NIMR

Annual Report
2014–15

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

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Preface

I am happy to present the activities and major achievements of National Institute of Malaria Research (NIMR) in the Annual Report of 2014–15. In line with the changing research needs related to prevention and control of malaria, NIMR has been actively involved in addressing the new challenges.

Working through its widespread network of 10 field stations across the country, diverse researches were carried out in various aspects of malaria and other vector borne diseases, which included vector bionomics and disease epidemiology, molecular and proteomic studies on vector-parasite interactions, insecticide resistance in malaria vectors, phase-III studies for evaluation of LLINs, malaria transmission dynamics, field testing of new vector control tools, studies on population genetic diversity of malaria parasites, drug resistance, efficacy of new antimalarials *etc*.

Vector biology and epidemiological studies were centered in different malaria endemic districts for mapping the distribution and biological attributes of malaria vectors, generation of malaria risk maps in context of climatic change, molecular characterization of novel haemocyte transcript, proteogenomic analysis of salivary microbiome, molecular population genetics of the NADPH cytochrome P450 reductase, phase-III studies for evaluation of NetProtect LLIN, and health impact assessment of various developmental projects. Research in Parasite biology included establishment of amplicon sequencing protocol for studying the complexity of parasitic infections, determination of the subpatent population of protozoan gametocytes and immune response studies on macrophage-T cells interaction in mouse models.

Regarding clinical studies, stress was laid on projects with operational and translational approach. A project was approved for establishment of NIMR as a WHO-recognized laboