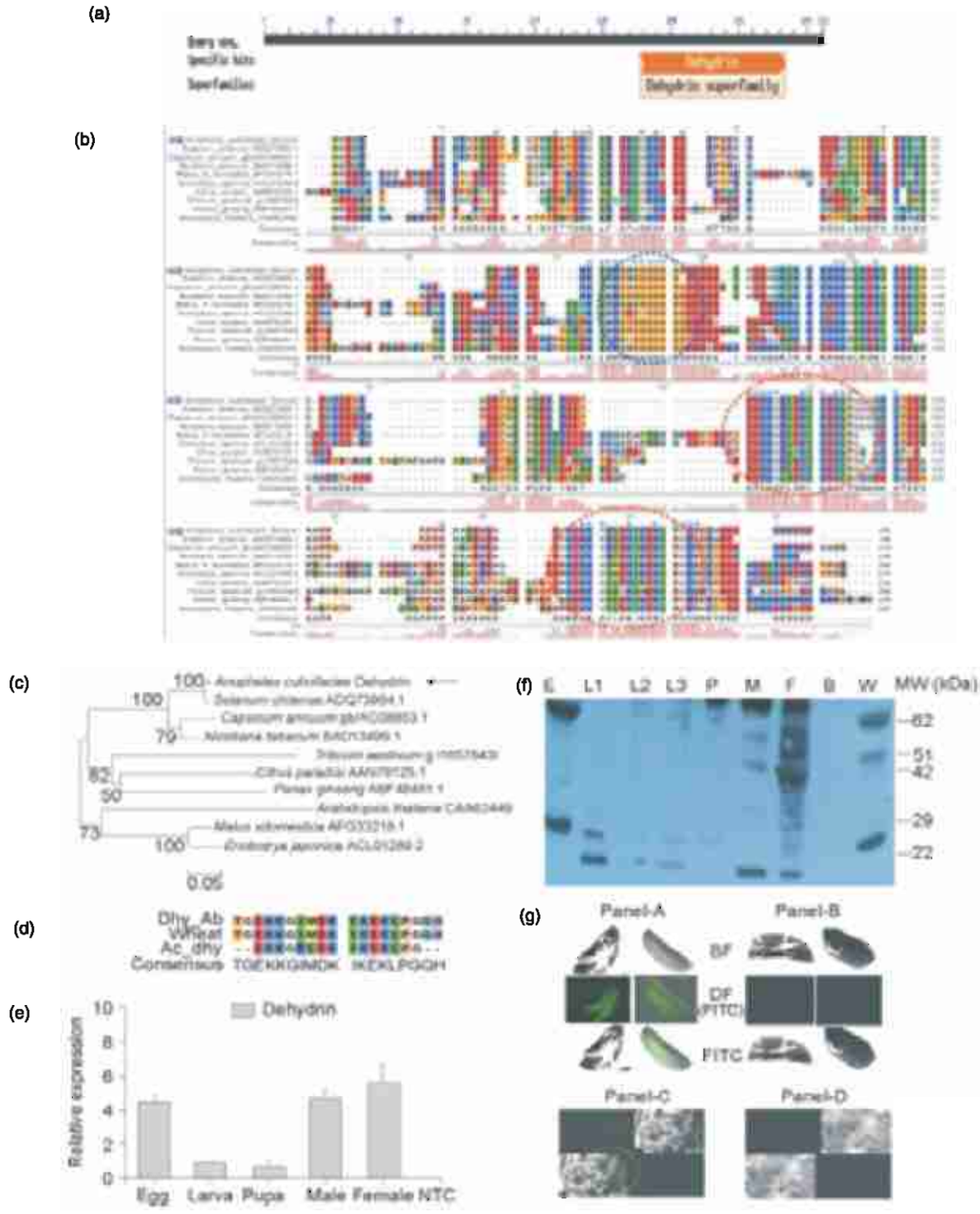

Fig.7: Functional validation of mosquito encoded plant homolog dehydrin: (a) Web-based functional prediction of putative domain of salivary transcript encoding plant homolog dehydrin-like protein; **(b-c)** Molecular and phylogenetic relation of mosquito encoded (Ac-Dehydrin with other plant dehydrins (dotted circle represents the conserved K/red circle and S/blue circle segments), a unique feature of plant dehydrins (see text); **(d)** Mosquito dehydrin alignment of k-segment sequence with wheat and synthetic Dehydrin sequence used for antibody generation; **(e)** Real-Time PCR-based developmental expression analysis of AcDehydrin; **(f)** Immunoblot analysis of AcDehydrin expression during the development of the mosquito: Anti-dehydrin antibody recognize three protein bands of expected size (28,52 & 63kDa) in the control wheat seedling samples (W). Mosquito samples included Egg, Larval stages (L1,L2,L3), pupa (P), male (M) and Female (F); **(g)** Immunofluorescence assay: IFA analysis of plant anti-dehydrin antibody binding and detection in the mosquito egg—**Panel-A &B** and pupa—**Panel-C&D**. The FITC labeled samples were observed and captured under bright field (BF), dark field (DF+FITC) and with FITC fluorescence signal only. Green fluorescence image (Panel-A or Panel-C) shows the presence of dehydrin expression in the mosquito. A corresponding imago (Panel-B or Panel-D) of the samples represents the negative control samples processed under identical conditions, excluding primary anti-dehydrin antibody treatment only.

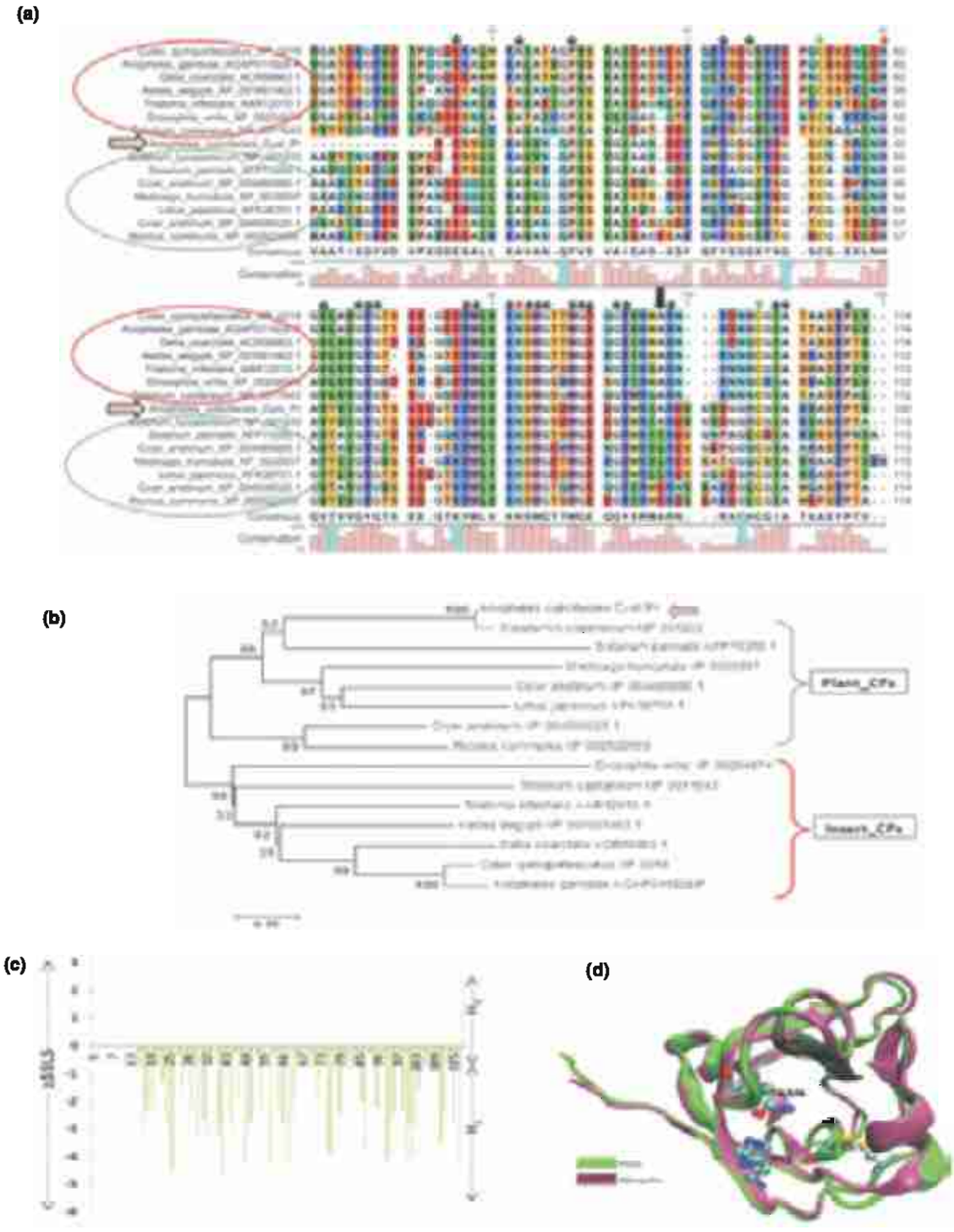

Fig. 8: Feeding associated molecular complexity of the mosquito salivary transcripts: **(a)** Molecular analysis of partial cDNA sequence encoding (100AA) Plant-like cysteine protease active domain: Multiple sequence alignment showing molecular relationship of AcSgCp with plant (Green circle) as well as insects (Red circle) cysteine proteases: conserved residues (marked as ★) as well as conserved active site residue (marked as ★). Green represents conserved cysteine residues, which enables disulfide formation. Upward arrow marks ⇧ represent unique plant-specific amino-acids residues also conserved in the *An. culicifacies*, while downward arrow mark ⇩ represents unique insect-specific residue conserved in *An. culicifacies* and only in *Solanum lycopersicum*; **(b)** The evolutionary history of AcSgCp inferred using the Neighbour-Joining method, favouring a clade formation with *S. lycopersicum* and other plant cysteine proteases; **(c)** Relationship between strength of convergent evolution favouring adaptive significance of feeding associated PLTs: A maximum likelihood (ML) estimation was applied to calculate and compare the site-wise likelihood (ΔSSLS) values between two, species evolution (H_0) and convergent adaptive evolution (H_1) hypothesis, for cysteine protease (see text for details); **(d)** Structural comparison between predicted 3D structure of the mosquito, and solved structure of the plants cysteine protease. Aspargine (ASN) and Histidine (HIS) indicate conserved residue of the active site.

Feeding associated molecular complexity of 'salivary-sugar-microbe': A tripartite interaction

Evolution of herbivores insect-plant association represents one of the dominant interactions over millions of years. These interactions are thought to play an important role in the co-evolution of molecular effector arms, enabling effective adaptation over each other. Uncovering of the

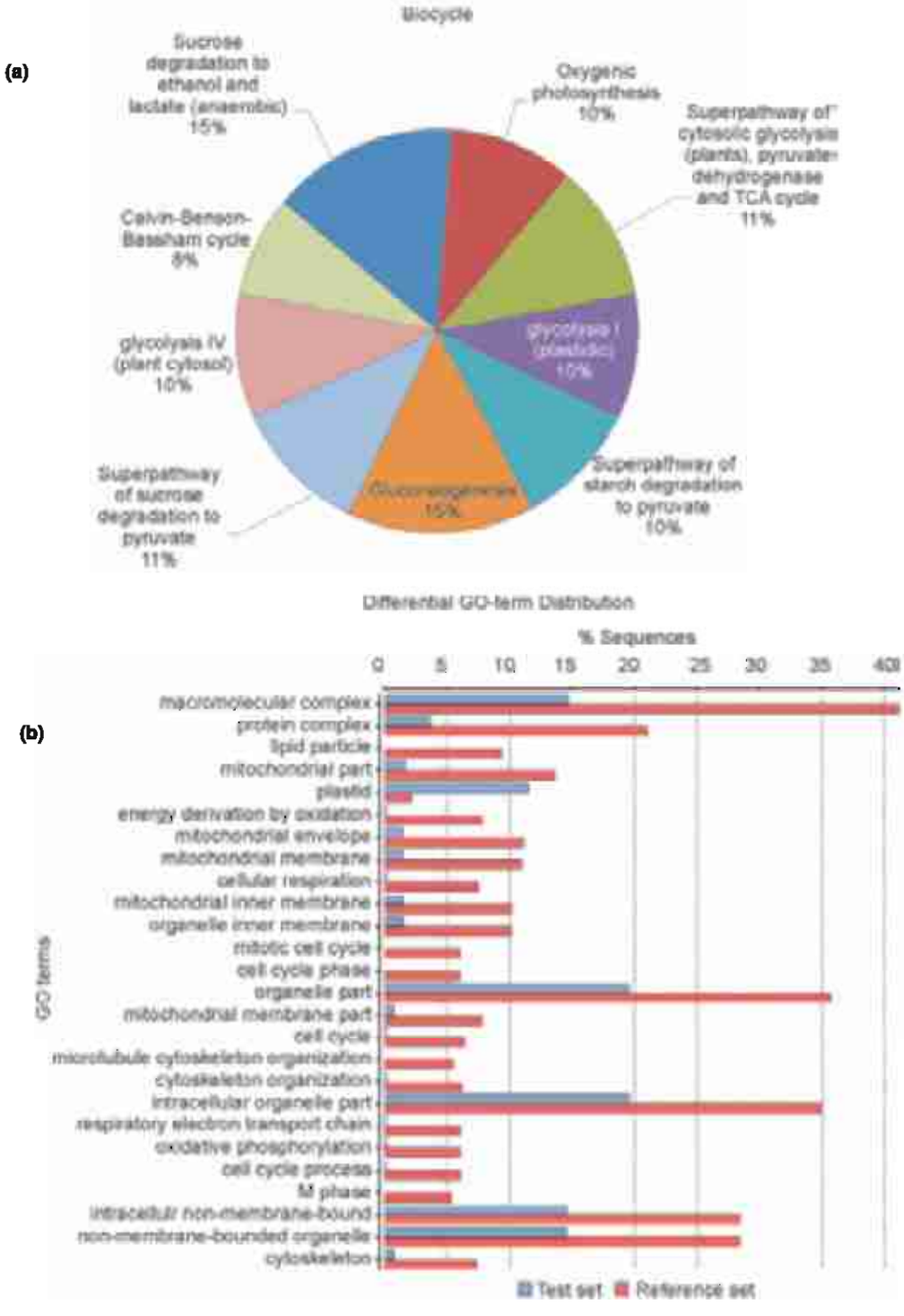

Fig. 9: Identification and characterization of plant photosynthetic machinery-like mosquito salivary components: KOBAS analysis revealed a total of 18 salivary transcripts (sugar-fed library) of 'Plastid' origin, involved in Carbon fixation and Metabolism **(a)**; and Comparative GO term-based differential expression analysis confirmed that at least 11 transcripts matching to PLASTIDs categories (Fisher test/*p-value* ≤0.001) differentially expressed in the sugar-fed library **(b)**, encoding putative enzymes linked to "carbon fixation in photosynthetic organism" pathway.

molecular mechanisms of the herbivores insect-plant interaction has greatly facilitated the design of molecular strategies to save the valuable crops from insect pests. However, such studies have not been given special attention to mosquitoes. From the unexpected findings of the mosquito PLTs, we interpreted that either studies in relation to the sugar feeding associated biology have largely been ignored or the mosquito *An. culicifacies* may have evolved with more complex genetic architecture favouring evolution of environmentally-guided several traits, *viz.* carbon metabolism; light mediated photo conditions for mating, feeding, survival *etc.* Therefore, to predict sugar metabolism associated molecular and functional relationship of salivary PLTs, initially we analyzed all the putative plant-like transcripts against three databases (Reactome; KEGG and Biocycles) annotated for *Arabidopsis thaliana*, using KOBAS online software *http://kobas.cbi.pku.edu.cn/home.do*.

Notably, we observed that 18 transcripts encoding proteins related to at least five Biocyclic pathways linked to photosynthetic organelles, *viz.* plastid in plants (Fig. 9a). To verify above predicted 'plastid' related salivary transcripts, Fisher's exact test analysis was performed, revealing a pool of 11 transcripts differentially expressed in the sugar-fed mosquitoes (Fisher test $p<0.001$; Fig. 9b.); encoding important enzymes/proteins, associated with one of the key pathway "Carbon fixation in Photosynthetic Organisms" (Fig. 10a). Further, we also identified four unique salivary transcripts encoding different enzymes linked to three other secondary metabolite synthesis pathways, namely 'Terpenoid Backbone Biosynthesis' (4-hydroxy-3-methylbut-2-enyl diphosphate reductase/ E.C.1.17.1.2, LYTB); 'Carotenoid Biosynthesis' (Phytoene Synthase/E.C.2.5.1.32, PS); and 'Flavonoid Biosynthesis' (3-dioxigenase/E.C.1. 14.11.9 & 3' beta-hydroxylase/E.C.1.14.13.88) pathways restricted to the plants. A comprehensive molecular and phylogenetic analysis of few selected transcript, encoding an enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate reductase/ E.C.1.17.1.2 (LYTB) and phytoene synthase/ E.C.2.5.1.32 (PS); exclusively revealed unique evolutionary relationship to the cyanobacteria, algae, plants and aphid *Acyrithosiphon pisum* (Fig. 10 b & c). In fact, during its early development mosquito larvae start to feed diverse micronutrients, e.g. bacteria, algae, fungi *etc.*, and switch to feed on nectar sugars in adult mosquito stage. Thus, it could be possible that a long association and regular microbes-mosquito-plant interactions, might have favoured insect/mosquito to adapt, feed, digest sugar and selective synthesis of secondary metabolites/pigments, essential for specific phenotype, e.g. visual pigmentation/dark body coloration. In their natural habitat, mosquitoes are regularly exposed to several environmentally guided abiotic as well as biotic factors, affecting their reproduction, survival and vector competence. Gut bacterial endosymbionts have now been shown to play many key insect's functions such as food digestion and metabolism, reproduction, and fighting against pathogens.

Our recent metagenomic analysis of salivary microbiome identified several unique bacterial phyla, including Chlorobium, Cyanobacteria, Nitrospira and other phototrophic bacteria associated with salivary glands (Fig. 10d), but absent in the gut of the laboratory reared 3-4 days old adult female mosquito *An. culicifacies*. Indirectly, above findings further support the hypothesis that mosquito may have feeding associated distinct plant like molecular machinery components, partly shared by residing symbiotic bacterial community for diverse carbon/nitrogen rich plant sugar source metabolism. With the current data we hypothesize that HGTs in metazoans may also play important role in the evolution and acquisition of beneficial traits that facilitate feeding and survival adaptation over diverse ecologies. This hypothesis is further strengthen by our following new observations: (a) that PLTs expression seems to be restricted to the tissues, *i.e.* the feeding machinery components that facilitate digestion and metabolism, e.g. salivary glands, midgut olfactory tissues in case of the mosquito (Fig. 11); (b) absence of PLTs from other non-digestive tissues, e.g. haemocyte (mosquito blood cells/ data unpublished); (c) the finding of dominantly associated unique bacterial species to the mosquito digestive tissues, *viz.* salivary gland and midgut, e.g. *Acidobacteria* (sugar metabolism); *Agromonas* bacteria, a soild oligotrophs (nitrogen fixing bacteria) that usually grow at extra low nutrient environments of the paddy field, complementing the high larval density of the mosquito *An. culicifacies* in paddy fields of the rural India.

Furthermore, in mosquitoes it has long been accepted and proved that a significant variation exists in the chromosomal DNA as well as genome

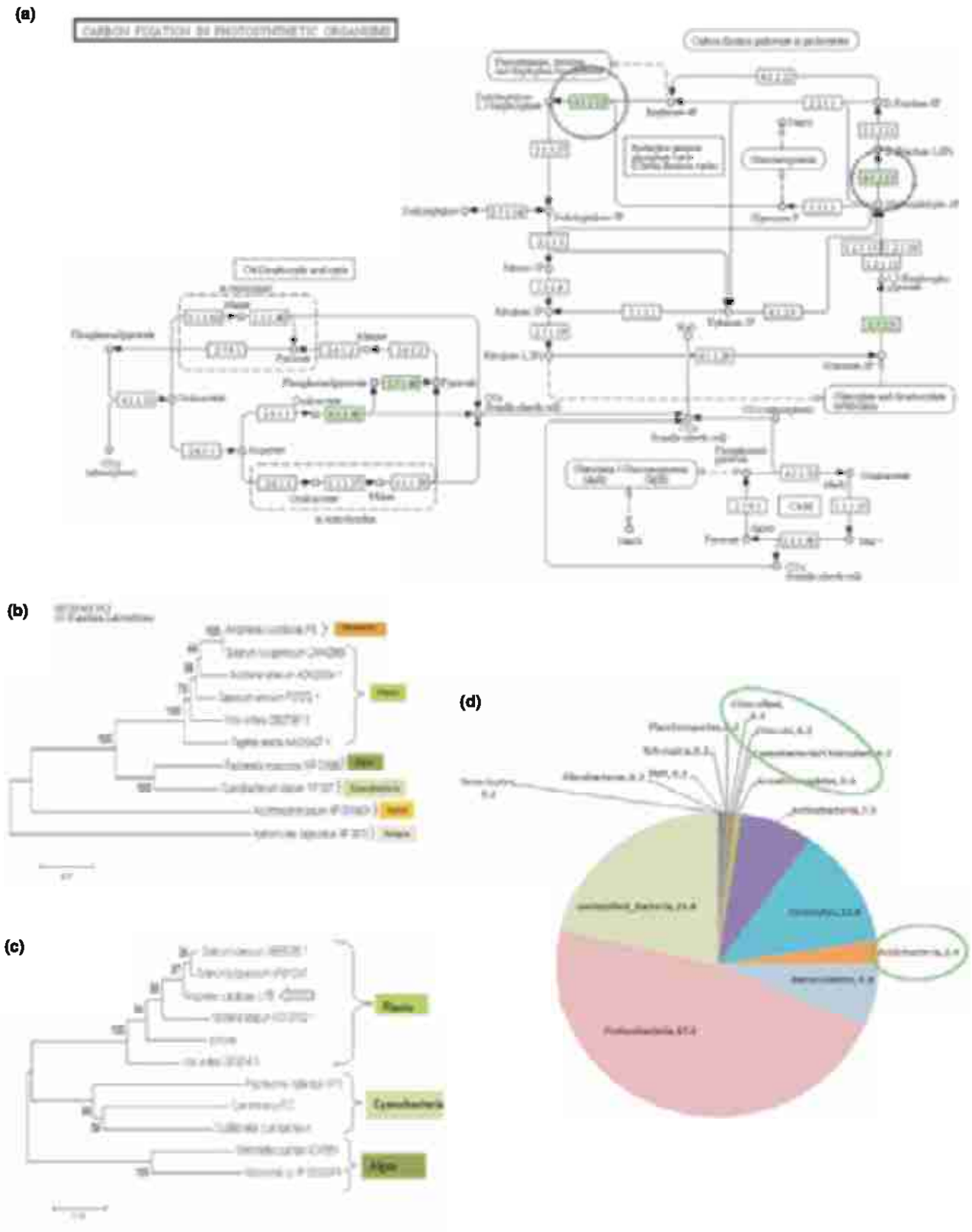

Fig.10: Molecular evidence that mosquito encodes plant-like photosynthetic machinery components partly shared by symbiotically-associated salivary bacteria for carbon fixation and metabolism: **(a)** KEGG prediction of salivary transcripts (differentially expressed/ Fisher test p <0.001) encoding enzymes (green); **(b)** Phylogenetic analysis of a unique mosquito salivary transcript, encoding a plant homolog 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; **(c)** phylogenetic analysis of a unique mosquito salivary transcript, encoding a plant homolog phytoene synthase; and **(d)** Identification of symbiotically-associated salivary microbial flora predominated and unique bacteria (marked green circle), probably assisting mosquito to adapt, feed and metabolize diverse carbon rich sugar sources of plant origin.

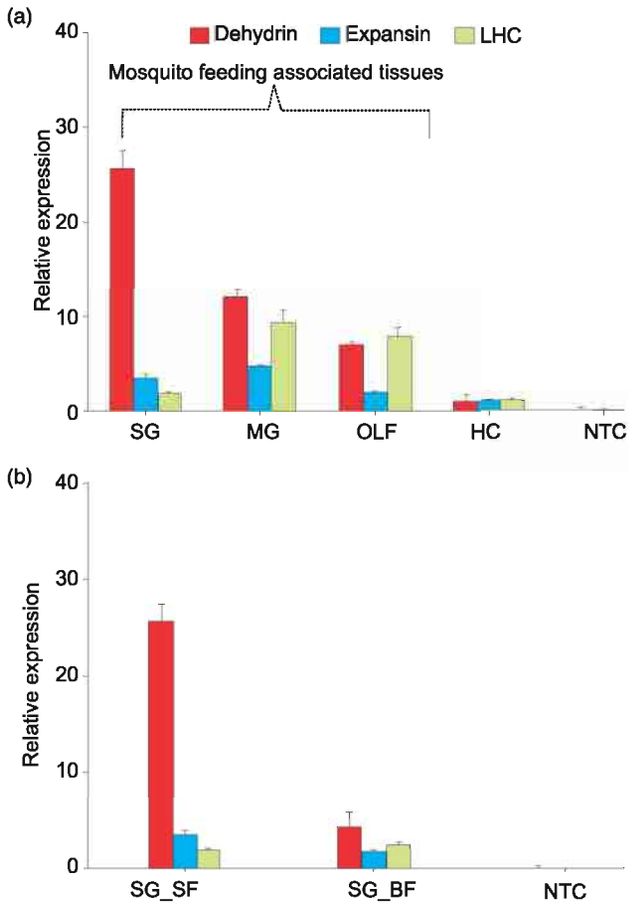

Fig. 11: PLTs dominantly express in feeding associated tissues: **(a)** Tissue-specific expression of PLTs; **(b)** Blood meal response of salivary glands PLTs, *viz.* dehydrin, expansin and light harvesting complex (LHC) in the adult female mosquito. SG_SF: Salivary gland sugar-fed; SG_BF: Salivary gland blood-fed; MG: Midgut; OLF: Olfactory; HC: Hemocyte; NTC: No template control.

size within *Anopheline* and other mosquito species, but how these variations differentially affect the mosquito biology, *viz.* behaviour, physiology, immunity and vectorial capacity etc., are poorly understood at the molecular level.

1.1.5 Molecular analysis of mosquito olfactory system in *Anopheles culicifacies*

One of the key molecular strategies to fight VBDs may rely on disorienting/altering the adult-female mosquito host seeking behaviour. Unlike adult male mosquitoes, evolution and adaptation to blood feeding is a unique property in adult female mosquitoes for egg maturation and Oviposition. To meet the demand of blood meal, it need to manage multiple behavioural co-ordinates including searching, locating, landing over suitable host, followed by tracking the proper site to pierce and suck the blood within two minutes. It is the salivary glands which mediates first biochemical interaction with human host blood and facilitate successful blood meal uptake. Our recent study in Indian malarial vector *An. culicifacies*, suggest that adult female mosquitoes salivary glands are evolved with unique ability of gene expression switching to manage meal specific responses. However, it remains unknown that how adult female mosquitoes regulate host seeking behavioural co-ordinates and the salivary actions.

Blood meal cause minor shift in olfactory proteins: To unravel this molecular complexity, in the present investigation we performed RNAseq transcriptomic analysis of the olfactory system in the adult female mosquito *An. culicifacies*. We generated a total of ~122 million Illumina sequencings reads from the mosquito olfactory system collected under distinct feeding status, *i.*e. Naïve (N-UF), 30 min post-blood-fed (30M-PBF) and 30 hour post-blood-fed (30H-PBF) of the mosquitoes. Following quality filtration and *de novo* assembly, an extensive molecular and functional annotation is under progress as indicated eariler in Table 2. An ongoing preliminary annotation of RNAseq analysis unraveled a limited but remarkable change in the nature and regulation of unique set of olfaction gene repertoire in response to distinct feeding status (Fig. 12).

Molecular characterization of odorant binding proteins (OBPs): Insects are able to detect and discriminate thousands of odor molecules by the specialized olfactory receptor moieties expressed on the specific chemo-sensitive neural cells which reside within the hair like sensilla scattered over the surface of the antennae and maxillary palps. Odorant binding proteins (OBPs), which are bathed within the sensillum lymph, are low molecular weight soluble proteins that mediate the first interaction of the olfactory system with the external world, are shown to play important role in the biology of olfactory system in many insect species and mosquitoes, but the associated complexity of OBP molecules influencing major behavioural properties of host seeking, blood feeding and Oviposition site finding are yet to be clarified. Toward this goal, currently we are cataloging the OBPs from the olfactory transcriptomic databases. An ongoing annotation analysis predicts a total of 65 OBPs, which could be classified in three major

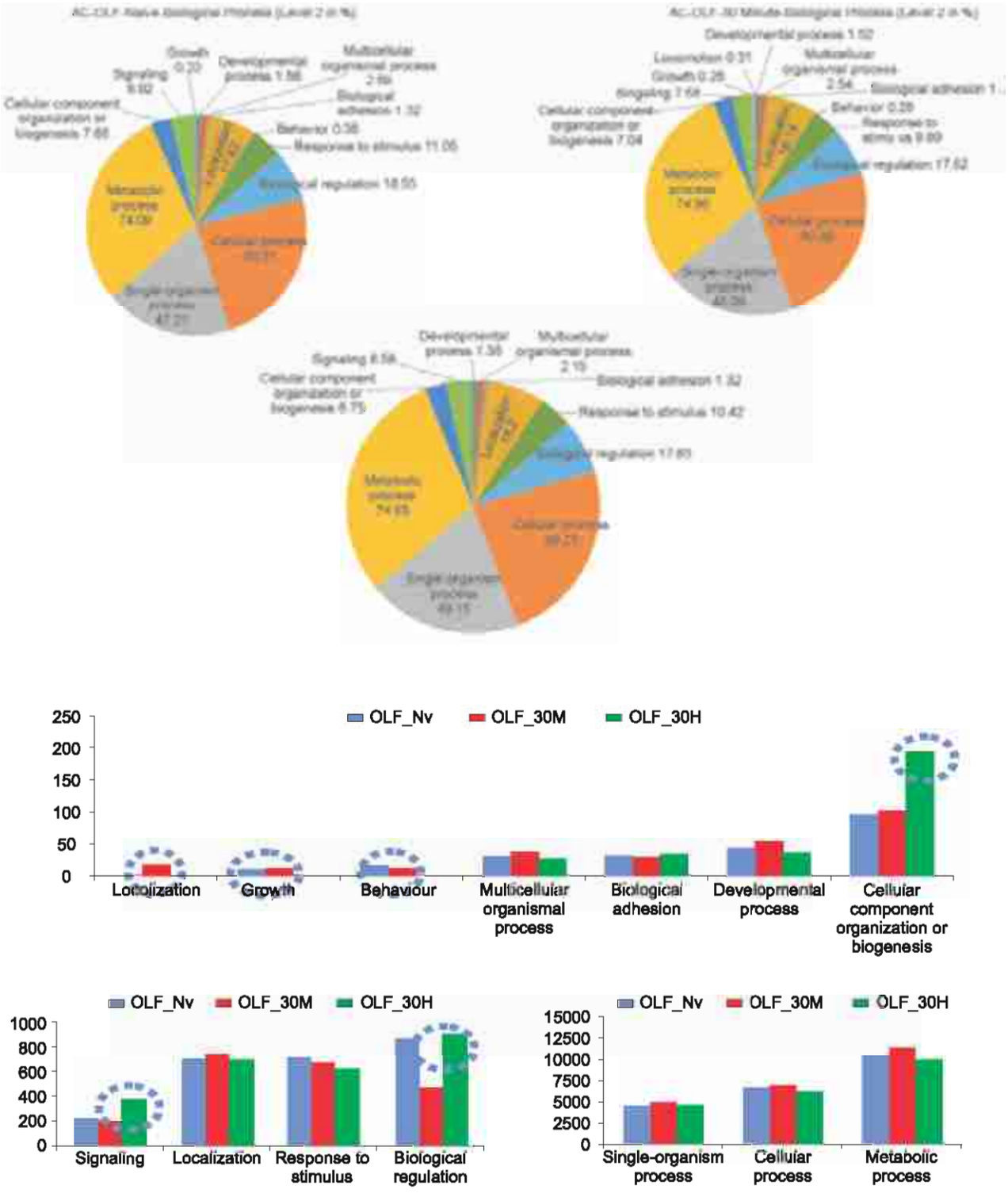

Fig. 12: Preliminary functional prediction and comparative analysis of mosquito olfactory RNAseq databases. Pie chart graph shows GO annotation catalog of mosquito olfactory encoded proteins, engaged in different biological process, in response to differential feeding status. Bar graphs show differential score distribution of selected gene family members. Marked circles indicate major changes associated with particular functional catagories.

categories: Classic OBP, Plus-C OBP and Atypical OBP. A preliminary phylogenomic analysis of Classic OBPs reveal conserved molecular signature of six cysteine residue within insect OBP domains, having strong phylogenetic relation within mosquito species (Fig. 13).

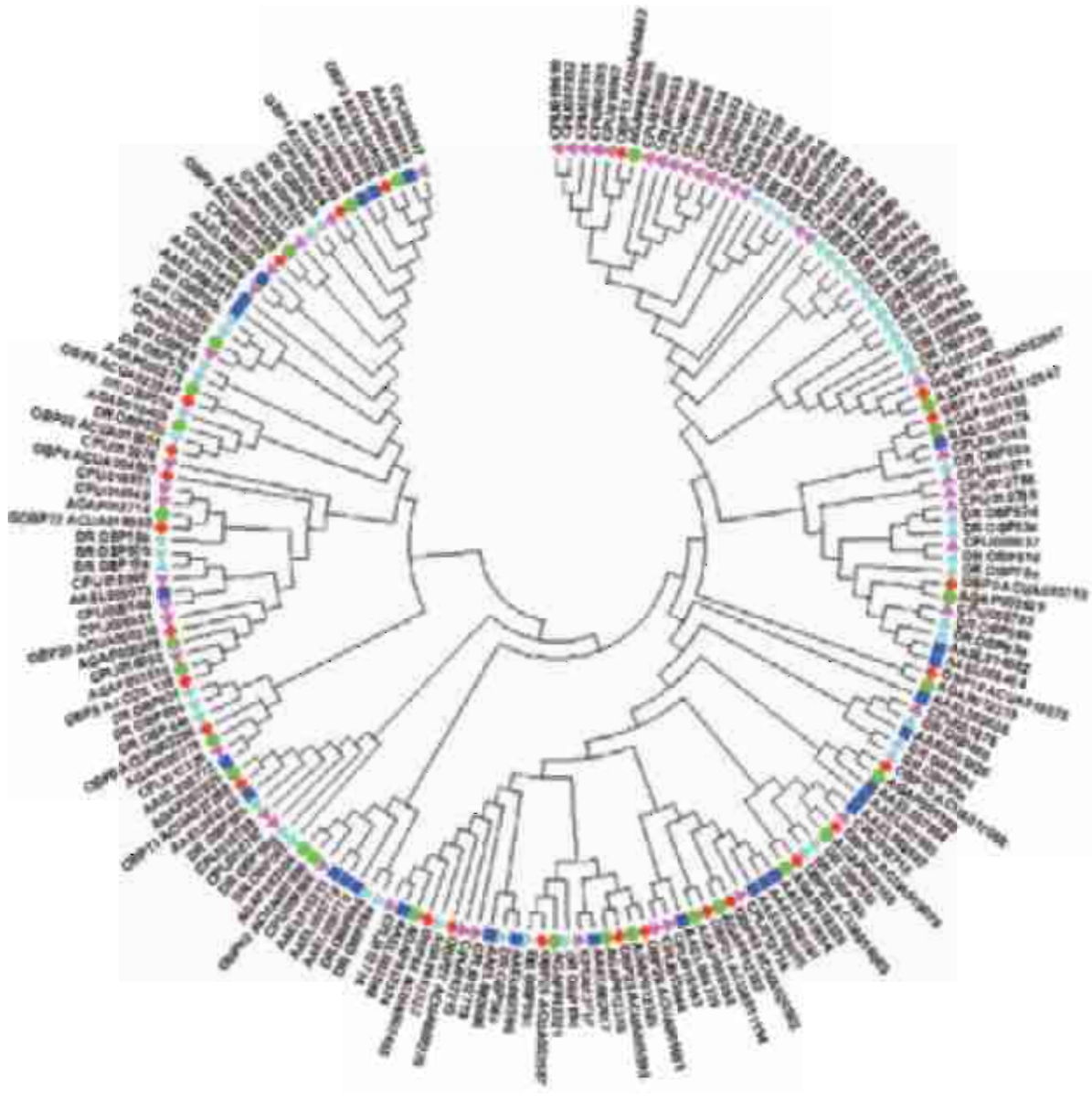

Fig. 13: Molecular phylogenetic analysis of classic OBPs identified from the Olfactory system of *An. culicifacies* by maximum likelihood method.

1.1.6 Impact of thermal conditions on survival and susceptibility of mosquito vectors to temephos

The present study was aimed to find out the role of rearing temperature and container material in larval development of *Aedes aegypti* mosquito. Three types of container material plastic, iron and earthen material were selected for the study. At temperatures >22°C, 50% pupation in earthen pot was completed within 4.3±0.6 – 6.3±0.6 days followed by plastic containers (5±0 – 8±0 days) and iron containers (6±0 – 9±0 days). Out of the three containers, pupation did not occur in the iron containers at 38°C while at 40°C there was no pupation in any of the three types of containers. A significant variation was found between the temperature of water in three types of containers and the temperature of environmental chamber ($p<0.05$). The temperature of water in all the containers was found lower than the temperature of environmental chamber. The difference in temperature between water and environmental chamber was highest in case of earthen pots (1.9–4.1°C) followed by plastic (1.2–3.3°C) and iron containers (0.8–2.5°C). More than 35°C temperature of water was found inimical for pupal development. Larval susceptibility studies with

larvae reared at experimental temperatures against temephos are in progress.

1.2 Vector Control

1.2.1 Reducing dengue transmission in Najafgarh zone of Delhi- An integrated vector management approach

A study was undertaken in 24 lanes of F-block, Raj Nagar II, Najafgarh zone, New Delhi. KAP, socio-economic and entomological data were collected. The study indicated the followings:

1. Analysis of entomological data indicated that the highest breeding was recorded from houses with tanker water supply (multiple water storage containers) and solid waste items (Figs. 14 & 15). Container index, house index and pupal index indicated indigenous transmission in the area.

Figs. 14 & 15: Multi-water storage containers and solid waste in Raj Nagar II.

2. A GIS-based risk map was generated based on these identified ecotypes.
3. Dengue cases recorded in NIMR clinic were geo-referenced using GPS and a dengue case map was generated.
4. Validation of GIS risk map was done with respect to dengue case map which indicated that cases clustered in and around houses with tanker water supply (multiple water storage containers).
5. Houses with tanker water supply and those within 20 m radius of it were found contributing around 60% of the dengue cases.
6. Socio-economic data was classified which indicated that households with low income group were having multiple water storage containers and solid waste stored on roof tops and courtyards.
7. Analysis of KAP data helped to identify target group in the community for conducting IEC activities which was organized during the month of October 2015. A live demonstration of different stages of *Ae. aegypti* was also conducted (Figs. 16 & 17). RWAs, residents, ward councilor and students attended IEC programme and people were shown the pictures of their households and various containers where breeding was recorded. Stakeholders appealed the masses to eliminate these breeding, in their households on regular basis.
8. Source reduction is being taken up in order to reduce dengue transmission in the study area.

Figs. 16 & 17: IEC activities in Raj Nagar II, New Delhi.

1.2.2 Evaluation of NetProtect LLIN (impregnated with deltamethrin) against malaria vectors in the states of Haryana, Uttar Pradesh and Jharkhand

Phase-III studies were conducted for the third year of the trial as per the standard protocol. During last one year, bioefficacy studies were performed every month on community used nets under field conditions and the bioefficacy of the nets was drastically reduced against *An. stephansi*. Keeping in view results of the trial, it has been established that NetProtect LLIN failed to fulfill WHO criteria for long-lasting insecticidal nets. However, the positive aspect of the trial was high compliance rate of the net usage and collateral benefits experienced by the community.

1.2.3 Evaluation of the efficacy of PermaNet®3.0 long-lasting insecticidal nets in areas with variable deltamethrin-resistant malaria vectors in India

This study was intended to study the efficacy of a combination LLIN containing deltamethrin and PBO. Altogether 21 districts in five states were selected for the study. Bengaluru Field Unit have been undertaking the study in 15 districts in three states, namely Karnataka (10 districts), Andhra Pradesh (3 districts) and Telangana (2 districts). Susceptibility status of *An. culicifacies* was carried out in 6 districts at 10 sites in the state of Gujarat out of which 4 sites from 3 districts were selected for the study. Similarly 3 districts were selected in Chhattisgarh with variable deltamethrin resistance

and efficacy of PBO. Mosquito collections and insecticide susceptibility tests are in progress at Chittoor, Tumkur, Bijapur, Bangalore rural, Mangalore and Bagalkot districts. Further studies are in progress.

1.3 Insecticide Resistance

1.3.1 Impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in India: A multidisciplinary approach (WHO project)

A study was initiated in 2012 to assess the impact of insecticide resistance in malaria vectors on the effectiveness of combination of indoor residual spray (IRS) and long lasting insecticidal nets (LLINs) in India. Data on insecticide susceptibility to two insecticides, deltamethrin and bendiocarb was generated in all the 80 study villages. LLIN or Combination interventions (IRS and LLINs) was assigned to study clusters based on the susceptibility status and malaria incidence by restricted randomization method with data on insecticide resistance and malaria incidence. May 2015—continuous fortnight active surveillance of study cohort households (by Mitanins) and all other non-cohort household surveillance (by malaria surveillance workers) was established and are in place. August 2015- mass blood collection of study cohorts was carried out to assess the subclinical infection among study children. Overall positivity rate has been reduced to 1.4 compared to 7.4% during recruitment of children. August-December 2015—LLIN survey to assess the LLIN uses and physical integrity after one year of distribution in the study area (As per RBM guidelines) was carried out. A total of 3035 HH were surveyed and 15,461 persons' data were collected from 80 villages. Data are being analyzed. Online and offline epidemiology and entomology data entry is ongoing in web-based database management programme built with support from NIC, Raipur. Till date 1.46 million study data have been entered in to the database programme. All study cohort households will be geo-spatially linked with coordinates, 33/80 villages have been linked so far. In December 2015—A cross-sectional study was carried out to assess the prevalence of malaria post-LLIN distribution in all the study villages. In January 2016—An evaluative study to assess Mitanins work in malaria surveillance has been done. A total of 157 Mitanins were interviewed from 80 villages. Data are being analyzed. A study on submicroscopic malaria in tribal and forested malaria endemic villages during non-transmission season is planned and to be carried out in May 2016. Behaviour study is ongoing in selected villages. Collected data in relation to insecticide resistance and malaria infection are being analyzed. All relevant entomological and epidemiological techniques are standardized and studies are in progress. Evaluation studies on impact of interventions on malaria transmission are in progress.

1.3.2 Studies for development of method(s)/strategy for the management of pyrethroid resistance in malaria vectors in India using insecticides with novel mode of action

The present study was proposed to develop method(s)/strategy for control of pyrethroid resistant malaria vectors using insecticides with novel mode of action using insecticides that show negative cross-resistance. Pathway prediction analysis identified three detoxification enzymes: malathion carboxylesterases (EC 3.1.1.1), pyrethroid hydrolase (EC 3.1.1.88) and unspecific monoxygenase (EC 1.14.14.1) followed by *in silico* molecular modeling and docking results showed common active binding site for pyrethroids and pro-insecticides.

Further, biotransformation pathway routes were predicted for IRAC Mode of Action classified 210 insecticides and 6 synergists, namely PBO, TPP, DEF, DEM, verapamil and ethacrynic acid. This study showed similarity in biotransformation rules in addition it was found that specific biotransformation rules were involved in insecticidal activity while others for detoxification. In addition, these detoxification enzyme were identical to the activation enzyme of pro-insecticides and for synergist.

It was found that several insecticides followed single biotransformation rule for insecticidal activity. These insecticides with single biotransformation and different MoA may be promising when used in rotation/ mixture/ combination. Other insecticides exhibited dual biotransformation rule where one is responsible for insecticidal activity/ susceptibility and the second for detoxification/ resistance. Here, excluding

insecticides with similar/same detoxification/ enzyme route will help avoid cross-resistance when used for rotation for managing insecticide resistance. These combinations will exhibit negative correlated resistance.

Topical assay studies showed an increase of intrinsic toxicity to chlorfenapyr in pyrethroid resistance strains. Native PAGE studies showed both qualitative and quantitative levels of intensified esterases in *An. culicifacies* field collected wild population than in *An. culicifacies* susceptible laboratory strain. This study also showed that malathion comparatively inhibited more esterases than deltamethrin. Biochemical analysis also showed that esterases was comparatively higher in resistant strains while, mono-oxygenases and GST where equivalent to susceptible strains.

Planning and implementation of insecticide resistance management strategy is crucial to prevent selection of insecticide and development of resistance. This information may unravel the metabolic resistance mechanism and provide an insight to design strategies for IRM.

1.3.3 Field validation of bottle assay for monitoring insecticide resistance in adult mosquito vector species

The bottle assay method developed by NIMR was validated for routine insecticide resistance monitoring of lab and field mosquito vector species. The study was validated at VCRC, Puducherry and the NIMR Field Units at Raipur, Bengaluru and Chennai using laboratory/field collected mosquitoes. Insecticide solution and bottles (without impregnation) for the assay were provided to the investigators by NIMR. Validation was done for the determined diagnostic dosages of 10 µg/bottle for anopheline species and 2 µg/bottle for *Aedes* species (Fig. 18). The assay will be standardized for other insecticides and synergists and validated in the field and adapted for large-scale use. Efforts will be made to adapt for use in the programme.

1.3.4 A laboratory simulation study on suppression of resistance genes by differential exposures to insecticide in *Anopheles stephensi* Liston

This study was performed to simulate conditions for differential exposure. For this, mosquitoes were exposed to differentially masked DDT 4% impregnated papers with Whatman no.1 filter paper resulting in a simulated exposure of known areas, viz. 25, 50 and 75%. Mosquitoes were exposed following WHO adult susceptibility test. The changes in the susceptibility status in 3-day old glucose-fed mosquito strain were assessed at each generation by exposing them to the masked DDT 4.0% impregnated papers. The mosquitoes at each generation were exposed to the same, *i.e.* 25, 50 and 75% masked areas to simulate the insecticide sprays in the field, *i.e.* simulating variable coverage due to uneven spray that generally lead to variable selection. This variable simulated differential exposure has resulted in precipitation of increased resistance while, complete exposures resulted lower levels of resistance.

1.3.5 Comparative genomics of insecticide resistance gene families in mosquito vector

Identification, classification of detoxification enzyme family *CYP 450*, *GST* and *COE* genes in *An. culicifacies* and *An. stephensi* are completed. The present study is a first attempt to identify the detoxification genes that belong to *CYP 450*, *GST* and *COE* gene families from the first Genome assembly of *An. culicifacies* and *An. stephensi* species from the Vector base database. In *An. culicifacies* 105 sequences were found. In GST family 24 sequences, CYP 450 family 61 sequences and COE family 20 sequences were found. In the gene sequence exon-intron organization was elucidated. In *An. stephensi*, Indian strain, 72 sequences were found. In GST family 24 sequences, CYP 450 family 41 sequences and COE family 7 sequences were found. In *An. stephensi* SDA 500 strain 133 sequences were found. In GST

Fig. 18: Validation of bottle assay technique.

family 33 sequences, CYP 450 family 92 sequences and COE family 8 sequences were found. The work is in progress. The end point utility of the work after completion by the year 2017 will be to gain deep insights into the gene families involved in insecticide resistance. The outcome of the present investigation will give a broad view of the organization and evolution of the insecticide resistance conferring gene families on different mosquito species. This will be also further useful in understanding to design an insecticide with novel modes of action and provide valuable information for designing molecular biology, genomics, transcriptomics and proteomics-based experiments efficiently.

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Parasite Biology

2

2.1 Molecular characterization of 4-diphosphocytidyl-2C-methyl-d-erythritol (IspE) kinase gene from *Plasmodium vivax*– Ligand recognition in a template for antimalarial drug discovery

In continuation to previous work on *Plasmodium vivax*, our present study includes the collection of clinical parasite samples of *P. falciparum* from different geographical regions of India in order to study the genetic polymorphism of *IspE* kinase gene. *IspE* gene of 1614 bp has been amplified and sequenced among all the samples using IspE primers designed by taking gene of *PfIspE 3D7* as a control. Further bioinformatics analysis using Blast shows 100% homology among isolates of *P. falciparum* collected from different geographical regions of India. Due to 100% similarity of nucleotide sequence, representation of only one strain of *Pf*IspE (India) is shown (Fig. 1).

Further 3-D model of *Pf*IspE protein was generated using deduced amino acid sequences (537) with the help of I-TASSER active site of IspE are depicted (Fig. 2).

Next to that IspE orthologous inhibitor library of 73 known compounds are prepared. *Pf*IspE protein models are molecularly docked with all the 73 inhibitors by submitting the structures to the Patchdock server. Best 1000 dockings by Patchdock were further refined by resubmission in Firedock. On the basis of docking parameters, *i*.e. 'global free energy', 'attractive & repulsive Van der Waals forces' and 'area of interaction' compounds were shortlisted. The deciding factor, however, the

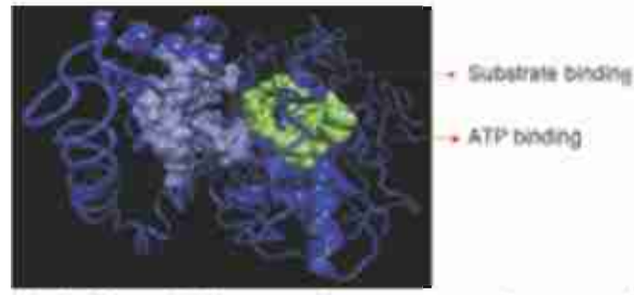


Fig. 2: 3-D model with structural representations of active site of *Pf*IspE.

Fig. 1: Nucleotide sequence of Indian *P. falciparum IspE* gene with deduced amino acid sequence.

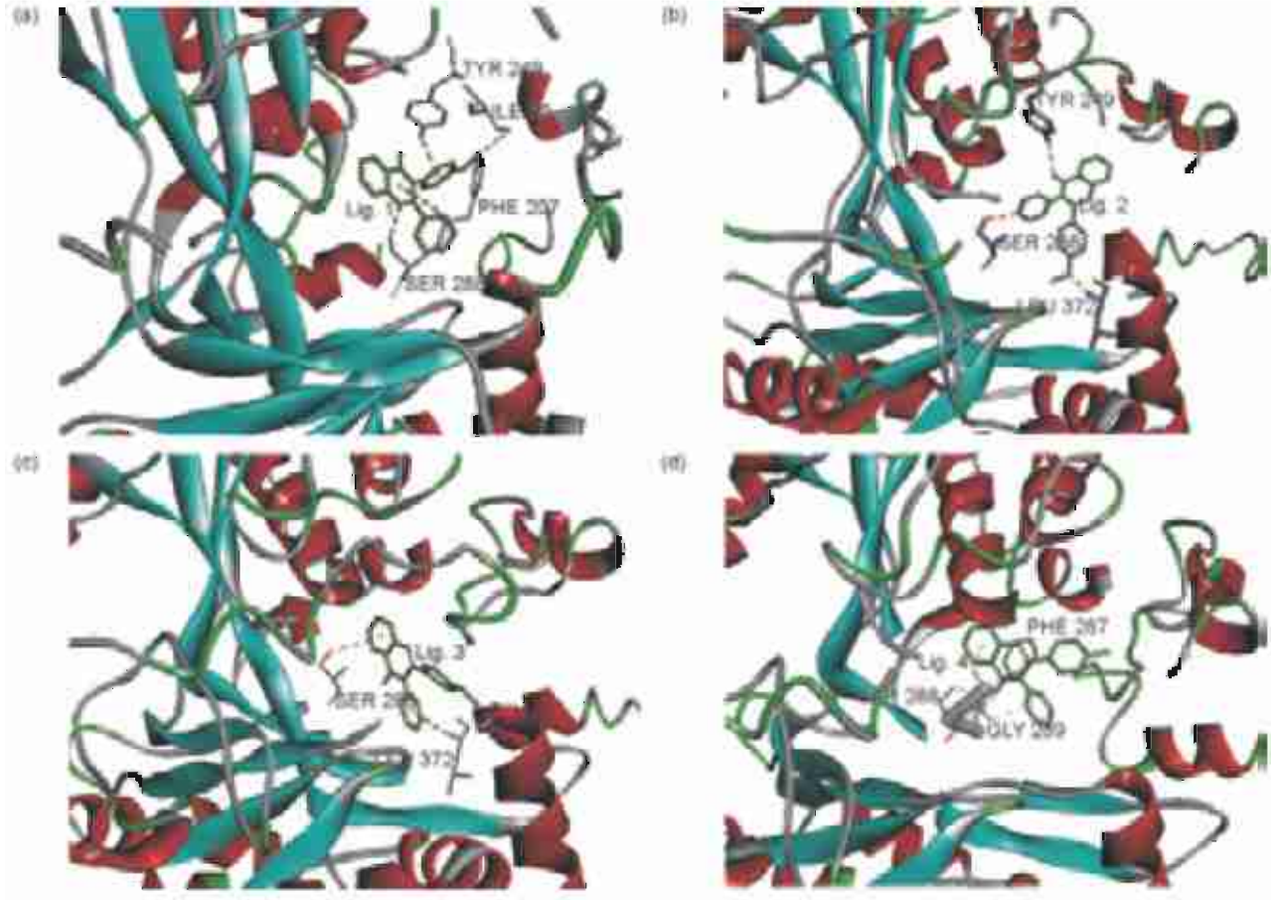


Fig. 3: Illustration depicting docking of selected four inhibitors (a, b, c, d) with *Pf*IspE 3-D model.

interaction with the active site determined by visual inspection of docked compounds by VMD software (Fig. 3). *In silico* ADME was performed for all of the compounds using Osiris property explorer for descriptors such as partition coefficient (aqueous solubility and intestinal absorption), solubility, carcinogenicity, mutagenicity and reproductive/ developmental hazards. After rigorous screening, finally we narrowed down four lead compounds that shown promising results with *P. falciparum* IspE enzyme (Table 1).

These 4 compounds are found to be non-toxic and had a very good drugablity score. Further optimization and testing of these compounds are in process that may lead to the development of novel antimalarials.

Table 1. Molecular property (ADMET analysis) of selected compounds

Compound ID	A	B	C	D
structure				
cLogP	2.88	2.88	3.24	3.3
Solubility	–3.91	–3.91	–4.44	–4.24
Mol weight	329	329	329	313
TPSA	54.79	54.79	54.79	45.56
Drug likeness	1.14	3.03	3.9	1.4
Drug score	0.69	0.76	0.71	0.67

2.2 Molecular characterization of *Plasmodium falciparum phosphoethanolamine methyltransferase (PfPMT)* gene: A novel antimalarial drug target

Phosphatidylcholine (PC) synthesis is the most essential phospholipid synthesized through serine-decarboxylase-phosphoethanolamine-methyltransferase (SDPM) pathway in *P. falciparum*, at very fast rate for the rapid multiplication of the *P. falciparum* within human host. Phosphatidylcholine is the most abundant phospholipid in *Plasmodium* membranes. Parasite requires phosphatidylcholine for growth, rapid multiplication at blood stages (rings, trophozoites, and schizonts) and for gametes development within the host.

Two set of primers were designed for amplification of *PMT* gene of 1382 bp. Gene amplifica-

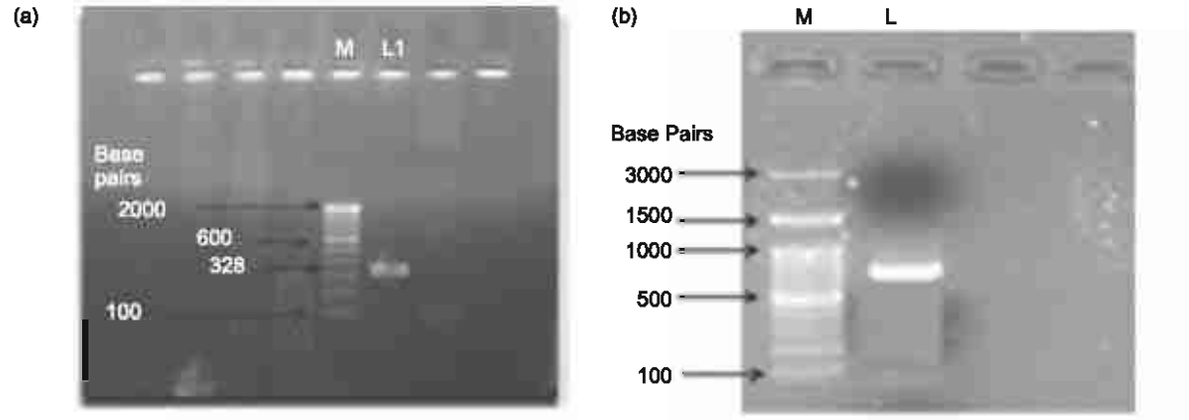


Fig. 4: PCR amplified product of *PMT* gene of *P. falciparum*. M – Master mixer (100 bp DNA Ladder): (a) L1- *PfPMT* gene amplified product (328); and (b) L - *PfPMT* gene amplified product (743 bp).

tion of *PfPMT* gene of *P. falciparum* culture (parasite lab, NIMR) has been carried out by one set of primer and amplified in two parts, *i.e.* 328 and 743 bp (Figs. 4a and b), respectively.

Further multiple alignment of PMT protein of different *Plasmodium* strains showed that almost all the PMT proteins are conserved among each other. Fully conserved amino acids are shown in red colour (Fig. 5). This conserved region of *PfPMT* implied that PMT protein is functionally as well as structurally conserved among all the plasmodium strains.

Phylogeny of *PfPMT* (Indian) and its PMT orthologues obtained from KEGG, EuPathDB, other plant orthologues and PtdEtPMT of human were studied using MEGA 6.0. The studied sequences

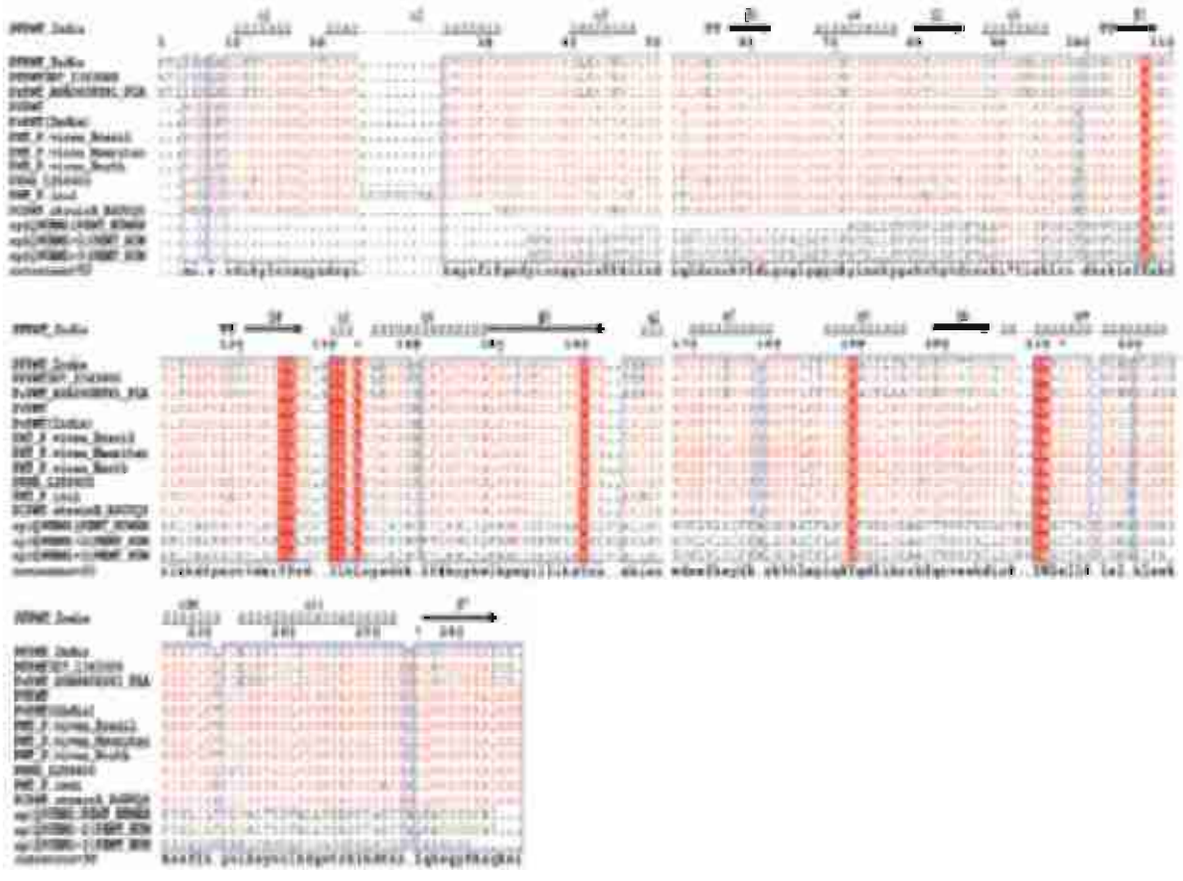


Fig. 5: Multiple alignment of *PfPMT* (India) with other orthologs of *Plasmodium* and human phosphatidylcholine ethanolamine methyltransferase (PEMT) enzyme isoforms.

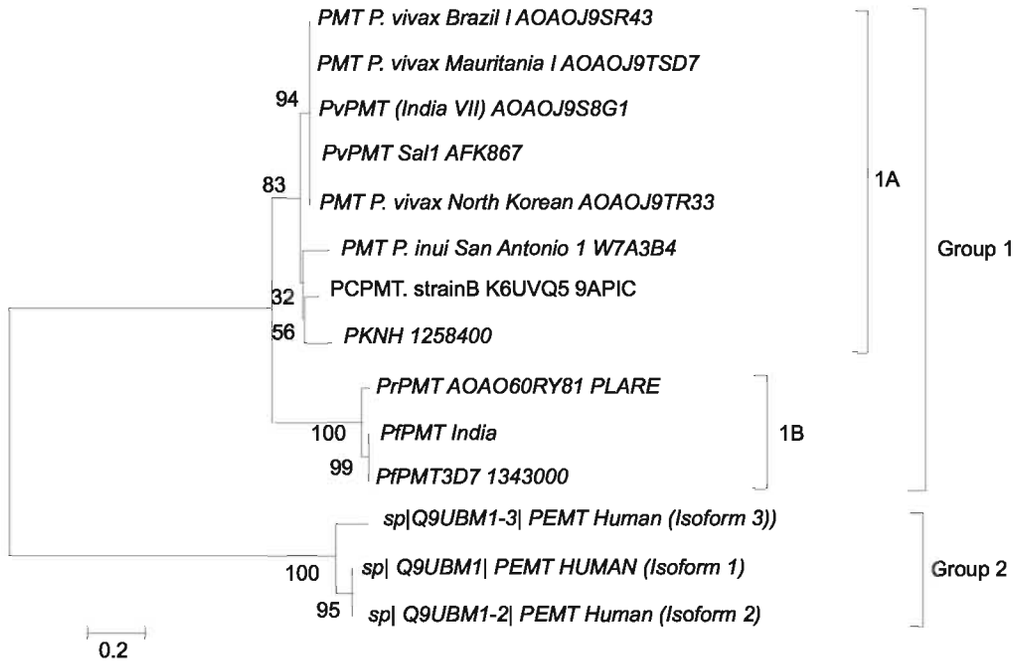


Fig. 6: Phylogenetic analysis of Indian *PfPMT* with other pmt orthologues in *Plasmodium* spp using neighbour joining method.

were aligned according to the ClustalW and the phylogenetic tree was constructed based on the neighbor joining method. The bootstrap confidence replication value was kept 10,000 (Fig. 6).

Phylogenetic tree showed that all studied proteins members clustered into two major, *i.e.* Group 1 cluster of PMT protein of all *Plasmodium* spp. isolates globally and Group 2 is a unique distant cluster of human PtdEPMT isoforms and subdivided into inimitable related family members of phosphoethamolamine methyltransferase protein.

Further modeling and docking studies are in process. Procurement of identified compounds and *in-vitro* parasite culture testing are in progress. Compounds with good inhibitory profile may provide lead for further antimalarial drug development.

2.3 Polymorphisms in gametocyte gene *Pfg 377* in *Plasmodium falciparum* field isolates

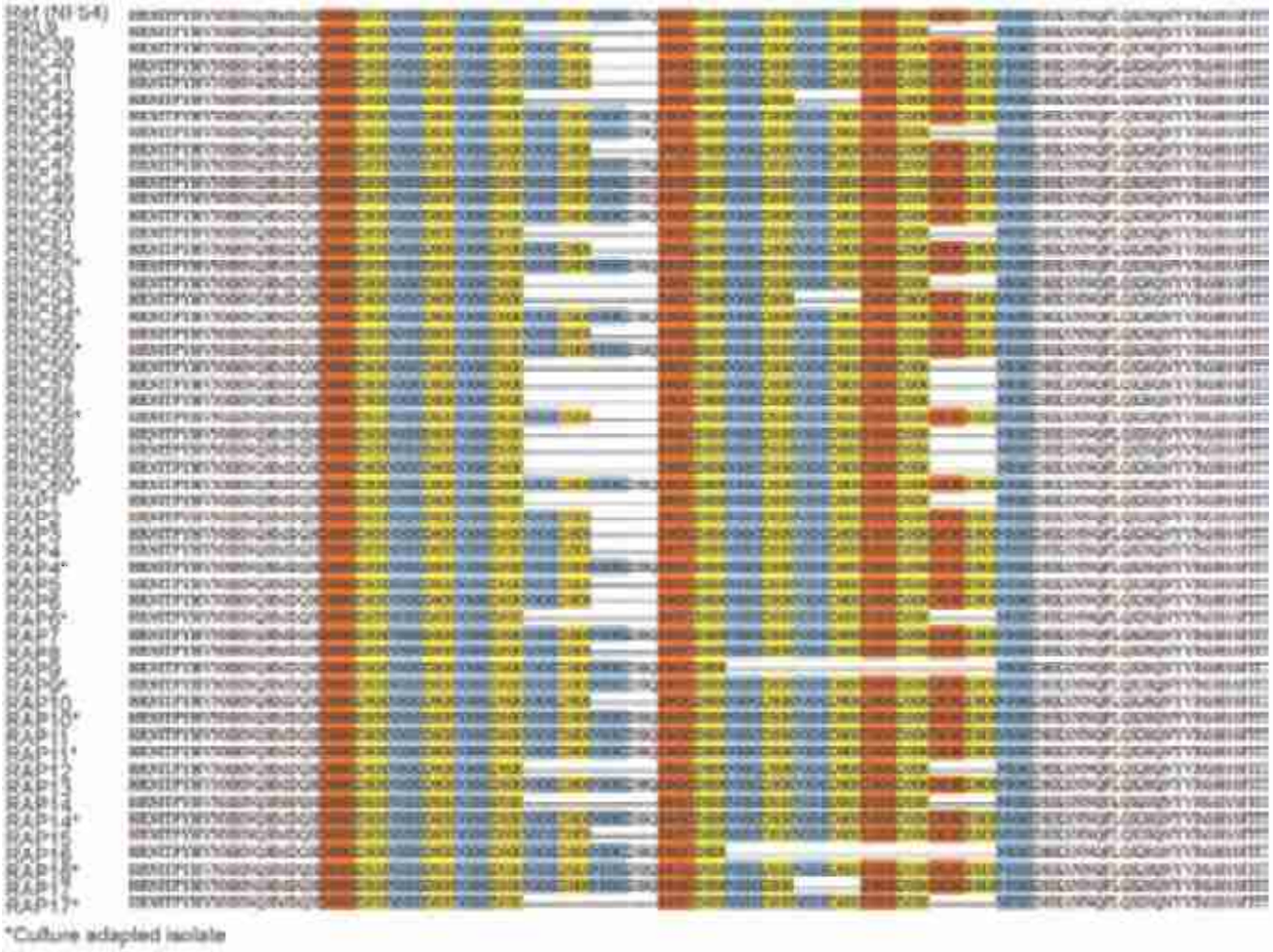
The malaria parasite lifecycle requires the production of gametocytes and transmission of gametocytes from human host to the vector. Gametocytes ensure the transmission of malaria to the mosquito although they are not responsible for clinical symptoms. The information available on gametocytogenesis in natural infections is limited till date. There have been reports indicating that some correlation might exist in drug sensitivity and gametocytemia in natural infections. To understand the complex gametocytogenesis process the analysis of gametocyte gene *Pfg377* involvement is crucial. The purpose of this study was to type the *Pfg377* genes in the field isolates of *P. falciparum*. Allelic typing of *Pfg377*gene in region 3 was compared between genomic DNA of field isolate and culture adapted samples. Different allelic type in region 3 of *Pfg377* was detected in field isolates and culture adapted samples. Total of three different alleles were detected which based on size polymorphisms in the 39 field isolates of *P. falciparum*. Out of 39 isolates, 6 possessed only one allelic type (*i.e.* 63bp deletion), 9 isolate possessed two different allelic types (*i.e.* 21 bp and 4 bp deletion). We identified three allelic types of *Pfg377* as A, B, and C, as distinguished by size polymorphism of PCR amplified fragments. The sizes of PCR products alleles A, B, and C, as observed by 1.5% agarose gel electrophoresis, were 292, 324, and 355 bp, respectively with the most common allele being 355bp (Fig. 7). After analysis of the identified amino-acid sequence, six polymorphic amino-acid identified (H-Q/I-M/H-Q/N-D/Q-H/D-N/H-Q/N-D/D-N) at different codons 36, 38, 40, 42, 55, 56, 62, 63, 77, respectively.

Fig. 7: Lanes 1-7 show field samples positive for *Pfg377* gene after PCR assay; and Lane 8 shows 100bp DNA ladder.

The cryopreserved and adapted samples of the same isolates showed difference in their genotypes on allele typing indicating the presence of multiple clones in the same sample on sequence comparisons (Fig. 8). It is important to analyse the functional role of *Pfg377* gene to understand its role in transmission. We were able to detect gametocytes in most blood samples isolated from patients during *P. falciparum* infection by a sensitive PCR detection method.

2.4 Immunomodulatory role of mesenchymal stem cells in the pathogenesis of malaria infection

Infusion of Mesenchymal Stem Cells (MSCs) derived from malaria infected animals to naïve animals protected against *P. berghei* infection. Further, we were interested to understand the mechanism underlining by which mesenchymal stem cells are able to protect the animals from malaria infection. Therefore, we intended to look at what is the fate of these Sca-1$^+$CD44$^+$CD29$^+$ CD34$^-$ cells, after adoptive transfer. In contrast to our expectation of increase in number of these cells by infusing mice, we found the number of Sca-1$^+$CD29$^+$ cells decrease on 7th day of post-infection compared to infected mice as deduced by FACs analysis. This observation was further confirmed by Immuno-histochemistry (IHC) by staining with anti-CD29 antibody (Fig. 9). The numbers of CD29 positive cells were observed less in spleen section from mice that were previously



*Culture adapted isolate

Fig. 8: Sequence results of cryopreserved and adapted samples of field isolates allele typed for *Pfg377* gene.

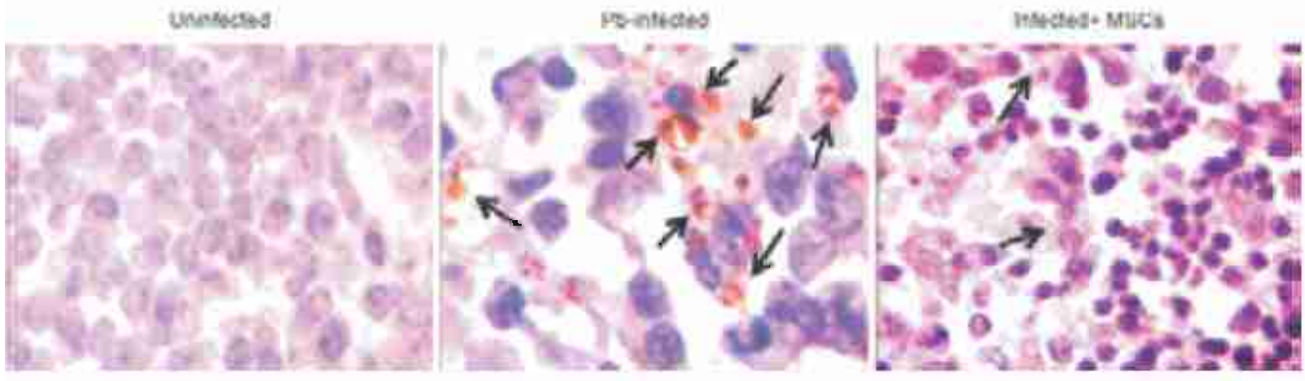


Fig. 9: Immunohistological analyses of spleen from uninfected, infected (IP) and mesenchymal stem cells infused (IV) mice. Sections were cut at 0.5 µ and stained with anti-CD29 antibodies. Black arrows indicate CD29+ cells in the spleen section of mice at 100 x magnification.

infused with Sca-1$^+$CD29$^+$ cells following malarial infection than that of malaria infected animals. These results indicated that these mesenchymal stem cells might be differentiated to other lineage. As mice infused with these cells were able to recover from malarial infection, we wondered whether these cells are able to differentiate into CD34$^+$ haemopoetic cells and / lymphopoitic cells. Therefore, we analysed the presence of these cells in lymphoid organs like spleen, lymph nodes and thymus. Surprisingly, we found significant increase in the number of CD34$^+$ in spleen as well in lymph node and thymus (Fig. 10). The number of lymphocytes (both CD4$^+$ and CD8$^+$ cells) decreased in mice after infection with malaria parasite while the number of lymphocytes (both CD4$^+$ and CD8$^+$ cells) were observed to increase equal to that of uninfected control wild type mice indicating that mesenchymal stem cells may be helping in rescuing the lymphocytes. Some reports indicate that mesenchymal stem cells can differentiate into haematopoietic stem cells and or lymphopoietic cells. The signalling pathway of mesenchymal stem cells for lymphopoiesis and haematopoiesis has been shown to be mediated by NOTCH-1. Further, we would like to examine whether NOTCH-1 has any role in this pathway.

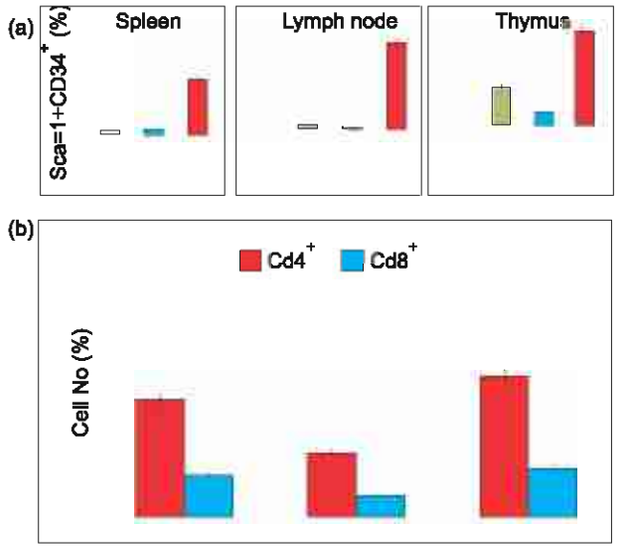


Fig. 10: Cells isolated from different organs of uninfected, *Pb*-infected and mesenchymal stem cell infused mice stained with specific antibodies for cell surface marker and analysed. The bar graph in upper panel: (a) represents Sca-1$^+$CD34$^+$ cells in different organs. Bar graph in lower panel; (b) represents CD4$^+$ and CD8$^+$ T cells in spleen of uninfected, *Pb*-infected (infected) and mesenchymal stem cell infused mice.

2.5 Next generation sequencing to study antimalarial drug resistance in the Indian *Plasmodium falciparum* population

Drug resistance is one of the most important challenges in containment of *falciparum* malaria. Thus it is of utmost importance to identify the emergence of new resistance genotypes as well as to have a thorough knowledge about the status of mutations associated with drug resistance in the population. In order to do that, we would need to genotype the genome of the parasites to unearth the mutations. Ion torrent PGM platform was used for genotyping, as this technology provides high resolution, scalability and sensitivity. An amplicon sequencing protocol have been established to examine genetic diversity in five genes (*pfk13, pfcrt, pfdhfr, pfdhps* and *pfmdr1*) implemented in resistance against various effective antimalarial drugs in field isolates. Three sites exhibiting different malaria epidemiology were selected for the study, i.e. Rourkela, Nadiad and Chennai. In total, 124 isolates (Rourkela = 75, Nadiad = 31 and Chennai = 18) have been sequenced for this panel

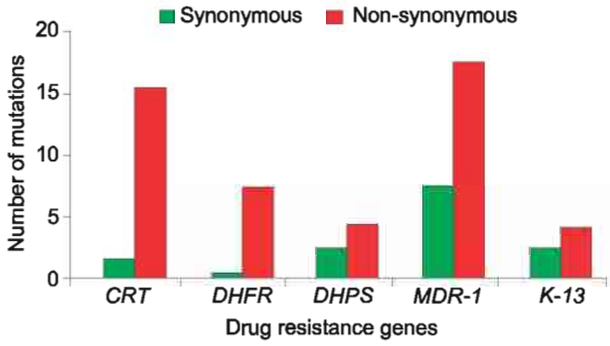


Fig. 11: Distribution of synonymous and non-synonymous mutation in various drug resistance genes.

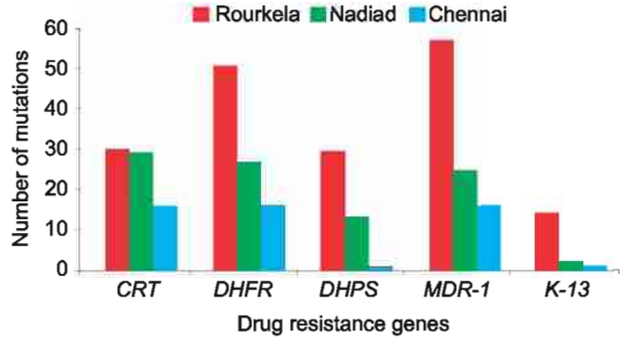


Fig. 12: Distribution of mutations in various drug resistance genes at different field sites.

of genes. The sequencing identified 58 mutations (synonymous = 12 and non-synonymous = 46) in above five discussed genes. Highest number of mutation were observed in *pfmdr-1* > *pfcrt* > *pfdhfr* > *pfdhps* > *pfk-13* gene (Fig.11). The field sites also showed marked disparity in the distribution and frequency of mutations; highest in isolates from Rourkela > Nadiad > Chennai (Fig. 12).

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Epidemiology

3

3.1 Epidemiological studies for establishing immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions in India

This is an international collaborative project between India and Denmark and involved multi-institutions from India. The Indian part of the studies are being funded by the Department of Biotechnology, Government of India. NIMR's role in the project is to study epidemiological parameters in high and low transmission areas for which field sites are located in high (Jharkhand) and low (Haryana) malaria endemic areas. These areas were selected based on preliminary data collection regarding demographic profile, topography, parasite load and transmission potential. As per detailed work package, sample size of the study population was worked out based on incidence rate. Accordingly, a cohort of 300 children (3-15 years of age) and 100 adults (>15 years of age) were enrolled in the study from high and stable malaria area of Jharkhand and 300 individuals of all age groups were enrolled from low endemic area.

Detection of malaria cases through active and passive surveillance was carried out at regular intervals through project staff. Malaria prevalence surveys during low transmission season were carried out in low endemic study area of Mewat, Haryana during April-May 2015 and in high endemic study area of Jharkhand during June-July 2015. During cross-sectional surveys, 148 individuals from low (Mewat, Haryana) and 258 persons from high transmission area (Ranchi, Jharkhand) were examined for malaria parasite irrespective of any clinical symptoms. The parasite rate during low transmission season in the low and high endemic area was found to be 1.4 and 19.4% respectively. Cross-sectional surveys during high transmission season in low and high endemic areas revealed that the parasite rate was 17.2 and 19.6%, respectively. Blood samples were also collected for separation of plasma and blood cells and also on filter papers for parasite genotyping, host immune response and functional bioassays etc as per protocol. Longitudinal entomological surveys were also conducted in both the study areas. Mosquito samples of *An. culicifacies*, *An. fluviatilis* and *An. annularis* were assayed for blood meal analysis and infection rate. However, more samples are being processed to determine the role of these species in malaria transmission in the study area. Further studies on the malaria transmission dynamics are in progress.

3.2 Comprehensive case management pilot programme in Odisha

Comprehensive Case Management Programme (CCMP) is an operational research study under programmatic conditions. It is being carried out by Government of Odisha, National Institute of Malaria Research and Medicines for Malaria Venture. It aims to assess the impact of early diagnosis and treatment, supported by a strong surveillance system, on the incidence of malaria in different transmission settings in the state of Odisha: Dhenkanal (meso-endemic), Bolangir (low endemic), Angul (high endemic) and Kandhamal (hyperendemic) (Fig. 1). Each district includes an intervention and control block (population of about 100,000).

The core components of case management are similar in the intervention and control blocks, however their implementation is more rigorous in the intervention areas. The following additional services are provided in the intervention block

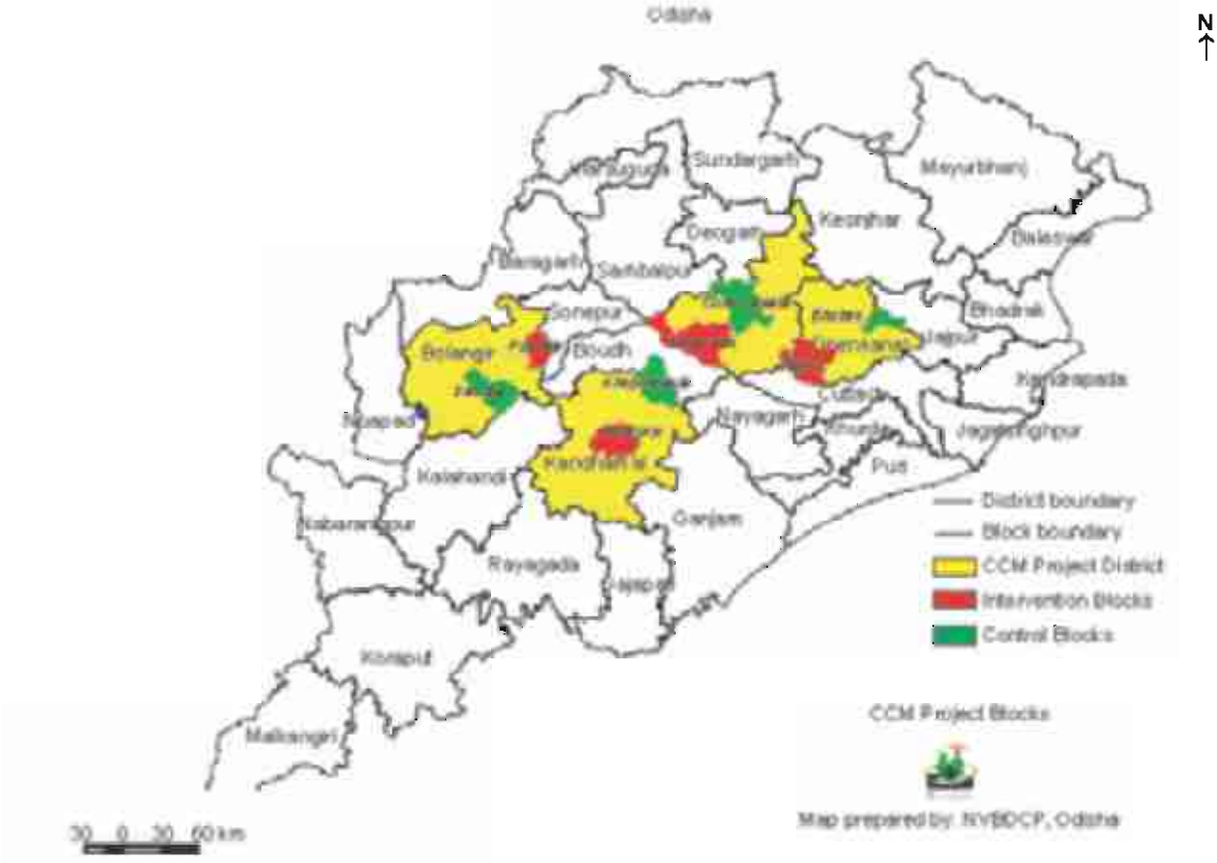


Fig. 1: Study areas for CCM programme in Odisha.

namely introduction of microscopy at the primary health care centres, use of patient cards for follow up, buffer stocks of rapid diagnostic tests and ACTs at the CHC level to avoid stock outs and training of accredited social health activitists (ASHAs) to detect primaquine-related adverse events.

Surveillance has increased in all intervention blocks and control blocks post-intervention period in 2014 and 2015 as compared to 2013 (except Bolangir) (Fig. 2). But the percent increase in the surveillance in the intervention blocks of Dhenkanal, Angul and Kandhamal is more as

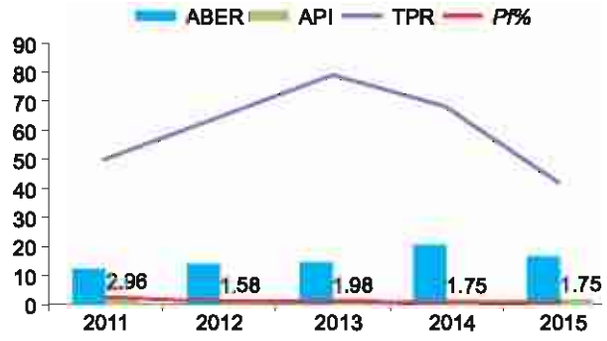


Fig. 2: Malaria epidemiological indicators in Puintala intervention block of District Bolangir.

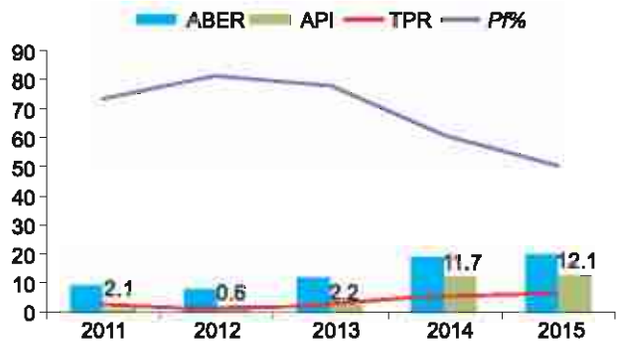


Fig. 3: Malaria epidemiological indicators in Hindol intervention block of District Dhenkanal.

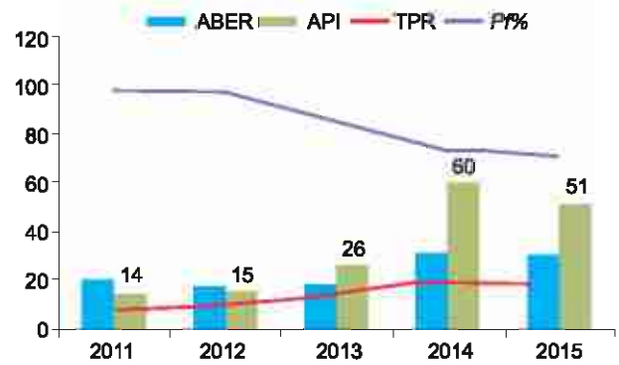


Fig. 4: Malaria epidemiological indicators in Athamallik intervention block of District Angul.

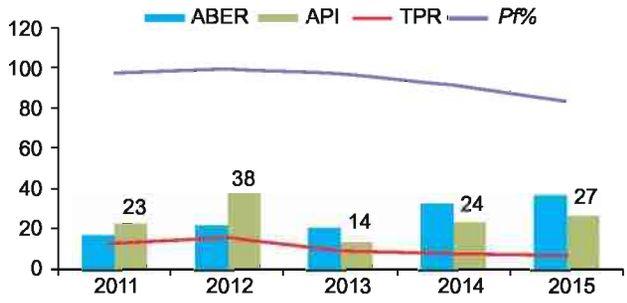


Fig. 5: Malaria epidemiological indicators in Nugaon intervention block of District Kandhamal.

compared to the control blocks (Figs. 3, 4 & 5). There has been a significant decrease in the malaria incidence in the intervention blocks of Dhenkanal and Angul in 2016 after initial rise in 2014 and 2015 the decrease in the intervention blocks is much higher as compared to the decrease of malaria incidence in the control block in 2016 as compared to 2015. The comparison between the intervention blocks and control blocks is difficult at this stage as the risk factors for malaria are differentially distributed amongst them. In Bolangir intervention block the surveillance is maintained in post-intervention period and there has not been much difference in the case incidence post intervention period.

More than 90% of malaria patients were followed up for complete treatment. Most cases are now diagnosed and treated at the ASHA level. The time from onset of fever to treatment has decreased with the larger proportion receiving treatment within 24 hours of onset of symptoms. Figure 6 shows the proportion of patients receiving diagnosis and treatment within 24 hours of onset of the fever.

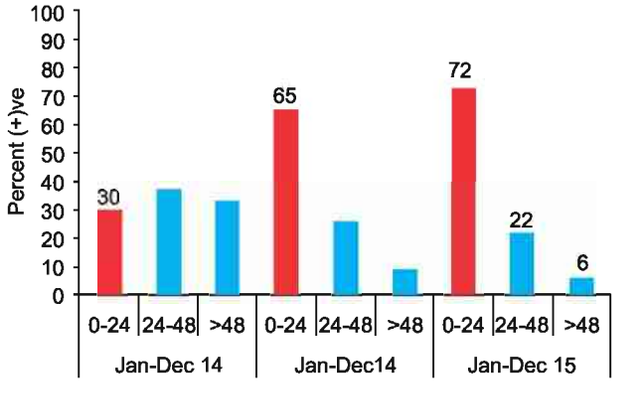


Fig. 6: Time lag of onset of fever to treatment—Percent positive cases receiving treatment within different duration of fever in intervention block of Kandhamal 2013–15. CCMP has led to a significant increase in access to diagnosis and treatment in all the intervention areas.

3.3 Health impact assessment of Narmada Basin dams and resettlement & rehabilitation colonies in Madhya Pradesh

Health Impact Assessment was initially started in 2004 in 3 major dam areas in Madhya Pradesh, which was extended further for 5 years in 2010 to cover entire Narmada Basin. Three study centres each working at Narmada Nagar, Bhopal and Jabalpur carried out entomological, parasitological and microbiological surveys in the affected area of Narmada Basin.

In 2015, NIMR Study Centre Narmada Nagar, Khandwa surveyed 226 villages of 6 districts under 10 dam projects in Narmada Basin area. In a cross-sectional survey, 542 blood slides were examined. Rapid Diagnostic kit is also being used for diagnosis of symptomatic patients. Total 40 malaria cases (31 *Pf* and 9 *Pv*) were found positive which were treated immediately and report was discussed with concerned Health Centre for follow up. Highest density of *An. culicifacies* (MHD) was recorded in Lower Goi project area (96). Total 20 drinking water samples were tested using HiWaterTM Test Kit (HiMedia), out of which only 16 were found positive with *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus*. A total of 223 ASHAs/ANMs were given training to control vector borne diseases in projected area.

The NIMR Study Centre Bhopal surveyed 218 villages of 7 districts under 10 dam projects in Narmada Basin area. In cross sectional survey, 682 blood slides were examined. Rapid Diagnostic kits were also used for diagnosis of symptomatic patients. Total 28 malaria cases (16 *Pf* and 12 *Pv*) were found positive which were treated immediately and reports were discussed with concern Health Centre for follow up. Highest density of *An. culicifacies* density (MHD) was recorded in Sher project area (60). Total 8 drinking water samples were tested using HiWaterTM Test Kit (HiMedia), out of which 4 were found positive with *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus* which were recommended for chlorination. In all, 51 ASHAs/ANMs were given training to control vector borne diseases in projected area.

The NIMR Study Centre Jabalpur surveyed 108 villages of 4 districts under 7 dam projects in

Narmada Basin area. In cross sectional survey, 288 blood slides were examined in the villages by NIMR team, in 2015. Rapid Diagnostic kit is also being used for diagnosis of symptomatic patients. Total 19 malaria cases (7 *Pf* and 12 *Pv*) were found positive which were treated immediately and report was discussed with concern Health centre for follow up. Highest density of *An. culicifacies* (MHD) was recorded in Matiyari dam project (50). Total four drinking water samples were tested using HiWaterTM Test Kit (HiMedia), all samples were found positive with *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus*. Total, 55 IEC activities were held in different villages and in different dam projects. Also 29 ASHAs/AWWs were given training to control vector borne diseases in projected area.

Engineering problems were found as dam seepage, damaged canals and blockage with vegetation and stones in the command areas of all the projects. Other domestic problems were mosquito breeding in stagnant pools, cemented tanks, and absence of drainage system, swamps and water logging near hand pumps and in gutter surrounding the houses etc. It results in high breeding potential of vectors and vector borne disease. Mitigation measures were suggested to NVDA and State Health Department, *i.e.* deweeding, introduction of larvivorous fishes, channelization of pools in main river and larvicidal spray to control the breeding. Health camps were organized involving Health Department for the awareness of vector borne diseases and their possible controls.

The information of blood slides and water testing was given to concerned Health Centre for immediate action. In each survey, detailed recommendations were submitted to NVDA and State Health Department for necessary action for control of vector borne diseases. No other vector borne disease was reported in any dam area.

3.4 Studies on health impact assessment of Sardar Sarovar Project (SSP) in command area of Rajasthan

Since the survey (November–December 2010) in 22 villages situated nearby NMC distribution network system, 13 surveys have been carried out so far (up to 2015) in selected 64 villages within the command areas of NMC encompassing two control villages, Meerpura and Gundaau (not falling in the command area of canal) in diggies, sump wells, minors, sub-minors, PHD points, outlets, escape water channels and distributaries.

The release of Gambusia fishes in diggies, sump wells, outlets and excess escapes water sites have been recommended. The releasing of larvivorous was started in February 2012. Meetings were organized with the concerned authorities to follow up the mitigating measures and to pursue recommendations which are continuing in year 2015 according to expansion of canal irrigation sites.

Based on the observations during December 2010–February 2012, intervention has been initiated in 251 diggies with 231 positive (92%) and having larval density of 17.73 per dips, by introduction of larvivorous fishes in February, 2012. In subsequent rounds carried out in February, 2012 to August, 2015, positivity was found in to reduced from 92% to about 13.4% in December 2015. However due to excessive rains in July to October 2015 the breeding was found to increase up to 21% due to flooding of diggies and sump wells which reduced after re introduction fishes in those diggis after rains. Similarly the dip index was reduced from 15.44 in February 2012 to 5.9 in February 2014 to 0.60 in December 2015. Similarly intervention has also been initiated in 251 sump-wells with 243 positive (97%) and having larval density of 10.64 per dips, by introduction of larvivorous fishes in February, 2012. to 0.07 per dips in December 2015.

About 10% of diggies and 5% of sump-wells were either dried or found without fishes during subsequent surveys fish introduction was carried out in sumpwells and diggies (Fig. 7) where fishes were not found during our surveys. The epidemiological data collected from the concerned state health departments showed that malaria cases were 245 in 2010; 204 in 2011; 190 in 2012 and 17 in 2013 and further reduced to one case till July 2015.

Fig. 7: Larval positivity in diggis (left) and sumpwell (right).

The cases increased slightly to 6 cases till December 2015 (due to excessive rains and flood in the area during July to September 2015).

3.5 Spatio-epidemiological analysis of dengue in Delhi (*Aedes*)

Dengue epidemiological study was carried out in Delhi which includes active human surveillance with virological and vector surveillance.

On the basis of active human surveillance, it was demonstrated that NS1 and IgM sero-prevalence with or without IgG evidenced high proportion of asymptomatic dengue infection. Serological tests (NS1, IgM & IgG Rapid Diagnostic Test) were conducted on family members for Household Index and Neighbourhood Index case study (around 50 index cases) from 18 localities among High, Medium and Low income group of Delhi (Fig. 8). We estimated the proportion of asymptomatic cases to be 63% among individuals of all 18 localities of Delhi. Specifically to three population groups, proportion of asymptomatic infections in localities of HIG, MIG and LIG were 60, 56 and 70%, respectively.

A prospective field study was conducted to detect dengue virus with the help of immuno fluorescence assay (Fig. 9) in adult *Ae. aegypti* mosquitoes collected from 18 localities represented by different socio economic groups in Delhi. Out of 2408 female *Ae. aegypti*, 14 were found positive with MIR of 5.8. LIG localities showed highest mosquito infectivity (9.8) followed by MIG localities (6.2) and least in HIG localities (1.3) which clearly showed that the risk of acquiring dengue infection is higher in residents of LIG in comparison to MIG and HIG.

Fig. 8: Serological testing.

Fig. 9: Fluorescent microscope.

· An entomological survey was also carried out in above mentioned localities of Delhi during transmission (June to November) and non-transmission season (December to May), with a view to study the prevalence and distribution of *Ae. aegypti* mosquito for appropriate interventions. Both immature and adult stages *Ae. aegypti* were collected from these localities. The occurrence of vector breeding was pre-dominant in localities with LIG and MIG as compared to the HIG. There was a significant difference in House Index (HI), Breteau Index (BI), Container Index (CI) of all the three income groups during transmission and non-transmission season.

3.6 Studies on transmission of complex malaria in Odisha

Studies were undertaken to: (i) quantify the role of environmental conditions in determining malaria transmission intensity (risk) in different eco-epidemiological contexts, including the future effects of climate change; and (ii) evaluate the evolutionary responses of key mosquito vectors to the increasing adoption of insecticide-based interventions, quantifying the implications for malaria transmission in different eco-epidemiological contexts. Results are summarized below:

Indoor resting mosquitoes were collected monthly between 0600 to 0800 h in each village from 4 human dwellings and 4 cattlesheds. In total, 5504 female anopheline mosquitoes representing 16 *Anopheles* species were collected from the plain, riverine and forest areas. *Anopheles culicifacies* (32.23%) was the dominant species followed by *An. annularis* (26.10%) and *An. pallidus* (18.1%). The percentage of *An. fluviatilis* was 3.07%.

Females of *An. culicifacies* and *An. fluviatilis* identified morphologically were used for identification of sibling species and blood meal source. Genomic DNA samples of the species were

subjected to allele specific PCR assay using species specific assay. Midgut blood smears of *An. fluviatilis* and *An. culicifacies* specimens identified to sibling species were subjected to blood meal source identification by PCR using an established protocol.

Sibling species composition

The sibling species type composition revealed by PCR is given in Table 1. Of the total *An. culicifacies* sampled, 99.8% were types BCE. *Anopheles culicifacies* A and D-type were encountered very infrequently (0.2% of the total species).

Anopheles fluviatilis species complex comprised types S and T only (no U and V-types were found). However, T-type was numerically dominant in the study villages (86.4% T type, and 13.6% S type) (Table 1).

Table 1. The sibling species type composition of *An. culicifacies* and *An. fluviatilis*

Species	Percent (Type)	
An. culicifacies	99.8 (BCE)	0.2 (AD)
An. fluviatilis	86.4 (T)	13.6 (S)

Host feeding behaviour

Blood meal source was determined by PCR in individual *An. culicifacies* identified to sibling species type. Of the 4 AD samples (0.2% of the total *An. culicifacies* collected), all were positive for mixed meals of bovine and human blood. The BCE types showed no clear feeding preferences, whether mosquitoes were collected from human dwellings or cattlesheds (Table 2).

Table 2. Host blood meal analysis of *An. culicifacies*

An. culicifacies	Percent				
	Bovine	Human	B+H	Unfed	Others
AD HD	0	0	0.05	0	0
AD CS	0	0	0.17	0	0
BCE HD	0.17	2.50	0.84	1.80	0
BCE CS	24.80	11.40	27.50	26	4.50

Blood meal analysis of *An. fluviatilis* provided limited evidence for exclusive human feeding (even for the anthropophilic S-type) with high amounts of cow feeding and mixed cow-human feeding for overall for both types across all blood fed *An. fluviatilis* collected, regardless of whether mosquitoes were sampled from cattlesheds or human dwellings (Table 3).

Table 3. Host blood meal analysis of *An. fluviatilis*

An. fluviatilis	Percent				
	Bovine	Human	B+H	Unfed	Others
S HD	0	0.6	0.6	0.6	0
S CS	4.1	5.3	1.2	1.2	0
T HD	3	5.3	0	1.2	0
T CS	17.1	4.7	49.1	3.5	2.3

Temperature and larval diet effects on development rate and survival of *Anopheles culicifacies*

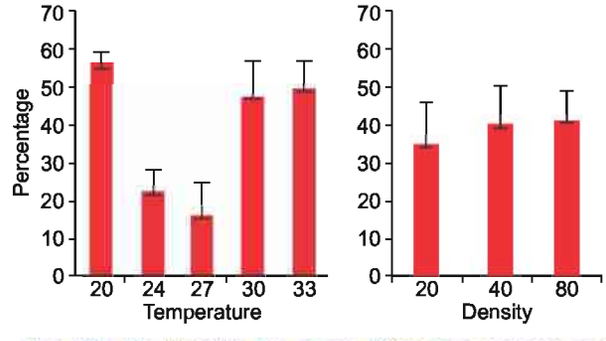
F_1 generation of field collected *An. culicifacies* from field were reared under standard insectary conditions. Larval development rate and survival were quantified in artificial containers over gradients of temperature, food concentration (mg/ml/day). Performance across conditions was evaluated by measuring mortality rates during development (dead individuals/cup), time to pupation (days since hatching), and time to emergence (days since hatching).

From the data it is observed that good larval survival (70-80%) at peak transmission temperature. Survival was slightly better at 30°C than 27°C (possibly higher optimum than expected). Low food reduces survival (predictably) but no evidence of food and temperature interaction (Table 4).

Temperature and density effects on development rate and survival of *Anopheles culicifacies*

Larvae were reared at five temperatures (20, 24, 27, 30 and 33°C) and three densities (20, 40 and 80). The effects of density and temperature strongly interacted to determine the mosquitoes' life-history parameters.

Survival was highest at the intermediate temperature of 27°C (Fig.10). The differences between the temperatures increased with increasing density. Survival decreased as density increased, but at decrease density led to higher survival (Fig. 11).

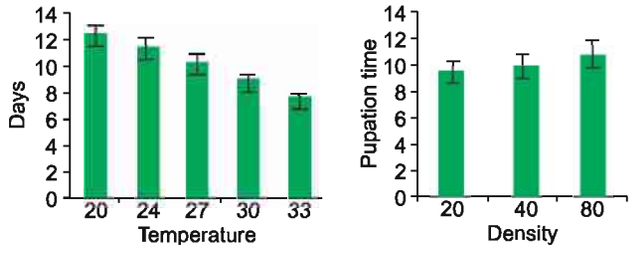


Figs. 10 & 11: Mortality rate across different temperature and initial density (bars indicate mortality rare).

Table 4. *Anopheles culicifacies* larval to adult survival at two temperatures representative of peak transmission season and with high and low food

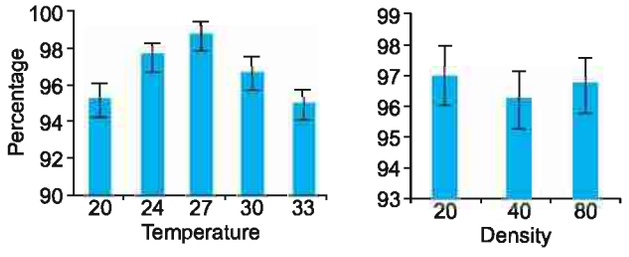
Temperature	Food concentration	Replicate No.	No.of 1st instar larva	Adult (%)		
				Pupation	Emergence	Mortality
27°C	0.6 mg	1	50	76	94.80	28
		2	50	86	93	20
		3	50	78	100	22
		4	50	58	100	42
		5	50	70	94.30	34
		Total	250	73.60	96.20	29.2
	0.2 mg	1	50	78	84.70	34
		2	50	64	96.90	38
		3	50	56	92.90	48
		4	50	66	97	36
		5	50	44	95.50	42
		Total	250	61.60	92.90	42.8
30°C	0.6 mg	1	50	80	97.50	22
		2	50	94	100	6
		3	50	90	100	10
		4	50	64	96.90	38
		5	50	76	100	24
		Total	250	80.80	99	20
	0.2 mg	1	50	70	100	26
		2	50	56	96.50	46
		3	50	48	95.90	54
		4	50	72	100	28
		5	50	60	100	40
		Total	250	62	98.70	38.8

Time of pupation increased as temperature decreased from 33 to 20°C (Fig. 12) and density increased from 20 to 80 (Fig. 13).



Figs. 12 & 13: Pupation time across different temperature and initial density (bars indicate days and pupation time).

The adult percentage showed high in 27°C and low in 20 and 33°C (Fig. 14). The high adult percentage was observed in low density (Fig. 15).



Figs. 14 & 15: Adult percentage across different temperature and initial density (bars indicate adult percentage).

When we compare the overall adult percentage across different temperature and initial density we observed that high adult percentage was in 27°C in density 20 and low adult percentage was observed in 20°C with density 40 and 33°C with density 80 (Fig. 16).

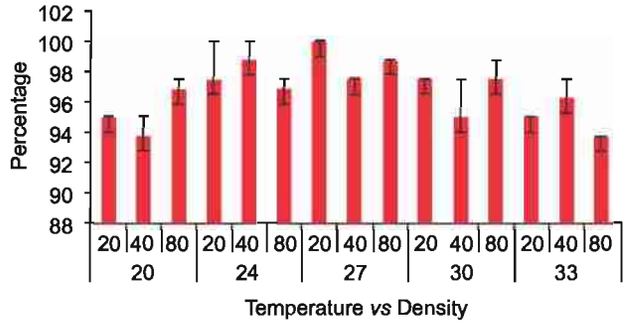


Fig. 16: Overall adult percentage across different temperature and initial density (bars indicate adult percentage).

3.7 Mapping of malaria risk in the context of climate change in India

The objective of this project is to generate risk maps of malaria in all states of India from the view point of climatic determinants, malaria prevalence, anopheline vectors distribution and ecological conditions. Mapping of *P. vivax* and *P. falciparum* malaria at district level was done taking average of

three years of data. Distribution of *An. culicifacies* and *An. stephensi,* the major rural and urban malaria vectors in India from published records were mapped. The highlights of achieve-ments are: Prepared distribution map of vectors of malaria in India highlighting the need of undertaking survey even in high endemic areas. Based on Case Mean Ratio and properties of Sine curve, a mathematical model for determination of outbreak and early warning has been developed. The rainfall cut-off for different regions indicated that in states like Assam, 100 mm rainfall in the month of March while in Maharashtra state, >200 mm cumulative rainfall in the month of July- Sept-ember could result into outbreak in the month of December. District level scenario of projection of windows of malaria in view of climate change has been generated. Climate suitability maps for malaria transmission based on climatic parameters (temperature & Relative Humidity), malaria endemi-city and other geographical features have been generated. The reasons of low malaria endemicity in Kerala and Southern India were identified.

3.8 Relationship between El Nino and malaria outbreaks

The main aim of this project is to identify early warning tool for malaria outbreaks. Based on the Oceanic Nino Index (ONI), the predicted SST anomaly values for November, December 2015, January and February 2016 and annual malaria cases in different states of India, a map was generated to show the impact of El Nino on malaria. As per available projections, the ONI values suggest strong El Nino conditions in first half of 2016. It means that the states of Odisha, Jharkhand, Chhattisgarh, Goa, Uttarakhand, and parts of Andhra Pradesh, Madhya Pradesh, Maharashtra, Uttar Pradesh and Meghalaya are likely to experience increased malaria from the climatic conditions point of view, while in the event of La Nina conditions (as predicted from July 2016 onwards), the states like Rajasthan, Gujarat, parts of Karnataka, Mizoram and Nagaland are likely to experience malaria outbreaks.

Further work is in progress so as to provide forecast at finer resolution.

❑

Clinical Research

4

4.1 Establishment of a WHO-recognized laboratory for quality assurance of malaria RDTs

NIMR is carrying out lot testing of malaria RDTs procured by NVBDCP since 2009, following the WHO FIND SOPs. Panels of parasitaemia of 2000 and 200 parasites/µl are prepared from endemic areas. The laboratory passed the proficiency testing for microscopy, PCR and lot testing and also the assessments, based on which WHO recognized the laboratory as a lot testing laboratory.

Dr Poonam Khetrapal Singh, Regional Director, WHO SEARO handing over certificate.

4.2 Quality assurance of malaria RDTs

Quality Assurance Programme was started by NIMR and NVBDCP jointly in August 2009. Phase-III of the project 'Quality Assurance of Malaria Rapid Diagnostic Tests' was initiated in year 2015 to assess the quality of RDTs procured and supplied by NVBDCP. The NVBDCP is the nodal centre and NIMR is National Referral Laboratory for this project. The major components of the quality assurance of RDTs for malaria include preparation of Quality Control (panels, pre-dispatch QC, post-dispatch QC, External Quality Assurance Scheme and Internal QC.

The NIMR has been identified as the National Referral Laboratory for the Quality Assurance of malaria RDTs. The National Vector Borne Disease Control Programme is the nodal agency.

Preparation of QC panels

Malaria patients from different areas were selected as donors. Parasitized blood was diluted with O (+)ve blood cells and AB (+)ve fresh frozen plasma to attain parasite density of 200/µl (low positive) and 2000/µl (high positive). On the same lines, negative controls were also prepared.

Testing of RDTs using panels

It is important to assess the quality of the RDTs with known low and high positive samples before they are supplied to the testing laboratory to confirm that they are free from any manufacturing defects and have desired sensitivity and specificity. This was achieved by lot testing of the kits. From each RDT lot, 58 RDTs were drawn and tested using the WHO protocol (Methods manual for laboratory quality control testing of malaria rapid diagnostic test, Version 7, June 2014). Ten negative and 4 sets of positive panels for *P. vivax* and *P. falciparum* were used to test RDTs. Thus, a total of 58 RDTs (10 negative, 24 *Pf* panels and 24 *Pv* panels) were tested. In the year 2015, 3048 RDTs were tested as a part of pre-dispatch QA. Post-dispatch testing was carried out on, 461 RDTs.

QA of RDTs used by health workers at periphery

On arrival of the RDT lot to each district, 26 RDTs were drawn at random and sent to NIMR where they were tested using positive (low and high parasitaemia) and negative controls for immediate QC. For long term quality assurance, 19 RDTs were drawn every quarter till the expiry date of the kit.

RDT samples were drawn from representative

Primary Health Centres/Subcentres/ASHAs and tested for their quality. The District Malaria Officers (DMOs) have been collecting RDT samples from their districts and sending the same to the referral laboratory every quarter. Figure given below gives the representative example of a district for picking up the RDTs. From each district, one PHC was selected every three months. One RDT was picked up from the PHC, two from two subcentres and four RDTs from ASHAs. In the next quarter, another PHC was selected and the procedure repeated.

Progress

Till date, 49 QC panels were prepared including 26 panels in 2015. Before testing the RDTs, panels were validated using at least two reference kits. The RDTs received from the field were tested for their quality by using negative panel and positive panels with 200p/µl following WHO protocol.

As a part of pre-dispatch QA, 3248 RDTs were tested in year 2015 and 1134 RDTs in year 2016. Post-dispatch testing was carried out on 489 RDTs in the year 2015 and 816 RDTs in the year 2016 till date. These RDTs were received from 45 districts of 11 states. The panel detection score of these RDTs was 100%. There were 8 invalid results but no false positives. The details are as follows:

State	No. of samples	RDTs tested	Concordant results	
			200p/µl	Negative
Assam	91	73	57/58	15/15
Madhya Pradesh	387	291	231/232	59/59
Chhattisgarh	19	15	12/12	3/3
Maharashtra	144	113	88/92	21/21
Goa	19	15	12/12	3/3
Odisha	401	276	224/225	51/51
Karnataka	118	98	86/86	12/12
Nagaland	34	32	23/23	9/9
Telangana	46	35	28/28	7/7
Arunachal Pradesh	8	5	4/4	1/1
Tripura	38	30	24/24	6/6

The WHO has recognised the NIMR as QA laboratory for lot testing and malaria RDTs. The data generated show that the malaria RDTs being used in the programme are satisfactory.

4.3 A Phase-IIIb, open label trial to assess the safety, tolerability and efficacy of DHA/piperaquine in paediatric patients with uncomplicated *Plasmodium falciparum* malaria

This trial is being carried out in children under 18 years with uncomplicated *P. falciparum* malaria to assess the safety and tolerability of DHA Piperaquine. It is being carried out at two sites: Rajendra Institute of Medical Sciences, Ranchi and Wenlock Hospital, Mangalore. There, 36 patients have been recruited so far.

4.4 A multi-centre, open-label randomised trial to assess the efficacy, safety and tolerability of triple ACTs compared to ACTs in uncomplicated falciparum malaria

This trial, funded by University of Oxford is being carried out to assess the efficacy and safety of triple ACT (artemether-lumefantrine + amodiaquine) compared to ACT (artemether-lumefantrine) at three sites: Agartala Government Medical College, Medical College Midnapur and Ispat General Hospital Rourkela. Eleven patients have been recruited so far at two sites.

4.5 Active pharmacovigilance for primaquine radical cure for the treatment of *Plasmodium vivax*

The project is a substudy of the project Comprehensive Case Management of malaria. It tries to assess the drop of haemoglobin and recovery follow-ing 14-day primaquine treatment for *P. vivax* radical cure.

Thirty-nine patients have been enrolled in the study so far. No haemolytic symptoms were observed in enrolled patients. Average haemoglobin fall was 1.1 g%; haemoglobin levels were recovered by Day 28.

4.6 Malaria Clinic

The Malaria Clinic at Delhi provides diagnosis and treatment of malaria. This also serves as resource of biological material for research. In the year 2015, 75 malaria cases: 70 *P. vivax*, 4 *P. falciparum* and 1 mixed infection were reported (Fig. 1).

The NIMR Delhi is one of the sentinel sites for diagnosis of dengue. In the year 2015, a total of 3534 samples were tested for dengue, of which 935 were confirmed dengue cases (Fig. 2). About 70% of the total dengue cases reported in the month of September. Serotyping performed in 18; serotype 2 was detected in 17; while as one sample had serotypes 2 and 3 (Fig. 3).

Follow up of dengue cases was also carried out.

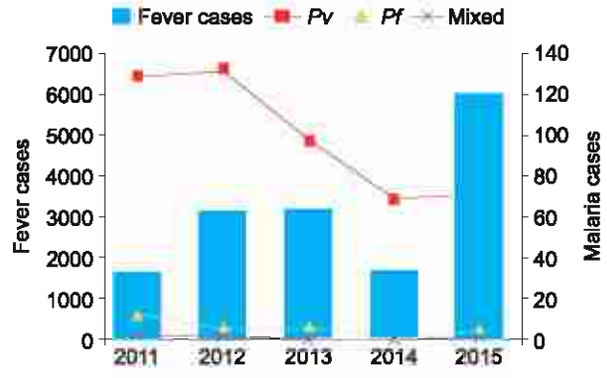


Fig. 1: Malaria cases in Delhi in the year 2015.

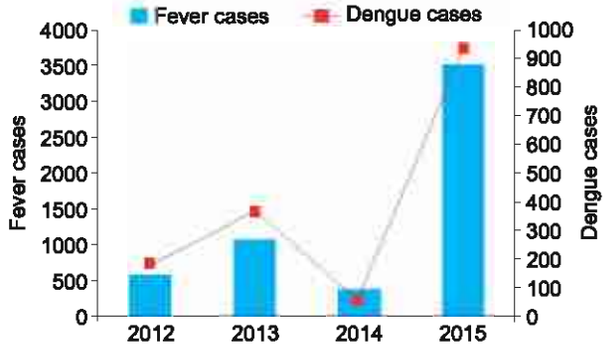


Fig. 2: Dengue cases in Delhi during 2012-15.

Fig. 3: Serotyping of dengue.

Of the 580 cases contacted, 42 required hospitalization. No death was reported. Vector surveillance also carried out in houses of 101 dengue cases. *Aedes* breeding was observed in 14 houses in solid waste, plastic containers, OHTs, bird pots, coolers etc.

4.7 Monitoring of therapeutic efficacy of antimalarial medicines in India

The problem of antimalarial drug resistance remains a challenge for the control of malaria in the country. NIMR in collaboration with NVBDCP has been carrying out operational research by monitoring the therapeutic efficacy of antimalarials used in the National Programme.

The joint NVBDCP-NIMR surveillance system with several innovations has been developed since 2009. The results of these studies have guided the national drug policy for malaria and based on observed high treatment failure of recommended ACT (AS+SP), the National drug policy on malaria for northeastern states has been revised to artemether + lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria since 30th April 2013. However, artesunate plus sulfadoxine-pyrimethamine (AS+SP) remains the first line of treatment for uncomplicated falciparum malaria in rest of the country. Till 2012, the efficacy of AS+SP has been found to be above 95% at all sites except Northeastern region. As these sites are spread across the country and there are chances that the recommended ACT (AS+SP) in other parts of country may show increasing treatment failure, there is a need to have continuous monitoring at the sentinel sites developed under the collaborative work.

A launch meeting on Operational Research Projects at NIMR, New Delhi on 8 May 2015 was organized under the Chairmanship of Dr Jagdish Prasad, DGHS, Govt. of India (Fig 4). Following

Fig. 4: Launch meeting of operational research projects held at NIMR, New Delhi on 8 May 2015.

Fig. 5: Technicians training at NIMR, New Delhi from 25–29 May 2015.

the recommendations of the launch meeting and the need of centralized training for generating quality data, a five-day orientation training was organized at NIMR, New Delhi from 25–29 May 2015. A total of 22 laboratory technicians from 9 study sites participated in the orientation training. Faculty of NIMR and NVBDCP were involved in imparting the training. The training included methodology to assess the therapeutic efficacy of antimalarials, microscopic examination of blood smears, quantification of the malaria parasites, data entry from the Case Record Forms, reporting of Adverse events and management of the study patients, etc. Hands on training to various procedures including molecular studies were also provided to the participants.

To develop capacity at the study sites, orientation meetings were organized at all the study sites. Medical officers, technicians and concerned staff involved in the study were part of the orientation trainings (Fig. 5). Resource persons from NIMR and NVBDCP, New Delhi were also part of the orientation meeting as per the need and availability.

The project was initiated on 1 April 2015 and field investigations were carried out at 11 study sites out of the total 13 study sites. These include Chhattisgarh (Balod), Jharkhand (Latehar), Karnataka (Mangalore-2), Madhya Pradesh (Betul), Maharashtra (Gadchiroli), Odisha (Angul), Gujarat (Surat), Mizoram (Lawngtlai), Meghalaya (West Garo Hills), Tripura (Dhalai) (Fig. 6).

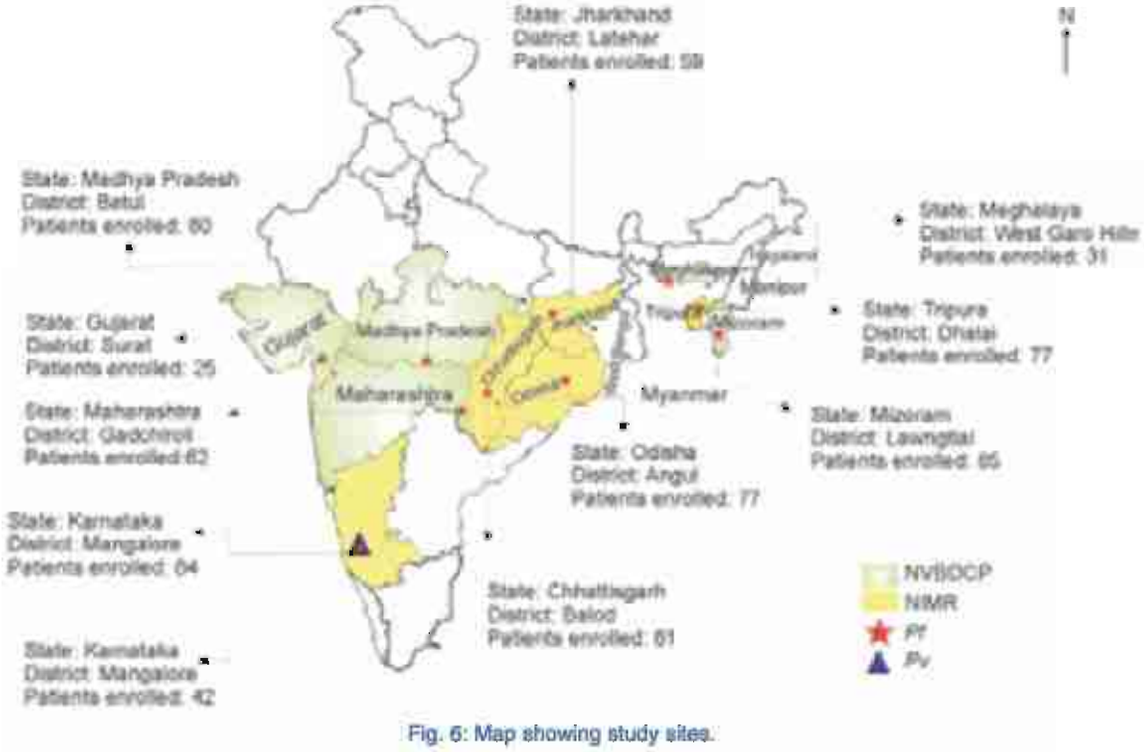


Fig. 6: Map showing study sites.

A total of 683 cases have been enrolled during the study period. All the eligible patients were between the age group of 2 to 70 years. To monitor the partner drug resistance, point mutations in chloroquine resistance transporter (*Pfcrt*; K76T) and multidrug resistance transporter 1 (*Pfmdr*1; N86Y, Y184F, D1246Y) in *P. falciparum* malaria patient samples were studied from the three northeastern sites. Similarly for sulfadoxine-pyrimethamine (SP) resistance, point mutations in *dhfr* and *dhps* gene as molecular markers were analyzed for the samples obtained on Day 0 as well in all the treatment failure samples.

The baseline parasitaemia ranged between 330-95625/µl of blood and 51.8% patients had fever on Day 0. Over all the studies conducted during this period showed high efficacy after 28 days of follow up; 100% for chloroquine in *P. vivax* at Mangalore, Karnataka and 100% efficacy of Artemether-lumefantrine (ACT-AL) in *P. falciparum* malaria at three sites in northeastern region of country. The efficacy of AS+SP in *P. falciparum* malaria in other parts of country ranged between 94.4–100% at seven sites.

The molecular studies indicate high prevalence of chloroquine resistance transporter *Pfcrt* K76T mutation ranging between 90.3 and 98.8% in north-eastern region and from 60–100% in other parts of the country. For *pfmdr*1, for codon 86, majority (80.7%) of the samples showed wild type pattern followed by 10.4% mutant and 7.8% mix type pattern. For codon 184, majority (96.4%) showed wild type pattern and only 1.6% samples showed mutant type. For codon 1246, the majority (99%) of the samples had wild type pattern. The *dhps* gene was wild (75.3%) during 2015 and triple mutation has been observed in 18.5% samples.

4.8 Effective and safe interventions for prevention of malaria in pregnancy in India: An assessment of malaria burden in pregnancy, implementability of a screening strategy and barriers to scaling-up interventions

The National Institute of Malaria Research (NIMR) in collaboration with the London School of Hygiene and Tropical Medicine has been carrying out the above mentioned study since January 2011. The component A of the study was a cluster randomized controlled trial to determine the effect of intermittent screening and treatment for malaria (IST) as part of the antenatal care on the risk of placental malaria and the incidence of clinical malaria. This trial is carried out in Gumla district (Basia and Kamdara blocks) and Simdega district Kolebira and Bano) in Jharkhand state.

The study was designed as a cluster randomized trial with two arms and was conducted in 4 blocks of Gumla and Simdega district (Bano, Kolebira, Basia, Kamdara). In each block, all sub-centres were randomly allocated to the control (PCD) or intervention arm (IST). Women attending antenatal care clinics in the control arm were tested for malaria only in case of reported illness (PCD). Women in the intervention arm were screened for malaria using an RDT test at each ANC visit. Women were offered free delivery in the study hospital (St. Ursula Hospital, Konbir Noatoli). All women and child pairs are followed up after 2, 4 and 6 months post-delivery.

A total of 6859 pregnant women were enrolled, out of which, 5441 women delivered and a total of 3136 (57.6%) placental biopsies were collected.

Data collection for component B has been completed. The health care personnel as well as the community of Murhu block showed a very positive response to IST implementation. Across all data collection methods, adherence to national guidelines for malaria case management and maternal health services were inadequate.

The preliminary findings suggest that compared to the Passive case detection (PCD) arm, the detection rate of malaria in pregnancy in the IST arm is approximately 10-fold higher at the first visit (enrolment and ANC1) and 5-fold higher at subsequent ANC visits. Also, the screening for malaria using an RDT during routine ANC can be done along with the HB testing during routine ANC.

In addition, routine and improved ANC services will increase ANC coverage and improve disease surveillance thereby reducing the burden of malaria in pregnancy. However, logistics and training for implementing this strategy will be required before adopting in health system. This will help the national programme by better informed decision making.

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Highlights of Research Activities under IDVC Project

5

5.1 Bengaluru (Karnataka)

- Larvivorous fish *Gambusia affinis* (~713700) and Guppies *Lebistes reticulatus* (~190060) were released in three JE endemic blocks Khorabar, Chagaon and Bhatat for vector control in JE prone areas in Gorakhpur district, eastern Uttar Pradesh. This led to gradual but sharp reduction on breeding index of the vector population.
- A WHOPES-sponsored study 'Field testing and evaluation of the efficacy and duration of effectiveness of a biolarvicide, BactiVec® SC (*Bacillus thuringiensis* var *israelensis* SH-14), was conducted in Bengaluru against immature stages of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* in their main natural habitats following the guidelines of World Health Organization Pesticide Evaluation Scheme.
- Therapeutic efficacy of chloroquine for *Plasmodium vivax* was initiated in Mangalore, Karnataka. A total of 71 patients were successfully followed-up the period of 28 days. All showed good response to chloroquine. Therapeutic efficacy of artemisinin + sulfadoxine-pyrimethamine for *Plasmodium falciparum* was also carried out along with *P. vivax*. There were less number of falciparum cases reported in Mangalore during the study period. A total of 36 patients were successfully followed-up to the period of 42 days. All responded to the ACT.
- In a collaborative project between NIMR and NCBS, Bengaluru on assay development for *P. vivax*-infected hepatocytes in micropatterned co-culture (MPCC) plates, successfully completed with four membrane-fed blood-infected with *P. vivax* to *An. stephensi*. About 55,000 sporozoites per mosquito were generated which is equivalent to other studies, so far. These sporozoites are used to infect *in vitro* cultured hepatocytes, namely HC-04 cell line.
- In a collaborative project between NCBS, Bengaluru, InStem, Bengaluru and NIMR, Bengaluru Unit to develop a sustainable *P. vivax* liver stage assay using human hepatocytes derived from induced pluripotent stem cells, peripheral monocyte cells from vivax-infected patients were successfully isolated and those undergoing differentiation of hepatocytes. These cells would be utilized for infection from vivax sporozoites.
- A Phase-IIIb, open label trial, to assess the safety, tolerability and efficacy of dihydroartemisinin-piperquine (Eurartesim) in Indian children and adolescent patients with acute uncomplicated *P. falciparum* malaria is underway in Wenlock District Government Hospital, Mangalore. A total of 15 children were recruited and 12 of them were successfully followed-up to the period of 63 days. All showed good response.

5.2 Chennai (Tamil Nadu)

- Given the intervention in force, high transmission of malaria was observed in districts of Mizoram and Tripura sharing international border with Bangladesh. Among two prevalent parasites, *P. falciparum* was the predominant infection (>80%). The remaining were *P. vivax* cases. Therapeutic efficacy based on 28-days follow up of investigations revealed good success rate (98%) to the given regime of AL (artemether + lumefantrine).

- Entomological investigation in Mizoram revealed that both *An. minimus* and *An. baimaii* were prevalent in the study area but *An. baimaii* was the predominant vector species. In addition, *An. maculatus* is suspected to play some role in malaria transmission.
- Questionnaire-based study from the medical practitioners revealed that prescriptions and diagnostics practices for malaria treatment were satisfactory. However, monotherapy still continues to be in practice.
- Other activities included providing technical support to the control programme, *i.e.* health education and capacity building measures, mass propagation and distribution of larvivorous fish (Guppy and *Gambusia*) in town areas, assessment of mass drug administration in filarial endemic districts and in providing technical expertise on long-lasting insecticide nets for procurement and supplies.

5.3 Guwahati (Assam)

- Monitoring therapeutic efficacy of antimalarials artemether + lumefantrine (AL) in the treatment of *P. falciparum* malaria in northeastern states was done. In Gandachara, of the total 77 subjects selected for 28 day follow up investigations, 98.7% (76/77) were ACPR. In Chawngte CHC, of the total 83 subjects enrolled 82 (98.8%) showed ACPR (subject to correction by PCR). In Darengeree PHC, as many 32 subjects were selected for follow up investigations all of which all showed ACPR except one which was lost to follow up.
- In order to study prevalence of malaria transmitting mosquitoes in Lawngtlai district of Mizoram, Indo-Bangla border in Northeast India, entomological investigations were undertaken in Chawngte (Lawngtlai district) of Mizoram during July–August 2015. Using different sampling techniques including day-resting mosquito catches in human dwellings, indoors, cattle biting catches in the evenings, overnight CDC trap collections and human landing mosquito catches, 9 anopheline mosquito species were collected including *An. aconitus*, *An. baimaii*, *An. jamesii*, *An. kochi*, *An. maculatus*, *An. minimus*, *An. nivipes*, *An. barbirostris* and *An. nigerrimus*. None of these mosquito species were encountered in day-resting catches in human dwellings indoors.
- A study was undertaken in five different block PHCs of Kamrup district of Assam to ascertain the malaria treatment practices in public and private healthcare providers and use of antimalarial medicines in Assam. Exit interviews were conducted and results have been discussed.

5.4 Hardwar (Uttarakhand)

- During the months July to October, a total of 1525 houses were surveyed, out of them 474 houses were found positive for *Aedes* breeding. Out of 4185 containers searched, 1034 containers were found positive for *Aedes* breeding. House index, container index and breteau index were 31.1, 24.7, and 67.8 respectively. Four *Aedes* species, namely *Ae. aegypti*, *Ae. albopictus*, *Ae. vittatus* and *Ae. pseudotaeniatus* were identified. Percent species composition of *Ae. aegypti* in Hardwar proper, Kankhal and BHEL Township was 98.5, 60.4 and 2, respectively. A total of 810 suspected dengue cases were recorded in District Hardwar, out of which 357 cases were confirmed by ELISA test and no death was recorded.
- In order to find out the risk factors of malaria in District Hardwar, four villages in Chanderpuri subcentre of Laksar CHC of population 6651 showing high malaria incidence have been selected and one subcentre (Shivgarh) in Bahadarabad CHC, showing low malaria incidence have been selected for demonstration of elimination of malaria. Prevalence of *An. culicifacies* was observed throughout the year and peak density was recorded during the months of July and August in both the areas. During the months of May to March, a total of 665 blood slides were collected in Chanderpuri out of which 78 cases were found positive for *P. vivax* and 5 cases for *P. falciparum*, SPR being 12.5, while 656 blood slides were collected from low malaria subcentre, out of which 16 slides were found positive for *P. vivax*, SPR

being 2.4 which coincided with high vector density. Density of *An. culicifacies* and total anophelines and malaria incidence in Chanderpuri was high as compared to Shivgarh.

- NIMR Field Unit has been working on Industrial malaria control since 1986 and successfully controlled malaria in BHEL, Hardwar. From April 2015 to March, 2016, a total of 2056 blood slides were collected, out of which 71 slides were found positive for *P. vivax* and 1 for *P. falciparum*, SPR being 3.5. Out of 72 positive cases, 25 were from Jwalapur, Kankhal and nearby villages and remaining 47 were from the BHEL township.

5.5 Jabalpur (Madhya Pradesh)

- In Phase-III trial of long-lasting insecticidal nets in Kundam (Madhya Pradesh) revealed that 20% NetProtect nets did not meet the Bioassay test as per WHO criteria. Chemical Assay Reports received from Gambloux showed that after one year of household use, the mean deltamethrin content in LifeNet is 4.42 g/kg corresponding to a loss of 55% of the original dose, 1.11 g/kg corresponding to loss of 38% in NetProtect and 0.92 g/kg corresponding to loss of 32% in PermaNet 2.0. The number of holes in nets were mostly found on lower side.
- Point prevalence study in District Betul where the risk factors associated with re-emergence of malaria showed SPR was 37 and *P. falciparum* infection 70%.
- Dengue transmission in Narsinghpur district showed container index 42%, breteau index 80% and breeding of *Ae. aegypti* was 94.5%.
- Bionomics of malaria vectors and their sibling species in Districts Bastar and Korea of Chhattisgarh showed 11 anophelines recorded from both the areas. *Anopheles culicifacies* C (38%) was dominant followed by species D (23%) and B (22%). *Anopheles fluviatilis* species T was dominant in the study area. Both the species were *P. falciparum* positive. *Anopheles culicifacies* was identified as species C.

5.6 Nadiad (Gujarat)

- A detailed study was initially carried out in Kheda, Surendranagar and Patan districts in Phase-II command area of Sardar Sarovar project. It was further extended to Morbi district of Saurashtra region. Narmada water had reached this region through canal for irrigation as well as for ceramic industries. Entomological activities in these two districts included mosquito collection, peri-domestic and intra-domestic larval surveys, host preference and survivorship of malaria vectors, cross-sectional mass blood survey in sentinel villages was done.
- A large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet 3.0 long- lasting insecticidal nets compared with PermaNet 2.0 in India was used by households under field conditions and to assess washing mode and washing habits of LNs by the householders, and to assess the community acceptability of LNs over three years.
- Bioassay, chemical assay and fabric integrity of Cohort nets were done.
- Transmission dynamics and control of malaria in tribal area of Gujarat India was initiated in June 2014 in Panchamahals district of Gujarat state. In total 30% population of this district is tribal. Three PHCs of Gogamba and two PHCs of Jambugoda Taluka were selected for this study. During this year entomological parameters such as adult mosquito density, larval density, parity, human blood index and human landing collection were monitored on bi-monthly basis. Supervision of indoor residual spray (IRS) activity was also done in the study area. During this year, bi-monthly intra-domestic breeding surveys were carried out. A total of 1026 houses were checked for mosquito breeding and out of these 44 houses were found positive for mosquito breeding (House index = 4.29). Container index (CI) was 3.65 and breteau index (BI) was 2.14.
- Centre for the Study of Complex Malaria in India had been launched in November 2012 in Gujarat with an aim to understand the complexity of malaria, including changing patterns of epidemiology. The objective of the project in Gujarat, is to collect blood samples from the patients with malaria symptoms, then

to identify the *Plasmodium* species in samples with three different diagnostic methods, *i.e.* microscopy, rapid diagnostic kits (RDTs) and polymerase chain reaction (PCR). After sample processing, the DNA samples were sent to NIMR, New Delhi for genomic studies.

- In the year of 2015, 203 suspected cases were enrolled for malaria diagnosis from the clinic of Civil Hospital, Nadiad and Vatva Urban Health Centre (UHC) of Ahmedabad Municipal Corporation, Ahmedabad. Initially these were examined by RDT kit (Zephyr Biomedical) which was followed by microscopy (as per the protocol). The blood samples were collected from the patients after taking informed consent for further molecular diagnosis. The DNA was isolated from the respective blood samples by QIAamp DNA mini kit and processed for the PCR diagnosis. The PCR products were analyzed by electrophoresis technique. Out of 203 cases, 138 were found positive by microscopy (*Pv* = 125, *Pf* = 9, Mixed = 4) as well as by RDT (*Pv* = 125, *Pf* = 10, Mixed = 3). PCR detected 132 cases positive (*Pv* = 119, *Pf* = 13) for malaria.
- Malaria diagnostic support was provided to the Civil Hospital, Nadiad and the data that was generated had been used for sentinel monitoring of malaria situation in the Kheda district of Gujarat. In 2015, 6583 febrile patients have been screened for malaria, of which 93 found positive for malaria infection (*Pv*=70, *Pf*=20 and Mixed=3). The slide positivity rate was 1.4. All the confirmed malaria patients were provided radical treatment by the Medical Officers of Civil Hospital, Nadiad. There is an increase in both *P. falciparum* and *P. vivax* malaria cases as compared to 2014. However, 3 cases of mixed infection have also been reported. The age-wise distribution of cases indicates highest malaria infection among 15 and above age group.

5.7 Panaji (Goa)

- Characterization of salivary gland proteome of dengue/DHF, chikungunya and yellow fever vector *Ae. aegypti* L was done. A large number of proteins were identified and catalogued and their functional analysis was performed.
- Proteomic analysis of urine of malaria patients using high resolution mass spectrometry was performed for identification of candidate biomarkers for *P. falciparum* and *P. vivax* infections. Promising results have been obtained.
- A study on the role of gut microbiota in modulation of longevity, fecundity and fitness of a major malaria vector *An. stephensi* was initiated.
- Larvicidal and pupicidal activity of leaf extracts of *IC_Goa* against *An. stephensi* Liston, *Cx. quinquefasciatus* Say and *Ae. aegypti* was determined and pupicidal activity at low doses showed that promising pupicidal compounds exist in the extract which are further being explored for translational research.
- Vector infection studies under epidemiology of malaria evolution in South Asia project funded by the National Institute of Health, USA were performed. *Anopheles subpictus* was found to play a major role in perennial transmission of malaria in Goa.
- A national multi-district study entitled, 'Estimation of malaria burden in India was launched and carried out in Kolhapur and Dakshin Kannada district NIMR Field Unit, Goa. Other sample districts in the country were Jaipur, Jhabua, Koraput and Chatra where similar exercise was undertaken by the NIMR scientists. The project activity included manpower recruitment and training, active surveillance, malaria incidence reporting from private, corporate, municipal and Govt. sectors covering all stakeholders and health providers. Verbal Autopsy of all death cases was carried out on population of 4 lakh per district.

5.8 Raipur (Chhattisgarh)

- Field evaluation of efficacy, fabric integrity and acceptability of Olyset LNs in 10 villages, Kanker (6) and Balod (4) districts of Chhattisgarh state.
- Monitoring of insecticide resistance in *An. culicifacies* against various insecticides in all

the 27 districts of Chhattisgarh state.

- Monitoring of impact of insecticide resistance in malaria vectors on effectiveness of combination of IRS and LNs in 80 clusters (villages) with population of 75,000 in Keshkal block of Kondagaon district, Chhattisgarh.
- Questionnaire-based surveys were undertaken to ascertain the presence of distributed nets, their physical integrity in 7 districts of Chhattisgarh state where LNs were distributed by the State/District Health Department through Public Distribution System (PDS).
- Undertook study with the objective to assess usability of PermaNet 3.0 in Kanker, Dhamtari and Janjgir-Champa districts of Chhattisgarh state with malaria vectors having different levels of deltamethrin resistance.
- Monitoring of therapeutic efficacy of ACT against uncomplicated *P. falciparum* in Balod and Kanker districts, Chhattisgarh and Gadchiroli district, Maharashtra.
- Provided technical support to the programme by cross-checking of malaria blood slides and by checking of filaria blood slides received from various districts of Chhattisgarh state.
- Imparted training in malaria and its control to M.B.B.S. students from Govt. Medical College, Raipur and B.H.M.S. students of Maharana Pratap Homoeopathic Medical College and Hospital, Raipur.
- Refresher training course in malaria microscopy and training in examination of blood slides for microfilaria to Laboratory Technicians of 10 and 7 districts, respectively of Chhattisgarh state.
- Participated in monthly review meeting of Chief Medical Officers/District Malaria Officers organised by the State Health Secretary at Raipur.

5.9 Ranchi (Jharkhand)

- Mosquito fauna survey was undertaken with reference to anophelines at Noamundi area of West Singhbhum district. Four recognised malaria vectors *An. culicifacies*, *An. annularis*, *An. fluviatilis* and *An. minimus* were recorded. Low density of *An. minimus* (MHD) 2–4 was recorded from Noamundi (Badajamda, Balijharan and Kadajamda villages) where high density (20–25) of *An. minimus* was recorded. Breeding was observed in hill tops slow moving streams, and pools. The feeding of *An. minimus* was observed in indoors and in outdoors (less numbers). Mosquito blood meal (MBM) analysis of *An. minimus* revealed high anthropophilic index 60% positive for human blood index.
- Susceptibility test of *An. minimus* using DDT (4%), malathion (5%) and deltamethrin (0.05%) was carried out in the villages of Noamundi (Barajamda, Balijharan and Kadajamda). *An. minimus* showed 95% mortality to DDT (4%) and 100% mortality to malathion (5%) and deltamethrin (0.05%). Four *An. minimus* were sequenced. Sequencing of 28r DNA confirmed that specimen identified morphologically as *An. minimus s.l.* were actually *An. minimus sensu stricto*.
- On the request of Air Force HQ, New Delhi an epidemiological and entomological investigation on malaria was carried out in and around Air Force unit, Singarsi of Pakur district, Jharkhand. In the present surveillance, mass blood survey smear examination result was presented. The result revealed slide positive rate (SPR) 37.9%. The highest SPR was observed in Singharsi (45.7%) and the lowest was observed in Madgama (19.0%); however, in the clinic at AF unit the SPR was 72.2% from Santhal and Pahadi tribes. *Plasmodium falciparum* was the dominating species recorded 86.5%. The *Plasmodium vivax* was 2.8 and 2% *Pf* cases showed gametocyte in the peripheral blood smear. The highest percentage of asymptomatic carrier of *Pf* was detected from the population that has no symptoms during a mass blood survey in all the villagers. Therefore, they act as a reservoir for transmitting the cases to the healthy persons through the vectors available in the local area. It was observed that the tribes were not using any protective measures for the control of malaria. Thus becoming vulnerable for malaria contact visa-à-vis transmitting malaria. The entomological survey revealed the mosquito comprise over

20 species. A total of 791 anopheles mosquito was collected. Major malaria vectors were An. *culicifacies* (39.9%) followed by *An. annularis* (7.2%) and *An. fluviatilis* (1.5%).

- Insecticide susceptibility test reported that *An. culicifacies,* and *An. annularis* were resistant to DDT (4.0%) and their susceptibility to malathion (5%) and deltamethrin (0.05%). However, it was observed that *An. fluviatilis* was susceptibility to DDT (4%).
- Suggested remedial measures were provided to the Air Force base. LLINS (long-lasting insecticide treated bednets) must be distributed and used covering all the population of Air Force, MES and civilians residing in AF units. All the three malaria vectors are susceptible to insecticides (malathion and deltamethrin). Therefore, it was suggested to overcome the entire problem, it is necessary to undertake proper surveillance, indoor spraying (IRS) of SP (synthetic pyrethroids) in the human dwelling and cattleshed at AF units. Active surveillance should be carried out the entire nearby village with the radius of 6 km weekly once and treatment must be provided to block the malaria transmission. Insecticide-treated nets (ITMNs) may be provided to all the nearby villages. Introduction of ACT in the AF unit and village level for more effective treatment.
- Monitoring of the therapeutic efficacy of ACT (artesunate + pyrimethamine and sulfadoxine) against uncomplicated *P. falciparum* malaria was carried out at Mahuadanr CHC of Latehar district, Jharkhand state. All the malaria positive cases were susceptible to ACT.
- Filariasis survey was carried out in the four districts of Jharkhand state (Simdega, Dhanbad, Palamu and Lohardaga). The district is dominated by Santhal, Munda, Oraons, Ho, Kharia, Karmali, Asur and Birhor tribes. The microfilaria rate was 6.07% in Bano PHC of Simdega district, 4.06% in Baliapur PHC of Dhanbad district, 4% in Mediningar PHC of Palamu district and 3.03% in Bhandra PHC of Lohardaga district. All the districts are in hotspot area. No MDA was carried out in Simdega and Palamu district and 9 and 10 rounds of MDA was carried out in Dhanbad and Lohardga districts. The study highlights the problem of filariasis in the Jharkhand state.
- To facilitate early diagnosis and prompt treatment, a malaria clinic function at NIMR, Field Unit, Itki, Ranchi. All the cases from Itki PHC and TB Sanatorium Hospital were diagnosed. A total of 248 patients attended the malaria clinic during the year 2015–16, out of which 14 cases were found to be positive for malaria of these 2 cases were positive for *P. vivax* and 12 cases were positive for *P. falciparum*. Overall percentage was—SPR 5.64, SfR 4.83 and *Pf* 85.71. One *P. falciparum* positive patients showed gametocyte in the peripheral blood.
- A filarial clinic is functioning at IDVC Field Unit, Itki, Ranchi. A total of 46 patients of filariasis attended the clinic during the year. Most of the cases were of old cases of filariasis. These cases were with acute manifestation of filariasis starting from hydrocele to elephantiasis. Two cases of epididymo-orchitis was observed. Five patients had multiple manifestations (10.86%).
- Support provided to NVBDCP and State Health Department: MDA evaluation, capacity building in the field of malaria entomology, microscopy and surveillance, insecticide resistance monitoring, evaluation of RDT kits, epidemic investigation for rapid response and management, quality control of laboratory services (diagnosis of malaria and filariasis and training for transmission assessment survey (TAS).

5.10 Rourkela (Odisha)

- MMV funded comprehensive case management programme launched in 2013 in four districts of Odisha with the primary objective to assess the impact of comprehensive case management system of uncomplicated malaria on its transmission in different transmission settings continued in Bolangir, Dhenkanal, Anugul and Kandhamal districts. The study was undertaken in collaboration with the Government of Odisha after completion of recruitment and training of project staffs as well as orientation training of the Medical Officers and other existing staffs of the Community Health Centres. The results were discussed.

- Monitoring the therapeutic efficacy of anti-malarial medicines in India. A multicentric study on therapeutic efficacy of artemisinin-based combination therapy (ACT) with the combination sulfadoxine-pyrimethamine + artesunate in uncomplicated *P. falciparum* malaria was carried out in Thakurgarh New PHC under Madhapur CHC in Angul district of Odisha. A total of 77 subjects who fulfilled all the inclusion criteria were enrolled in the study comprising of 35 (45.5%) females and 42 (54.5%) males.
- Eco-epidemiology and transmission of complex malaria in India (under NIH Sponsored CSCMi project) was carried out in 11 villages located in forest, plain and riverine areas under Bisra, Kuarmunda and Birkera CHCs of Sundergarh district, Odisha. In this study health seeking behaviour, diagnostic methods used, proportion of asymptomatic cases, mosquito fauna and composition of vector sibling species, host blood meal preferences and various environmental factors supporting growth of vector immature and adult survival were studied.

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Research Support Facilities

6

6.1 Animal House Facility

The animal house facility at NIMR is maintained as per the CPCSEA guidelines. Majorly it maintains small laboratory animals like mice and rabbits for research activities such as screening the antimalarials, parasite maintenance, insectary maintenance, immunological studies, etc. The projects involving the animals are only undertaken after their approval by the Scientific Advisory Committee (SAC) and Institute Animal Ethics Committee (IAEC) of the Institute. The new animal house is under construction and to be completed soon. The animal facility has dedicated technical staff for its smooth functioning.

6.2 Repository of Biological Materials

6.2.1 Mosquito species

The details of mosquitoes being maintained in the NIMR Insectary are furnished in Table 1.

6.2.2 Malaria Parasite Bank (MPB)

Malaria Parasite Bank at NIMR is functioning as a National Resource with a variety of human and non-human plasmodia (Table 2). *Plasmodium falciparum in vitro* cultivation, characterization of the isolates for susceptibility to different antimalarials; and cryopreservation of isolates adapted to *in vitro* culture and those non-adapted and their revival are routine activities at the Bank (Table 3).

Collection of biological materials

Till now, a total of 1434 isolates collected in the parasite bank, including 1030 *P. falciparum*, 399 *P. vivax* and 5 *P. malariae* has been depicted (Tables 4). Providing malaria parasites to the scientific community is one of the major activities of the Malaria Parasite Bank.

Supply of biological materials

Till now, 1434 vials/samples of positive sera/plasma and human and non-human malaria parasites have been supplied to 88 institutes/universities and research organizations.

Resource generation

As per the SAC recommendations we have already started charging fee for the biological

Table 1. Details of mosquito species being maintained in the Insectary of NIMR

Species	Strain/Origin	Year of establishment	Isolated from
An. stephensi	Sonepat	Since 2000	Haryana
	Nadiad	2007	Gujarat
	Panjim	2009	Goa
	Alwar	2013	Rajasthan
An. culicifacies	Burari	2013	Delhi
	Rameswaram	2013	Tamil Nadu
	Dehra	2013	Himachal Pradesh
	Dadri	2013	Uttar Pradesh
	Beel Akbarpur	2013	Uttar Pradesh
	Manki	2013	Uttar Pradesh
	Raipur	2013	Chhattisgarh
Cx. quinquefasciatus	RR Permethrin (0.05%)	1999	Mewat (Haryana)
	RR Lambdacyhalothrin (0.05%)	1999	Mewat (Haryana)
	RR Deltamethrin (0.05%)	1999	Mewat (Haryana)
	RR Malathion (5%)	2000	Mewat (Haryana)

Table 2. Non-human malaria parasites preserved in the Malaria Parasite Bank

Parasite	Species	Susceptibility to antimalarials
Simian malaria	*P. cynomolgi bastianelli* (CDRI)	Not done
	P. cynomolgi bastianelli (NICD)	Not done
	P. knowlesi (NICD)	Not done
	P. knowlesi (CDRI)	Not done
	P. fragile (CDRI)	Not done
Avian malaria	*P. gallinaceum*	Not done
	P. relictum	Not done
Rodent malaria	*P. berghei* (CDRI)	CQ-Resistant
	P. berghei	CQ-Sensitive
	P. berghei	Quinine-Resistant
	P. berghei ANKA	Not done
	P. berghei (NK65) PGI Chandigarh	Not done
	P. chabaudi (Paris)	Not done
	P. yoelii nigeriensis (ICGEB)	Not done
	P. yoelii nigeriensis (CDRI)	Multi-resistant
	P. yoelii nigeriensis (London S.H.T.M.)	Not done
	P. yoelii yoelii (265 By) Paris	Not done

Table 3. Details of characterized *P. falciparum* parasites

• Adapted isolates susceptible to chloroquine	54
• Adapted isolates resistant to chloroquine	52
• NF-54, an infective gametocytes producing strain of *P. falciparum*	1
• 3D 7A : A clone of NF-54	1
• Field isolates which can invade trypsin-treated erythrocytes	3
• Field isolates which can invade neuraminidase-treated but not trypsin-treated erythrocytes	3
• Field isolates which can invade normal erythrocytes but not in neuraminidase or in trypsin-treated erythrocytes	3
• Field isolates which can invade both in neuraminidase-treated and in trypsin-treated erythrocytes	5
• Field isolates which can form rosettes	3
• Field isolate which can bind to CSA	1
• Field isolates which can bind to CD36	9
• Field isolates which can bind to ICAM-1	2
• Isolates with isoenzyme profile of GPI, GDH, ADA & LDH markers	22
• Isolates with MSP-1, MSP-2 and GLURP markers	110
• Isolates genotyped for virulence genes	74
• Isolates genotyped for *msp3α* genes	46
• Isolates adapted *in vitro* producing gametocytes	5
• Isolates characterized for drug resistance genes	47
• Field isolates sequenced for various genes	92

Table 4. Year-wise total parasite samples collected

Year of collection	*P. falciparum*	*P. vivax*	*P. malariae*	Total
1992–2003	601	52	5	658
2004	17	1	—	18
2005	4	6	—	10
2006	59	9	—	68
2007	27	9	—	36
2008	55	88	—	143
2009	9	16	—	25
2010	42	75	—	117
2011	75	47	—	122
2012	11	45	—	56
2013	40	16	—	56
2014	27	5	—	32
2015	63	30	—	93
Total	1030	399	5	1434

materials supplied from Parasite Bank and till now ₹ 4,54,000/- has been collected up to 2015–16.

Manpower development

On the part of manpower development parasite bank is actively involved in imparting training to Scientists/Research Fellows/WHO Fellows /students in *in vitro* cultivation of *P. falciparum* and drug sensitivity testing. A total of 260 students have taken training from the Parasite Bank.

Training facilities available in Malaria Parasite Bank

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/ strains.
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*.
- Short-term cultivation of *P. vivax*.
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials.
- *In vitro* screening of medicinal plant extracts for antiplasmodial properties.

Cell lines available at Malaria Parasite Bank

- Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of exo-erythrocytic stage of malaria parasites.
- Myeloma cell line: SP2
- Hybridomas: 2A 10 (anti-*P. falciparum* sporozoite antibody secreting cells).
- 2 F2 1 A7 (anti-*P. vivax* sporozoite antibody secreting cells).

6.3 Library and Information Centre

Library and Information Centre at NIMR is a resource centre which provides an access to

literature and documentation in the field of malaria and other vector borne diseases. It serves as a bank of information.

The Library & Information Centre of NIMR endeavours to acquire process, organize and disseminate global information to fulfil the information needs of the administrators, policy makers, scientists, research scholars, outside visitors and foreign delegates. This centre uses LIBSYS software package, which consists of modules on acquisition, cataloguing, circulation, serial, OPAC, membership and article indexing. All the collections of this resource centre are completely computerized and indexed.

Library Timings

Monday to Friday— 0900 to 1730 hrs

Library collections

Books	8600
Journals (Online)	16
Newspapers	14
Magazines	20
CDs/DVDs	30
Reprint documents	290
Theses	29
Reports (National and International)	115

Special collections

- Census of India Publications
- WHO Publications
- National Survey Reports on Malaria and other Vector-Borne Diseases
- NIMR Publications

Library services

- Circulation of Books
- Inter Library Loan
- Document delivery
- Reference services
- Wi-Fi Internet Access Facility
- Current Awareness services of Journals
- Abstract services
- Photo Copying services
- Thesis Database
- Reading hall
- Non-Print Material Database
- Print + Online Journals

E-consortia for Journals

- ICMR e-Consortia
- ERMED Consortia

Documentation services

- Health News Repository on Malaria & other Vector-Borne Diseases
- New arrivals/Addition of books

Apprentice training

The NIMR Library & Information Centre trains and empowers students of library and information discipline by recruiting apprentices for one year. In the year 2015, three apprentice trainees were recruited and trained successfully.

Resource sharing

Library and Information Centre is an active member of Developing Library Network (DELNET) and shares its resources with 5535 member libraries and information centres across the Globe.

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Inter-Institutional Collaboration

7

The Institute collaborated with different national and international centres/agencies through different projects for wide coverage, protection and effective control of malaria and other vector-borne diseases:

1. Monitoring the therapeutic efficacy of antimalarial medicines in India in collaboration with NVBDCP, Delhi and State Health Authorities of Tripura, Mizoram and Meghalaya.
2. Quality assurance of malaria rapid diagnostic tests in India in collaboration with NVBDCP, Delhi.
3. Effective and safe interventions for prevention of malaria in pregnancy in India: An assessment of malaria burden in pregnancy, implementability of a screening strategy and barriers to scaling-up interventions in collaboration with the London School of Hygiene and Tropical Medicine (LSHTM), London.
4. Center for the Study of Complex Malaria in India (CSCMi) under the International Centers of Excellence for Malaria Research (ICEMR) program in collaboration with the New York University and the Pennsylvania State University (NIH project).
5. Mapping of malaria risk in the context of climate change in India in collaboration with NVBDCP, Delhi and Bhaskaracharya Institute for Space Applications and Geo-Informatics (BISAG), Gujarat.
6. Studies on Health impact assessment of Sardar Sarovar project in command area of Rajasthan in collaboration with the State Health Department, CE Narmada and SE Narmada, Rajasthan.
7. Reducing dengue transmission in Najafgarh zone of Delhi: An Integrated vector management approach in collaboration with Municipal Corporation of Delhi.
8. Development of a sustainable *Plasmodium vivax* liver stage assay using human hepatocytes derived from induced pluripotent stem cells in collaboration with NCBS, Bengaluru.
9. Epidemiological studies for establishing immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions in India in collaboration with Denmark and Department of Biotechnology, India.
10. Observational study to explore clinical and laboratory presentation of dengue patients with different serotypes, in collaboration with AIIMS, and Kasturba Hospital, New Delhi.

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Human Resource Development

8

8.1 Ph.D. Programme

NIMR provides facilities for pursuing Ph.D. degrees to the students. The Institute is affiliated to the Jiwaji University, Gwalior; Goa University, Goa; Gautam Buddh Technical University, Lucknow; University of Madras, Chennai; Kumaun University, Nainital; and Maharshi Dayanand University, Rohtak.

8.2 Students in Ph.D. Programme

Following students are completing their Ph.D. degree under the supervision of NIMR scientists: Ms Shalu Thomas, Ms G Sri Lakshmi Priya, Ms R Sangamithra, Ms Kavita Kadian, Ms Ritu Rawal, Mr Jagbir Singh, Mr B Prasad Rao, Mrs Vaishali Verma, Mrs N Elamathi, Mr Kona Madhavinadha Prasad, Ms Sneh Shalini, Ms Ruchi Gupta, Mr Kamlesh Kaitholia, Mrs Supriya Sharma, Mr Kapil Vashist, Mr Ram Suresh Bhatt, Mrs Baljinder Kaur Sandhu, Mr Rahul Pasupureddy, Mr Rewa S Thakur, Mr Vikky Awasthi, and Ms Rubika Chauhan.

8.3 M.Sc. Projects/Dissertations

The Institute also provides facility for fulfilment of Master's degree in Life Sciences/Biotechnology/ Bioinformatics, *etc.*

Several M.Sc. students, namely Ms Anshul, Ms Jyoti Rawat, Ms Surabhi Rawat and Ms Nidhi successfully completed their projects/dissertations under the supervision of NIMR scientists.

8.4 Training Courses/Workshops organized

NIMR has conducted regular training programmes as under:

Dr Mishra N

1. Organized a brain-storming meeting to disseminate the findings of the studies and on Possible contribution factors for antimalarial resistance with special focus on treatment practices including studies on new regimen at Agartala Govt. Medical College, Tripura on 24 February 2015.
2. Organized the Launch of operational research projects at NIMR, New Delhi on 8 May 2015.
3. Organized an orientation meeting of the project entitled "Monitoring the therapeutic efficacy of antimalarial medicines in India at NIMR/NVBDCP" at Tura, West Garo Hills, Meghalaya on 8 July 2015 and at Betul district, Madhya Pradesh on 3 July 2015.
4. Organized a Laboratory Technician training on Malaria microscopy and sampling techniques under the project on Monitoring the therapeutic efficacy of antimalarial medicine in India at NIMR, New Delhi from 25–29 May 2015.

Dr Nagpal BN

1. Coordinated one-day re-orientation training programme in strengthening of entomological surveillance in MCD funded by the Municipal Corporation of Delhi on 5 June 2015.

Dr Raghavendra K

1. Organized a training programme on Public Health Entomology for VBD Consultants/ DMO/IDSP Consultants/District Entomologists, in collaboration with Centre for Medical Entomology and Vector Management, National Centre for Disease Control from 23 February to 22 March 2016.
2. Organized a training course for District Vector Borne Disease Control officers (36 participants) as part of the WHO-sponsored Training course for Indoor residual spraying for control

of malaria and leishmaniasis in India at New Delhi from 2-7 November 2015 and in Patna from 30 November to 4 December 2015.

3. Organized a WHO-IVM training for trainees from North Korea on "Personal protection measures and vector control options", at VCRC, Puducherry on 12 August 2015.
4. Organized a workshop on malaria vector insecticide resistance monitoring and management in India, in collaboration with WHO and NVBDCP for 18 entomologists from different Indian states, 4 Institutional participants from India and 2 from Bhutan, at the National Institute of Malaria Research (ICMR), New Delhi from 7-11 December 2015.

Dr Valecha N

1. Organized ASEAN workshop on malaria research supported through AISTDF at NIMR, New Delhi from 10–15 May 2015.
2. Organized a meeting of Technical Resource Group (TRG) to observe the progress and timelines of population-based survey under GFATM supported by NVBDCP at NIMR, Delhi on 27 January 2015.
3. Organized India - Canada Malaria Consortium Project showcase on Development of Novel antimalarial drug at ICGEB, New Delhi on 30 January 2015.

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Research Papers Published
(January–December 2015)

9

1. Abdulla S, Binka F, Graves P, Greenwood B, Leke R, Malik E, Marsh K, Meek S, Mendis K, Schapira A, Slutsker L, Tanner M, Valecha N, While N, Alonso P, Bosman A, Cibulskis R, D'Souza B, Mnzava A, Ringwald P, Shutes E, Szilagyi Z. WHO Malaria Policy Advisory Committee and Secretariat. Malaria Policy Advisory Committee to the WHO: Conclusions and recommendations of seventh biannual meeting (March 2015). *Malar J* 2015; *14:* 295.
2. Anushrita, Nagpal BN, Kapoor Neera, Srivastava Aruna, Saxena Rekha, Vikram Kumar, Gupta Sanjeev, Jain JK, Valecha Neena. Prevalence of vector mosquitoes of major mosquito borne diseases in areas of Indira Sagar Project, Madhya Pradesh, India. *Int J Mosq Res* 2015; *2*(3): 182–7.
3. Binka F, Graves P, Greenwood B, Leke R, Malik E, Marsh K, Meek S, Mendis K, Schapira A, Slutsker L, Tanner M, Valecha N, White N. WHO Malaria Policy Advisory Committee and Secretariat. Malaria Policy Advisory Committee to the WHO: Conclusions and recommendations of sixth biannual meeting (September 2014). *Malar J* 2015; *14:* 107.
4. Carlton JM, Volkman SK, Uplekar S, Hupalo DN, Pereira Alves JM, Cui L, Donnelly M, Roos DS, Harb OS, Acosta M, Read A, Ribolla PE, Singh OP, Valecha N, Wassmer SC, Ferreira M, Escalante AA. Population genetics, evolutionary genomics, and genome-wide studies of malaria: A view across the international centers of excellence for malaria research. *Am J Trop Med Hyg* 2015; *93*(3 Suppl): 87–98.
5. Chaturvedi N, Bhandari S, Bharti PK, Basak SK, Singh MP, Singh N. Sympatric distribution of *Plasmodium ovale curtisi and P. ovale wallikeri* in India: Implication for the diagnosis of malaria and its control. *Trans R Soc Trop Med Hyg* 2015; *109*(5): 352–4.
6. Conn JE, Norris DE, Donnelly MJ, Beebe NW, Burkot TR, Coulibaly MB, Chery L, Eapen A, Keven JB, Kilama M, Kumar A, Lindsay SW, Moreno M, Quinones M, Reimer LJ, Russell TL, Smith DL, Thomas MB, Walker ED, Wilson ML, Yan G. Entomological monitoring and evaluation: Diverse transmission settings of ICEMR Projects will require local and regional malaria elimination strategies. *Am J Trop Med Hyg* 2015; *93*(3 Suppl): 28–41.
7. Das A. The distinctive features of Indian malaria parasites. *Trends Parasitol* 2015; *31*(3): 83–6
8. Dev V, Adak T, Singh OP, Nanda N, Baidya BK. Malaria transmission in Tripura: Disease distribution & determinants. *Indian J Med Res* 2015; *142* Suppl: S12–22.
9. Dev V, Barman K, Khound K. A cross-sectional study assessing the residual bio-efficacy and durability of field-distributed long-lasting insecticidal nets in malaria endemic ethnic communities of Assam, northeast India. *J Infect Pub Health* 2015; pii: S1876–0341(15) 00188–4.
10. Dev V, Sharma VP, Barman K. Mosquito-borne diseases in Assam, northeast India: Current status and key challenges. *WHO South-East Asia J Public Health* 2015; *4:* 20–29.
11. Dutta P, Khan SA, Chetry S, Dev V, Sharma, KC, Mahanta J. First evidence of dengue virus infection in wild caught mosquitoes during an outbreak in Assam, northeast India. *J Vector Borne Dis* 2015; *52*(4): 293–8.
12. Dykes CL, Kushwah RB, Das MK, Sharma SN, Bhatt RM, Veer V, Agrawal OP, Adak T, Singh

OP. Knockdown resistance (kdr) mutations in Indian *Anopheles culicifacies* populations. *Parasit Vectors* 2015; *8:* 333.

13. Elamathi N, Sreehari U, Raghavendra K. A note on new insights into vector control through *in silico* approaches. In: *Proceedings of Global meet of Biologists* organized by Department of Zoology, Osmania University and IICT Hyderabad. *Nat J Life Sci* 2015:*12*(3 Special Issue): 181–4.
14. Ghosh SK. Human dirofilariasis: A fast emerging zoonosis in India. *Indian J Med Microbiol* 2015; *33*(4): 595–6.
15. Gupta H, Jain A, Saadi AV, Vasudevan TG, Hande MH, D'Souza SC, Ghosh SK, Umakanth S, Satyamoorthy K. Categorical complexities of *Plasmodium falciparum* malaria in individuals is associated with genetic variations in ADORA2A and GRK5 genes. *Infect Genet Evol* 2015; *34:* 188–99.
16. Gupta P, Yadavendu V, Singh V. Assessing the gametocyte production in field isolates of *Plasmodium falciparum*. *J Bacteriol Parasitol* 2015; *6:* 250.
17. Gupta R, Mishra N, Kumar A, Rana R, Srivastava B, Tyagi PK, Anvikar AR, Valecha N. Monitoring artemisinin resistance in *Plasmodium falciparum*: Comparison of parasite clearance time by microscopy and real-time PCR and evaluation of mutations in *Pfatpase6* gene in Odisha state of India. *Parasitol Res* 2015; *114*(9): 3487–96.
18. Jain S, Rana V, Tridibes A, Sunil S, Bhatnagar RK. Dynamic expression of miRNAs across immature and adult stages of the malaria mosquito *Anopheles stephensi*. *Parasit Vectors* 2015; *8:* 179.
19. Kanchan K, Jha P, Pati SS, Mohanty S, Mishra SK, Sharma SK, Awasthi S, Venkatesh V, Habib S. Interferon-γ (IFNG) microsatellite repeat and single nucleotide polymorphism haplotypes of IFN-α receptor (IFNAR1) associated with enhanced malaria susceptibility in Indian populations. *Infect Genet Evol* 2015; *29:* 6–14.
20. Kanchan K, Pati SS, Mohanty S, Mishra SK, Sharma SK, Awasthi S: Indian Genome Variation Consortium, Venkatesh V, Habib S. Polymorphisms in host genes encoding NOSII, C-reactive protein, and adhesion molecules thrombospondin and E-selectin are risk factors for *Plasmodium falciparum* malaria in India. *Eur J Clin Microbiol Infect Dis* 2015; *34*(10): 2029–39.
21. Kleinschmidt I, Mnzava AP, Kafy HT, Mbogo C, Bashir AI, Bigoga J, Adechoubou A, Raghavendra K, Knox TB, Malik EM, Nkuni ZJ, Bayoh N, Ochomo E, Fondjo E, Kouambeng C, Awono-Ambene HP, Etang J, Akogbeto M, Bhatt R, Swain DK, Kinyari T, Njagi K, Muthami L, Subramaniam K, Bradley J, West P, Massougbodji A, Okê-Sopoh M, Hounto A, Elmardi K, Valecha N, Kamau L, Mathenge E, Donnelly MJ. Design of a study to determine the impact of insecticide resistance on malaria vector control: A multi-country investigation. *Malar J* 2015; *14:* 282.
22. Kumar A, Moirangthem R, Gahlawat SK, Chandra J, Gupta P, Valecha N, Anvikar A, Singh V. Emergence of sulfadoxine-pyrimethamine resistance in Indian isolates of *Plasmodium falciparum* in the last two decades. *Infect Genet Evol* 2015; *36:* 190–8.
23. Kushwah RB, Dykes CL, Kapoor N, Adak T, Singh OP. Pyrethroid-resistance and presence of two knockdown resistance (kdr) mutations, F1534C and a novel mutation T1520I, in Indian *Aedes aegypti*. *PLoS Negl Trop Dis* 2015; *9*(1): e3332.
24. Kushwah RB, Mallick PK, Ravikumar H, Dev V, Kapoor N, Adak TP, Singh OP. Status of DDT and pyrethroid resistance in Indian *Aedes albopictus* and absence of knockdown resistance (kdr) mutation. *J Vector Borne Dis* 2015; *52*(1): 95–8.
25. Lather M, Sharma D, Dang AS, Adak T, Singh OP. Isolation and characterization of polymorphic microsatellite markers from the malaria vector *Anopheles fluviatilis* species T (Diptera: Culicidae). *J Med Entomol* 2015; *52*(3): 408–12.
26. Mann R, Sharma S, Mishra N, Valecha N, Anvikar AR. Comparative assessment of genomic DNA extraction processes for *Plasmodium* - Identifying the appropriate method. *J Vector Borne Dis* 2015; *52*(4): 273–80.
27. Masur U, Kumar H, Kumar A. Evaluation of larvicidal effect of *Dysoxylum binectariferum* Hook against *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* L. *Int J Bioassays* 2015; *4* (12): 4683–8

28. Mishra N, Prajapati SK, Kaitholia K, Bharti RS, Srivastava B, Phookan S, Anvikar AR, Dev V, Sonal GS, Dhariwal AC, White NJ, Valecha N. Surveillance of artemisinin resistance in *Plasmodium falciparum* in India using the kelch13 molecular marker. *Antimicrob Agents Chemother* 2015; *59*(5): 2548–53.
29. Moss WJ, Dorsey G, Mueller I, Laufer MK, Krogstad DJ, Vinetz JM, Guzman M, Rosas-Aguirre AM, Herrera S, Arevalo-Herrera M, Chery L, Kumar A, Mohapatra PK, Ramanathapuram L, Srivastava HC, Cui L, Zhou G, Parker DM, Nankabirwa J, Kazura JW. International Centers of Excellence for Malaria Research: Malaria epidemiology and control within the International Centers of Excellence for Malaria Research. *Am J Trop Med Hyg* 2015; *93*(3 Suppl): 5–15.
30. Nagaraj VA, Mukhi D, Sathishkumar V, Subramani PA, Ghosh SK, Pandey RR, Shetty MC, Padmanaban G. Asparagine requirement in *Plasmodium berghei* as a target to prevent malaria transmission and liver infections. *Nat Commun* 2015; *6:* 8775.
31. Nagpal BN, Ghosh SK, Eapen A, Srivastava A, Sharma MC, Singh VP, Parashar BD, Prakash S, Mendki MJ, Tikar SN, Saxena R, Gupta S, Tiwari SN, Ojha VP, Ravindran KJ, Ganesan K, Rao AN, Sharma RS, Tuli NR, Yadav NK, Vijayaraghavan R, Dua VK, Dash AP, Kaushik MP, Joshi PL, Valecha N. Control of *Aedes aegypti* and *Ae. albopictus*, the vectors of dengue and chikungunya, by using pheromone C21 with an insect growth regulator: Results of multicentric trials from 2007–12 in India. *J Vector Borne Dis* 2015; *52*(3): 224–31.
32. Pandey S, Das MK, Singh RK, Dhiman RC. Anopheline mosquitoes in District Ramgarh (Jharkhand), India. *J Vector Borne Dis* 2015; *52*(3): 232–8.
33. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*—Authors' reply. *Lancet Infect Dis* 2015; *15*(6): 630–1.
34. Quiñones ML, Norris DE, Conn JE, Moreno M, Burkot TR, Bugoro H, Keven JB, Cooper R, Yan G, Rosas A, Palomino M, Donnelly MJ, Mawejje HD, Eapen A, Montgomery J, Coulibaly MB, Beier JC, Kumar A. Insecticide resistance in areas under investigation by the International Centers of Excellence for Malaria Research: A challenge for malaria control and elimination. *Am J Trop Med Hyg* 2015; *93*(3 Suppl): 69–78.
35. Roy M, Bouma M, Dhiman RC, Pascual M. Predictability of epidemic malaria under non-stationary conditions with process-based models combining epidemiological updates and climate variability. *Malar J* 2015; *14*(1): 419.
36. Sahu B, Barik TK, Dhiman RC. Analysis of sulphadoxine-pyrimethamine drug resistance in *Plasmodium falciparum* from Khurda district of Odisha, India. *Res J Pharmac Biol Chem Sci* 2015; *6*(3): 1270–77.
37. Savargaonkar D, Nagpal BN, Srivastava B, Anvikar AR, Valecha N. The footprints of relapsing malaria in South West Delhi. *J Vector Borne Dis* 2015; *52:* 287–92
38. Sharma D, Lather M, Mallick PK, Adak T, Dang AS, Valecha N, Singh OP. Polymorphism in drug resistance genes dihydrofolate reductase and dihydropteroate synthase in *Plasmodium falciparum* in some states of India. *Parasit Vectors* 2015; *8:* 471.
39. Sharma P, Das De T, Sharma S, Mishra AK, Thomas T, Verma S, Kumari V, Lata S, Singh N, Valecha N, Pandey KC, Dixit R. Deep sequencing revealed molecular signature of horizontal gene transfer of plant like transcripts in the mosquito *Anopheles culicifacies*: An evolutionary puzzle. *F1000Res* 2015; *4:* 1523.
40. Sharma P, Sharma S, Mishra AK, Thomas T, Das De T, Rohilla SL, Singh N, Pandey KC, Valecha N, Dixit R. Unraveling dual feeding associated molecular complexity of salivary glands in the mosquito *Anopheles culicifacies*. *Biol Open* 2015; *4*(8): 1002–15.
41. Sharma VP, Dev V. Biology and control of *Anopheles culicifacies* Giles 1901. *Indian J Med Res* 2015; *141:* 525–36.
42. Sharma VP, Dev V, Phookan S. Neglected *Plasmodium vivax* malaria in northeastern states of India. *Indian J Med Res* 2015; *141*(5): 546–55.
43. Singh AK, Rajendran V, Pant A, Ghosh PC, Singh N, Latha N, Garg S, Pandey KC, Singh BK, Rathi B. Design, synthesis and biological evaluation of functionalized phthalimides: A new class of antimalarials and inhibitors of

falcipain-2, a major hemoglobinase of malaria parasite. *Bioorg Med Chem* 2015; *23*(8): 1817–27
44. Singh N, Mishra AK, Chand SK, Bharti PK, Singh MP, Nanda N, Singh OP, Sodagiri K, Udhyakumar V. Relative abundance and *Plasmodium* infection rates of malaria vectors in and around Jabalpur: A malaria endemic region in Madhya Pradesh state, Central India. *PLoS One* 2015; *10*(5): e0126932.
45. Singh OP, Dykes CL, Sharma G, Das MK. L1014F-kdr mutation in Indian *Anopheles subpictus* (Diptera: Culicidae) arising from two alternative transversions in the voltage-gated sodium channel and a single PIRA-PCR for their detection. *J Med Entomol* 2015; *52*(1): 24–7.
46. Singh RK, Mittal PK, Kumar G, Karlekar RR, Dhole RB, Dhiman RC. Prevalence of *Aedes* mosquitoes in various localities of Gadchiroli district of Maharashtra state, India. *Int J Mosq Res* 2015; *2*(2): 38–41.
47. Singh V, Kumar A. The integral *Plasmodium* life cycle phenomenon: Gametocyte genes. *J Bacteriol Parasitol* 2015; *6:* 224.
48. Sohail M, Shakeel S, Kumari S, Bharti A, Zahid F, Anwar S, Singh KP, Islam M, Sharma AK, Lata S, Ali V, Adak T, Das P, Raziuddin M. Prevalence of malaria infection and risk factors associated with anaemia among pregnant women in semiurban community of Hazaribag, Jharkhand, India. *Biomed Res Int* 2015; *2015:* 740512.
49. Sreehari U, Verma V, Elamathi N, Velamuri PS, Raghavendra K. Adulticidal and larvicidal efficacy of three neonicotinoids against insecticide susceptible and resistant mosquito strains. *Indian J Med Res* 2015; *142* Suppl: S64–70
50. Srivastava B, Anvikar AR, Ghosh SK, Mishra N, Kumar N, Houri-Yafin A, Pollak JJ, Salpeter SJ, Valecha N. Computer-vision-based technology for fast, accurate and cost-effective diagnosis of malaria. *Malar J* 2015; *14:* 526.
51. Tennyson S, Ravindran J, Eapen Alex, William J. Larvicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Asian Pacific J Trop Dis* 2015; *5*(Suppl 1): S73–S76.
52. Tiwari S, Ghosh SK, Sathyanarayan TS, Nanda N, Uragayala S, Valecha N. Malaria outbreaks in villages in north Karnataka, India and role of sibiling species of *Anopheles culicifacies* complex. *Health* 2015; *7:* 946–54.
53. Tomar N, Singh Shiv Vardhan, Jain CL, Verma Gaurav, Pal A, Singh Vineeta. Synthesis and antiplasmodial activity of some novel chalcone derivatives. *Asian J Pharm Clin Res* 2015; *8*(2): 47–50.
54. Toure OA, Rulisa S, Anvikar AR, Rao BS, Mishra P, Jalali RK, Arora S, Roy A, Saha N, Iyer SS, Sharma P, Valecha N. Efficacy and safety of fixed dose combination of arterolane maleate and piperaquine phosphate dispersible tablets in paediatric patients with acute uncomplicated *Plasmodium falciparum* malaria: A phase II, multicentric, open-label study. *Malar J* 2015; *14*(1): 469.
55. Tripathi M, Das A. Genotyping malaria parasites with DNA barcodes. *Trop Med Int Health* 2015; *20* (12): 1636-8.
56. Tyagi S, Das A. Mitochondrial population genomic analyses reveal population structure and demography of Indian *Plasmodium falciparum*. *Mitochondrion* 2015; *24:* 9–21.
57. Uragayala S, Kamaraju R, Tiwari S, Ghosh SK, Valecha N. Small-scale evaluation of the efficacy and residual activity of alpha-cypermethrin WG (250 g a.i./kg) for indoor spraying in comparison with alpha-cypermethrin WP (50 g a.i./kg) in India. *Malar J* 2015; *14:* 223.
58. Verma V, Elamathi N, Velamuri PS, Sreehari U, Agrawal OP, Raghavendra K. Chlorfenapyr: Irritant effect compared to other insecticides and its intrinsic toxicity in multiple-insecticide-susceptible and -resistant *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis* 2015; *52*(1): 99–103.
59. Vijay S, Rawal R, Kadian K, Raghavendra K, Sharma A. Annotated differentially expressed salivary proteins of susceptible and insecticide-resistant mosquitoes of *Anopheles stephensi*. *PLoS One* 2015; *10*(3): e0119666.
60. Vikram K, Nagpal BN, Pande V, Srivastava A, Saxena R, Singh H, Anushrita, Gupta SK, Tuli NR, Yadav NK, Olivier T, Richard P, Valecha N. Detection of dengue virus in individual *Aedes aegypti* mosquitoes in Delhi, India. *J Vector Borne Dis* 2015; *52*(2): 129–33.
61. Vikram K, Nagpal BN, Pande Veena,

Srivastava Aruna, Gupta Sanjeev K, Anushrita, Singh VP, Singh Himmat, Saxena Rekha, Tuli NR, Yadav NK, Richard Paul, Valecha Neena, Olivier Telle. Comparison of *Ae. aegypti* breeding in localities of different socio-economic groups of Delhi, India. *Int J Mosq Res* 2015; *2* (2): 83–8.

62. Wilson ML, Krogstad DJ, Arinaitwe E, Arevalo-Herrera M, Chery L, Ferreira MU, Ndiaye D, Mathanga DP, Eapen A. Urban malaria: Understanding its epidemiology, ecology, and transmission across seven diverse ICEMR network sites. *Am J Trop Med Hyg* 2015; *93*(3 Suppl): 110–23.
63. WorldWide Antimalarial Resistance Network (WWARN) AS-AQ Study Group, Adjuik MA, Allan R, Anvikar AR, Ashley EA, Ba MS, Barennes H, Barnes KI, Bassat Q, Baudin E, Björkman A, Bompart F, Bonnet M, Borrmann S, Brasseur P, Bukirwa H, Checchi F, Cot M, Dahal P, D'Alessandro U, Deloron P, Desai M, Diap G, Djimde AA, Dorsey G, Doumbo OK, Espié E, Etard JF, Fanello CI, Faucher JF, Faye B, Flegg JA, Gaye O, Gething PW, González R, Grandesso F, Guerin PJ, Guthmann JP, Hamour S, Hasugian AR, Hay SI, Humphreys GS, Jullien V, Juma E, Kamya MR, Karema C, Kiechel JR, Kremsner PG, Krishna S, Lameyre V, Ibrahim LM, Lee SJ, Lell B, Mårtensson A, Massougbodji A, Menan H, Ménard D, Menéndez C, Meremikwu M, Moreira C, Nabasumba C, Nambozi M, Ndiaye JL, Nikiema F, Nsanzabana C, Ntoumi F, Ogutu BR, Olliaro P, Osorio L, Ouédraogo JB, Penali LK, Pene M, Pinoges L, Piola P, Price RN, Roper C, Rosenthal PJ, Rwagacondo CE, Same-Ekobo A, Schramm B, Seck A, Sharma B, Sibley CH, Sinou V, Sirima SB, Smith JJ, Smithuis F, Somé FA, Sow D, Staedke SG, Stepniewska K, Swarthout TD, Sylla K, Talisuna AO, Tarning J, Taylor WR, Temu EA, Thwing JI, Tjitra E, Tine RC, Tinto H, Vaillant MT, Valecha N, Van den Broek I, White NJ, Yeka A, Zongo I. The effect of dosing strategies on the therapeutic efficacy of artesunate amodiaquine for uncomplicated malaria: A meta-analysis of individual patient data. *BMC Med* 2015 *13:* 66.

Books

1. Joshi Pankaj, Anvikar Anup R, Jangale Neeta, Valecha Neena. Global malaria, HIV and tuberculosis diagnostics platforms. In: *Major tropical diseases: Public health perspective*. Panjim-Goa, India: Broadway Publishing House 2015; pp. 180–96
2. Kochar D, Das A, Maji A. Malaria. In: *Adult immunization 2014*, II ed. Eds. Muruganantan A, Mathai Dilip, Sharma SK. Association of Physicians of India 2015; pp. 149–54. ISBN: 978-93-5152-190-7.
3. Singh V. Molecular epidemiology. In: *The battle against microbial pathogens: Basic science, technological advances and educational programs*. Ed. Méndez-Vilas A Badajoz, Spain: Formatex Research Center 2015; *2:* 661–7.

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Other Activities

10

10.1 Information, Education and Communication

In order to create awareness about vector borne diseases a scientific tour for school children was organized. Students of 8th and 9th standard from four schools, visited NIMR's Parasite Bank, Insectory and other laboratories on 9 October 2015. An informal lecture-cum-discussion session was also organized for students. They were informed about mosquito vectors; their life cycles, and live larvae of *Anopheles* mosquitoes were shown to them. Positive slides of malaria parasites were also shown to them. Satisfactory answers were given to the queries raised by the students by technical staff as well as by scientists of NIMR.

General information about breeding sites of mosquito, malaria and dengue were imparted to locals of Raj Nagar, Part II, Sector 8, Dwarka, New Delhi on 1 April 2015. Attempts were made to reduce/eliminate source of breeding sites.

Students visiting Insectary of NIMR.

Imparting general information about dengue and malaria at Raj Nagar-II, Dwarka, Delhi on 9 April 2015.

Students visiting Parasite Biology laboratory.

Identification of mosquito breeding sites during dengue epidemic in Delhi and NCR.

10.1.1 Documentation Cell

In the Documentation Cell following tasks were carried out:

(i) Updating of various information for Research Projects undertaken by NIMR using information received through minutes of SAC meetings for the year 2015–16 as well as inputs provided by individual principal investigators (PI/Co-PI) was done. Status of Projects, whether completed or ongoing, budget and extension period (if any) granted, List of Project staff were also updated.

(ii) Typing work was carried out for new projects approved by SAC/RAC for the year 2015–16.

(iii) Intramural and extramural projects approved by 36th SAC Meeting were enlisted and necessary information were furnished, *viz.* name of funding agency, subject and the name of collaborators.

(iv) List of Research Papers published, trainings/ workshops/ conferences organized by NIMR for the year 2015-16 were added to the Master list of publication alongwith trainings/workshops/conferences attended, respectively.

The following services were provided to various Divisions of NIMR:

(i) Names of Principal Investigators of 150 Extramural projects were updated for Accounts Division/ Dr RC Dhiman, Scientist 'G'.

(ii) Information on Research publications of NIMR scientists for the last ten years (2005–2014) and on research projects (intramural-extramural, collaboration) were provided to Dr Anup Anvikar for preparing status report on NIMR for university status of ICMR.

(iii) List of new Projects from January-July 2015 was given to the Library Incharge for *Plasmodium* Newsletter preparation.

(iv) Project IDs were allotted to intramural/ extramural projects approved by SAC and information were given to PIs.

(v) Updated list of SAC approved intramural/ extramural projects was provided to the Library Incharge for Annual Report 2014–15 preparation.

(vi) List of collaborative Projects was given to the Publication Division of NIMR for Annual Report preparation.

(vii) List of new ongoing and completed projects carried out at Field Units of NIMR was provided to the Administration Officer, NIMR.

10.1.2 Photography & Videography

In the Photography Unit, the following photography work were carried out on various occasions including meetings/trainings/workshops/ field surveys and functions held at NIMR and ICMR.

IEC Photography Unit

1. Photography of different major scientific works undertaken in the laboratories of NIMR; and ICMR;
2. Coverage of training/workshop on Malaria Burden from 7–9 April 2015;
3. World Malaria Day held at NIMR on 25 April 2015;
4. Launch of Operational Research Project held at NIMR on 8 May 2015;
5. ASEAN–INDIA workshop on Malaria Research held at NIMR from 10–15 May 2015;
6. Laboratory Technician training from 25–29 May 2015;
7. Re-orientation training programme of Municipal Corporation of Delhi held on 5 June 2015;
8. Month-wise photography of *Swachh Bharat Abhiyan;*
9. Photographs of *Yoga Diwas;* and *Hindi Pakhwada* held at NIMR from 14–15 September 2015;
10. Launch of e-learning modules for registration of clinical trials in India at ICMR;
11. RAC & SAC meetings held at NIMR from 17–18 December 2015;
12. Workshop on Malaria vector insecticide resistance monitoring and management in India from 7–11 December 2015;
13. Joint workshop on System biology of antimicrobial resistance held at the India International Centre from 20–21 January 2016;
14. ICMR awards presentation ceremony held on 19 January 2016;
15. Training at NIMR for Health Department, Madhya Pradesh from 11–15 January 2016;
16. INDO-US Workshop on Air pollution and health from 24–26 Febuary 2016;
17. Inauguration event of WHO recognized Malaria RBT testing laboratory held on 7

March 2016; and

18. Dissemination workshop on ICMR-India DIABetes (INDIAB) study held on 15 March 2016.

IEC Video Unit

In the Video Recording section work was carried out on occasions of several meetings/workshops/ functions, health education camps and field work activities held at NIMR and at other places. Editing & special effects were applied on different video films related to *Swachh Bharat Abhiyan*.

Video Shows

NIMR-produced documentaries on malaria and other vector borne diseases were shown to the Trainees attending/participating in Trainings organized by the NIMR.

Distribution of Video DVDs

Video DVDs on malaria, life cycle of malaria parasites, dengue, chikungunya, and related subjects produced at NIMR were distributed to the participants of different training programmes organized by NIMR, NVBDCP and State Health Department, Madhya Pardesh. The CDs were also sent to the states and given to interested visitors.

10.1.3 *Swachh Bharat Abhiyan*

The Hon'ble Prime Minister of India, launched *Swachh Bharat Abhiyan* on 2 October 2014. Since then, the NIMR has been actively undertaking various activities under monthly schedule which include identifying unhygienic spots in Institute's premises and their cleaning and monitoring, handling and safe disposal of biohazardous materials in laboratories, organizing monthly voluntary *Kar Sewa/Shramdan* in and around NIMR research block, dissemination of IEC materials at various public places, like hospitals, schools, dispensaries, petrol pumps pertaining to health and cleanliness. Monthly public lectures organized at various places in Delhi. Projects have been developed for demonstration of cleanliness and hygiene in the nearby colony Raj Nagar. Weeding out and digitization of files are regularly carried out. Toilets/washrooms, corridors and other places were strictly monitored and kept hygienic. In addition to this some activities like invited lectures and drawing competition on the *Swachh Bharat* theme were conducted for school going children of surrounding localities of NIMR, Dwarka. The students who participated were given certificates and prizes on the basis of their performances. The Institute is also regularly uploading the reports of regular and specific drive activities to *Swachh Bharat* web portal. NIMR also received an appreciation letter for sending innovative and implementable idea for cleanliness and hygiene for community.

Monthly *Shramdan* activities inside NIMR campus and nearby locality Raj Nagar-Part II.

Public lecture and Institutional seminar on health and hygiene.

Drawing and painting competition for school going children held on 9 September 2015 and the prize winning painting.

10.1.4 Infrastructure strengthening

GLP accreditation of the NIMR facilities for insecticide testing and trials

Our Institute is WHO recognized collaborating centre for laboratory testing and evaluation of public health pesticides products and collaborating Institute for conducting efficacy of different insecticide intervention such as larvicides, adulticides and bednets. WHO has proposed to upgrade the laboratory and field sites for GLP compliance using quality management systems for quality control and

quality assurance. An expert from UK visited our Institute and together we had a meeting with DST India on the procedures for accreditation. WHO also proposed a five-day workshop in May 2016. The process for the GLP compliance will be initiated soon and will be followed up with necessary preparation and inputs to get the accreditation for testing and evaluation following GLP. The process may take around two years.

Experimental huts for Phase-II testing of anti-vector interventions

NIMR is presently recognized for Phase-I, Phase-II and Phase-III evaluation of Public Health Pesticide products. WHO has proposed to provide support to create "Experimental Hut Facility" for Phase-II efficacy trials following the WHO standards. This activity may take one year. Administrative clearances are being obtained from the local administration from Gujarat and WHOPES agreed to provide the funds.

The above two strengthening and upgradation activities of the infrastructure and facilities shall make the Institute an International facility for evaluation and testing of insecticides and will be of use nationally for providing appropriate Insecticide-based control products and will also be a facility for use Globally. This could be an important effort towards disease elimination.

10.2 Publication and Information Division

The P&I Division of the NIMR continued its multifaceted activities in the field of publication and information by publishing periodicals, books, newsletters, *etc.* meant for dissemination of scientific information generated through research to different target groups.

Journal of Vector Borne Diseases

The *Journal of Vector Borne Diseases* is a peer reviewed, open access, quarterly published biomedical journal dedicated to the publication of original research contributions in the field of vector borne diseases such as malaria, filariasis, Japanese encephalitis, dengue, chikungunya, crimean-congo haemorrhagic fever (CCHF), leishmaniasis, trypanosomiasis, *etc.* with the aim of their control and prevention.

The journal is indexed by the major abstracting agencies like Science Citation Index Expanded, MEDLINE/PubMed, Scopus, Scimago Journal Ranking, DOAJ, *etc.*

Quarterly issues of the journal were published regularly and timely during the reporting period. The full articles of the journal can be accessed online through the Institute's website (*http://nimr.org.in/jvbd.html*) as well as PubMed, DOAJ and other resources. At present, archives from the year 2003 are available on the website. The print version is available on subscription basis with discount to the scientific community and agencies.

Malaria Patrika

Malaria Patrika is a quarterly published popular Hindi magazine launched in 1993. The Division continued to publish the issues of *Malaria Patrika*, for educating the local as well as scientific community on malaria and other vector borne diseases for their control. The issues published in 2015 were focused on use of different antimalarials, intervention strategies and control methods for malaria.

Plasmodium Newsletter

Plasmodium Newsletter of the Institute, published bi-annually highlighted the recent research investigations and advancements in the field of malaria, focusing primarily on the news related to malaria drugs, diagnostic tools and techniques, and reported important activities of the Institute and its field units during the reporting period.

Annual Reports

In addition to above, the Division also published the Annual Reports of the Institute (NIMR) as well as IDVC project for the financial year 2015–16. The annual reports included all the research activities of the Institute, publications of scientists and researchers, details of inter-institutional collaborations, extramural funded projects, and other activities of the Institute.

10.3 Seminars/Conferences/Workshops Training Courses/Meetings attended

Eapen Alex

1. Attended and presented a poster on 'Urban Malaria and its control in Chennai' in the 'ASEAN-India Flagship programme on Science & Technology for combating malaria: A public health challenge' at NIMR, New Delhi, India on 13 May 2015.

2. Attended a training on 'Bottle assay–An indigenous method for laboratory and field monitoring of insecticide resistance' along with Lal OP at NIMR, Delhi from 22–24 June 2015.
3. Attended and presented an invited scientific paper in 'IV Symposium on perspectives on malaria elimination' at Cali, Colombia on 20 August 2015.

Dixit Rajnikant

1. Attended a conference on Understanding the molecular genetics of mosquito immunity and vector competency (Oral presentation): "IMMUNOCON-15" at RMRIMS, Patna from 9–11 October 2015.
2. Attended a conference on Insect biodiversity: Where does India stand in the Global map? in March 2016.

Kumar Ashwani

1. Attended and delivered lecture on Burden of malaria in India in the 'ASEAN- India Flagship programme on Science & Technology for combating malaria: A public health challenge' at NIMR, New Delhi, India on 13 May 2015.
2. Attended Scientific Advisory Group meeting of NIH sponsored project "Malaria evolution in South Asia" held at New York USA from 4–10 July 2015.
3. Attended Annual workshop of International Centers of Excellence for Malaria Research held at Cali, Colombia from 15–24 August 2015.
4. Attended Vector Control Advisory Group (VCAG) meeting held at WHO HQs in Geneva, Switzerland as Advisor from 15–19 November 2015.
5. Attended 4th Annual workshop of International Centers of Excellence for Malaria Research (ICEMR) at Cali, Colombia from 17–19 August 2015.
6. Participated and presented a talk on new vector control tools in 4th Symposium on Perspectives for malaria elimination in Latin America from 20–21 August 2015.
7. Attended a malaria meeting of Experts on "Tools for malaria elimination" at Washington, USA from 6–8 October 2015.
8. Attended conference on Entomology organized by Punjabi University Patiala during November 2015.
9. Attended meeting—Invite as Temporary Advisor in Vector Control Advisory Group (VCAG) by Department of Neglected Tropical Diseases at World Health Organization, Geneva from 16-18 November 2015.

Mishra N

1. Attended International training on "Ethics and IRB operations" sponsored by the National Institute of Health, USA (NIH) and the International Clinical Studies Support Centre (ICSSC), USA at Bangkok from 16–17 July 2015.
2. Attended International training on "Fundamentals of International Clinical Research Training" at Bangkok (Thailand) from 6–11 September 2015.
3. Attended "Expert committee meeting on Malaria diagnostic and chemotherapy and prospects of malaria elimination in the Country" NVBDCP, DMRC at Delhi IT Park on 27 November 2015.
4. Attended FRC meeting of Ph.D. students at Department of Biotechnology, Goa University on 11 December 2015.

Nagpal BN

1. Attended the meeting to Review the action plan and activities in GNCT Delhi for Prevention and control of dengue during the year 2015 under the Chairmanship of Director, NVBDCP at Delhi on 28 April 2015.
2. Attended the workshop on ASEAN-India Flagship programme on Science & Technology for combating malaria and delivered lecture on "Mapping of biodiversity of mosquitoes and health impact assessment of developmental projects" at New Delhi on 12 May 2015.
3. Attended the meeting to Review the status & preparedness of dengue and other vector borne diseases and delivered lecture-cum-demonstration on life cycles of mosquitoes organized by the Directorate of Health Services, Govt. of Delhi on 3 July 2015.
4. Deputed the surveillance team the request of CMO and NDMC for checking the *Aedes* mosquito breeding in NDMC area, *viz*. PM House, New Moti Bagh Complex, Netaji Nagar, Sarojini Nagar, Minister bungalows,

AIIMS, Safdarjung hospital, RML hospital and DIZ Area-Shaheed Bhagat Singh Marg on 9 July 2015.
5. Attended one-day training on Dengue & VBDs for MOs and delivered the lecture on "Entomology aspect of dengue and demonstration of vector" organized by DPMU North East District on 17 July 2015.
6. Attended the Sensitization programme for Principals/Nodal officers of schools/ colleges for containment of dengue/chikungunya organized by the Health Department, NDMC on 22 July 2015.
7. Attended workshop for councillors and RWAs to create awareness regarding Prevention of vector borne diseases organized by the South, North and East Delhi Municipal Corporations on 13 August 2015.
8. Attended meeting and delivered lecture on "Common breeding sites of dengue vector and their control" in a meeting of 180 medical officers on participation of health institutions in dengue control and other health programmes organized by the EDMC on 19 August 2015.
9. Attended meeting to Review current situation of dengue fever chaired by the DGHS and organized by DGHS, MOHFW (GOI) on 17 September 2015.
10. Delivered lecture for Awareness and prevention of dengue in ICMR HQs organized by ICMR on 29 September 2015.

Nanda N

1. Participated as faculty member in Laboratory technician training on malaria microscopy and sampling organized by the National Institute of Malaria Research in collaboration with NVBDCP from 25–29 May 2015.
2. Participated as faculty member in a Re-orientation training programme on Strengthening of entomological surveillance in Municipal Corporations of Delhi funded by South Delhi Municipal Corporation (SDMC) in June 2015.
3. Provided Hands on training in identification of *Anopheles culicifacies* sibling species using diagnostic inversions in polytene chromosomes to Mr Kona M Prasad (Research Assistant) during November-December 2015.
4. Participated in the meeting with Vice-Chairman DDA, regarding extension of time for construction of hostel, guest house, auditorium *etc.* held at NIMR campus on 8 January 2016.
5. Participated as faculty member in Training to Master Trainers to Control Vector Borne Diseases in Simhasta Mela during April - May 2016 in Ujjain, Madhya Pradesh, funded by Health Department of Madhya Pradesh from 11–15 January 2016.
6. Mr Tenzin Wangdi, Sr Medical Entomologist, VBDCP, MoH, Bhutan and APMEN Fellow was taught basic concepts and provided training in cytotaxonomic tools for identification of sibling species of malaria vectors for one week in February 2016.
7. Participated in the meeting of Parliament Committee regarding implementation of *Raj Bhasha* in official work of the Institute held on 20 February 2016.

Raghavendra K

1. Attended the meeting on "Use of larvicides and adulticides for mosquito control" at NVBDCP, Delhi on 15 April 2015.
2. Attended the workshop on ASEAN-India Flagship programme on Science & Technology for combating malaria and presented a presentation on "Insecticide resistance and its management in malaria vectors" at New Delhi from 10–15 May 2015.
3. Delivered a lecture on "Insecticide resistance in anophelines and management at inventions, innovations and regulations in crop sciences" (IIRCS 2015) at IICT, Hyderabad from 24–25 June 2015.
4. Delivered a lecture on "Challenges of malaria vector resistance to insecticides in SEAR" on World Malaria Day at SEARO, New Delhi on 1 May 2015.
5. Delivered a lecture on "Insecticide resistance and its management in malaria vectors" in ASEAN-India Flagship programme on Science & Technology for combating malaria: A public health challenge, ASEAN-India workshop on malaria research at the National Institute of Malaria Research (ICMR), New Delhi, India from 10–15 May 2015.
6. Delivered a lecture on various aspects of vector control, insecticide resistance and management in the WHO-sponsored

workshop on Malaria vector insecticide resistance monitoring and management in India at National Institute of Malaria Research (ICMR), New Delhi from 7–11 December 2015.

7. Presented a presentation on "Insecticide resistance and management" in the meeting of vector borne diseases science forum in the session—Malaria: Challenges and possible strategies for the achievement of elimination at ICMR, New Delhi on 2 February 2016.

Sharma SK

1. Participated in the foundation stone-laying ceremony of the Model Rural Health Research Unit (MRHRU) at Tigria village in Cuttack district of Odisha which was chaired by Dr VM Katoch, Secretary, DHR and DG, ICMR, and Member of Parliament from Cuttack, Member of Legislative Assembly, Odisha on 31 January 2015.
2. Participated in the State Action Plan meeting of 9 northern states at Chandigarh from 3-4 February 2015. The meeting was organized by the NVBDCP to Review progress and programme implementation plan (PIP) of these states.
3. Participated in the launching of UNIDO and UNEP/GEF project on Development and promotion of non-POP alternatives to DDT at UN House, New Delhi on 25 August 2015.
4. Attended Parliamentary Standing Committee meeting in the Parliament House Annexe on 23 September 2015 to discuss government preparedness to control vector borne diseases in general and outbreak of dengue in particular in the country.
5. Participated in the Expert Group meeting for the Use of adulticides and larvicides for the control of vector borne diseases held at NVBDCP, New Delhi on 15 February 2016.
6. Participated in the inception workshop on "Development and promotion of non-POP alternatives to DDT" organized jointly by UNIDO and UNEP/GEF at UN House, New Delhi on 25 February 2016.

Valecha N

1. Attended World Cancer Day and Foundation Day celebration at ICPO, Noida on 4 February 2015.
2. Attended meeting with Principal Secretary, Govt. of Uttarakhand in foundation for cooperation and joint action for establishing the Model Rural Health Research Unit (MRHRU) at Dehradun on 13 February 2015.
3. Attended meeting Tracking on resistance to Artemisinin collaboration II (TRACII) preparation meeting at Mahidol University, Bangkok, Thailand on 27 February 2015.
4. Attended meeting on Malaria Policy Advisory Committee (MPAC) held at World Health Organization, Geneva, Switzerland from 5–7 March 2015.
5. Attended meeting for Preparations of Joint commemoration of World Malaria Day 2015 at WHO-Regional Office for South East Asia, New Delhi on 13 March 2015.
6. Attended first meeting of the Regional Technical Advisory Group on Antimicrobial resistance (AMR) at WHO- Regional Office for South-East Asia, New Delhi from 16–17 June 2015.
7. Attended 5th Annual Scientific Advisory Group meeting (SAG) at New York University from 6-7 July 2015 & at University of Washington (Seattle, WA, USA) from 9–10 and 13 July 2015.
8. Attended *Plasmodium vivax* Global meeting and informal consultation on Adaptation of malaria global technical strategy at Oberoi Hotel, New Delhi from 29-31 July 2015.
9. Attended Project Advisory Committee (PAC) meeting for the project proposal entitled "Malarial parasite biology: An avenue to discover new drug targets (Phase-II)" at DBT, New Delhi on 28 August 2015.
10. Attended 19th meeting of the group under the chairmainship of Union Secretary (H & FW) to mandate the Use of DDT for the year 2015–16 at Nirman Bhawan, New Delhi on 1 September 2015.
11. Attended Malaria Policy Advisory Committee (MPAC) meeting held at the World Health Organization, (HQs), Geneva, Switzerland from 16-18 September 2015.
12. Attended meeting on Medicine for Malaria Venture (MMV) ESAC from 5–7 October 2015 and Product Management Advisory Committee (PMAC) at Geneva, Switzerland from 8–9 October 2015.
13. Attended Launch of state-level disease burden

initiative in collaboration with the Ministry of Health and Family Welfare at PHD House, New Delhi on 12 October 2015.

14. Attended Panel discussion on "Research and development needs for neglected tropical diseases in South Asia" organized by DNDi at India International Centre, New Delhi on 15 October 2015.
15. Attended Review meeting of the CCMP at Le Meridien Hotel, New Delhi from 17–18 November 2015.
16. Attended Workshop on multi-institutional synergy approach for pesticide-related issues in India at AIIMS, New Delhi on 21 November 2015.
17. Attended an Inter-academy panel meeting to prepare a vision document - A Road Map on 'Women in Science' in India at NIPGR, New Delhi on 23 November 2015.
18. Attended Expert group meeting on Malaria diagnostics and chemotherapy at NVBDCP, Delhi on 27 November 2015.
19. Attended first meeting of in-house sub-committee to consider the norms for Recruitment of project staff at ICMR (HQs), New Delhi on 23 December 2015.
20. Attended workshop on Malaria vector insecticide resistance monitoring and management in India jointly organized by NIMR and NVBDCP at Welcome Hotel, New Delhi on 10 December 2015.
21. Attended meeting for submission of report of "National list of essential medicines 2011" at Nirman Bhawan, New Delhi on 9 December 2015.
22. Attended Ethical Committee meeting of BL Kapur Memorial Hospital at BLK Hospital, New Delhi on 12 December 2015.
23. Attended public debate on Body Burden 2015: An effort to start the conversation and find solutions at India Habitat Centre, New Delhi on 15 December 2015.
24. Attended Selection Committee meeting to award for Post-doctoral fellowship (PDF) -12th Batch at ICMR (HQs), New Delhi on 21 December 2015.
25. Attended Brainstorming meet on the National framework for malaria elimination in India (2016-30) at Lalit Hotel, New Delhi on 21 December 2015.
26. Attended DBT-PAC meeting for reviewing the proposal "Malarial parasite biology: An avenue to discover new drug targets" at ICGEB, New Delhi on 22 December 2015.

10.4 Awards/Honours/Nominations

Dr Alex Eapen

- Selected as 'Chartered Biologist (CBiol)' by the Royal Society of Biology, London, UK.
- Selected as Fellow of 'Royal Entomological Society (FRES)', UK.

Dr Neena Valecha

Received ICMR's Dr MOT Iyengar Memorial Award from Honorable Union Minister for Health & Family Welfare, Shri J.P. Nadda.

10.5 Books/Manuals edited/published

- Dr Raghavendra K. contributed in the preparation of *"Operational Manual for Integrated Vector Management in India"*, 2015, published by the Directorate of National Vector Borne Disease Control Programme (NVBDCP), New Delhi.
- Dr Raghavendra K. contributed in the preparation of *"Operational Manual for Malaria Elimination in India"*, 2016 Version 1, published by the Directorate of National Vector Borne Disease Control Programme (NVBDCP), New Delhi.
- Dr Raghavendra K. (Ed.) Proceedings: *Global Meet of Biologists 2015: Review Articles* 2015. National Journal of Life Science (Special issue) Vol *12*(3).

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संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

11

संस्थान में वर्ष 2015-16 के दौरान राजभाषा अधिनियम के अनुपालन के उद्देश्य से राजभाषा हिन्दी के प्रगामी प्रयोग को बढ़ावा देने हेतु कई कदम उठाए गए जिसके अंतर्गत तिमाही बैठकों का नियमित रूप से आयोजन किए जाने के साथ ही, *मलेरिया पत्रिका* (हिन्दी) का प्रकाशन किया गया एवं राजभाषा विभाग द्वारा लागू प्रोत्साहन योजनाएं कार्यान्वित की गईं जिसके अन्तर्गत निदेशक महोदया द्वारा लागू की गईं अधिक शब्द सीमा की प्रोत्साहन योजना जारी रही एवं संस्थान के प्रवेश स्थल पर प्रतिदिन एक नया अंग्रेजी-हिन्दी शब्द व सुविचार लिखने की गतिविधि इस वर्ष भी जारी रही जो कि राजभाषा के प्रति रूचि जागृत करने का प्रयास है।

इसके साथ ही, प्रतिवर्ष की भांति इस वर्ष भी हिन्दी पखवाड़ा दिनांक 14 से 23 सितम्बर 2015 तक पूर्ण उत्साह के साथ मनाया गया जिसमें जहां एक ओर दिनांक 15 सितम्बर 2015 को प्रशासनिक वर्ग के अधिकारियों एवं कर्मचारियों के लिए अर्द्धदिवसीय हिन्दी कार्यशाला का आयोजन किया गया जिसका उद्घाटन डॉ. नीना वलेचा, निदेशक तथा संचालन श्री सी.एस. नम्बूदिरि, प्रशासन अधिकारी द्वारा किया गया। वहीं दूसरी ओर महत्वपूर्ण गतिविधि, पुरस्कार वितरण समारोह का आयोजन था, किंतु इसी के साथ निबन्ध प्रतियोगिता, वाद-विवाद (कर्मचारी), वाद-विवाद (अधिकारी) एवं टिप्पण प्रारूपण प्रतियोगिताओं का आयोजन भी किया गया।

इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 23 सितम्बर 2015 को एक और गतिविधि पुरस्कार वितरण समारोह का आयोजन किया गया जिसका संचालन सहायक निदेशक (रा.भा.) डॉ. वंदना शर्मा द्वारा किया गया। इस समारोह में दक्षिणी दिल्ली नगर निगम के माननीय महापौर श्री सुभाष आर्य जी को मुख्य अतिथि तथा डॉ. राजीव कृष्ण सक्सेना, उपकुलपति, साउथ एशियन विश्वविद्यालय को सम्मानित अतिथि के रूप में आमंत्रित किया गया था जो कि एक वैज्ञानिक होने के साथ ही एक कवि भी हैं।

इसके साथ ही संबंधित समारोह को रोचक बनाने एवं राजभाषा के प्रति रूचि जागृत करने के उद्देश्य से राष्ट्रीय-स्तर के कवि श्री दीपक गुप्ता को भी आमंत्रित किया गया था। इस समारोह का शुभारंभ मुख्य अतिथि, सम्मानित

हिन्दी कार्यशाला का शुभारंभ करती निदेशक महोदया

संबोधित करती हुई निदेशक डॉ. नीना वलेचा

मुख्य अतिथि श्री सुभाष आर्य जी संबोधित करते हुए

डॉ. बी.एन. नागपाल धन्यवाद ज्ञापित करते हुए

अतिथि, कवि और संस्थान की निदेशक महोदया को पुष्प भेंट कर किया गया। स्वागत समारोह के पश्चात् संस्थान के डॉ. अरूण शर्मा द्वारा क्रमशः माननीय अतिथि, सम्मानित अतिथि एवं कवि महोदय को शॉल भेंट कर सम्मानित किया गया और संस्थान की निदेशक महोदया द्वारा सभी अतिथियों को स्मृति चिन्ह प्रदान किए गए। इसके बाद संस्थान की निदेशक महोदया ने अपने संबोधन में सर्वप्रथम हिन्दी पखवाड़े के दौरान होने वाली प्रतियोगिताओं के विजेताओं को बधाई दी और कहा कि राजभाषा की प्रेरणा एवं प्रोत्साहन नीति के अंतर्गत प्रत्येक वर्ष हिन्दी पखवाड़े का आयोजन किया जाता है जिसका उद्देश्य होता है राजभाषा हिन्दी में कार्य करने के लिए प्रोत्साहित करना। राजभाषा हिन्दी में काम करना हमारा संवैधानिक दायित्व है और इस पखवाड़े के आयोजन की सार्थकता तभी है जब हम अपना सरकारी कामकाज अधिक से अधिक हिन्दी में करें। इसके साथ ही निदेशक महोदया ने बताया कि हमारी माननीय महानिदेशक महोदया डॉ. सौम्या स्वामीनाथन द्वारा हिन्दी दिवस के अवसर पर राजभाषा हिन्दी में अधिकाधिक कार्य करने की अपील की गई है।

निदेशक महोदया के संबोधन के पश्चात् पुरस्कारों का वितरण किया गया। तत्पश्चात् संस्थान द्वारा आमंत्रित सम्मानित अतिथि डॉ. राजीव कृष्ण सक्सेना ने सभा को संबोधित करते हुए बताया कि वैज्ञानिक होते हुए भी उनकी रूचि सदैव से ही हिन्दी में रही है। उन्होंने साहित्य को समर्पित अपनी वेबसाइट 'गीता-कविता' के बारे में भी बताया। उनके द्वारा श्रीमद् भागवत गीता के 700 संस्कृत श्लोकों का अनुवाद छंदों में 'गीता काव्य माधुरी' नामक पुस्तक में किया गया है। उन्होंने कुछ छंदों का कविता पाठ कर सभी को भाव-विभोर कर दिया।

इसके पश्चात् मुख्य अतिथि श्री सुभाष आर्य जी ने सभा को संबोधित कर राजभाषा हिन्दी पर चर्चा करते हुए बताया कि हिन्दी भाषा ही एक मात्र वैज्ञानिक भाषा है जिसमें जैसे बोला जाता है वैसे ही लिखा जाता है। हिन्दी भाषा अभिव्यक्ति का सशक्त माध्यम है और यह भाषा सभी भाषाओं की शब्द संपदा का समावेश करने की क्षमता रखती है। उन्होंने ऐसी समृद्ध भाषा का अधिकाधिक प्रयोग करने हेतु सबको प्रेरित किया।

संबंधित पुरस्कार वितरण के पश्चात् आमंत्रित हास्य कवि श्री दीपक गुप्ता ने विभिन्न समसामयिक विषयों पर अपनी हास्य एवं व्यंग्यपूर्ण कविताओं से आनन्द-विभोर कर दिया। अंततः कार्यक्रम का विधिवत् समापन संस्थान के डॉ. बी.एन. नागपाल, वैज्ञानिक 'एफ' द्वारा किया गया।

यहां यह भी बताना उल्लेखनीय होगा कि संस्थान ही नहीं वरन् संस्थान की क्षेत्रीय इकाइयों में भी राजभाषा कार्यान्वयन के प्रति जागृत करने के उद्देश्य से हिन्दी दिवस के उपलक्ष्य में विभिन्न प्रतियोगिताओं का आयोजन किया गया, जिसमें नडियाद, बंगलुरू, जबलपुर एवं गुवाहाटी मुख्य हैं। इस प्रकार यह कहने में कोई अतिशयोक्ति नहीं होगी कि वर्ष 2014-15 के दौरान संस्थान एवं क्षेत्रीय इकाइयों में राजभाषा के प्रयोग को बढ़ावा देने हेतु सृजनात्मक, रचनात्मक एवं व्यावहारिक कार्य एवं कार्यक्रमों के माध्यम से हर संभव प्रयास किया गया। संस्थान एक विज्ञानीय अनुसंधान संस्थान होने के साथ ही राजभाषा नियम-अधिनियमों का अनुपालन करते हुए राजभाषा के प्रयोग को बढ़ावा देने में प्रयासरत है और इसका साक्षात् प्रमाण राजभाषा संबंधी गतिविधियों का उल्लेखित सारांश है जो इसके बहुमुखी विकास का प्रतिरूप है।

❑

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12

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12.5 Human Ethics Committee

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12.6 Animal Ethics Committee

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Dr Neena Valecha

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Dr SK Ghosh
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Dr K Raghavendra
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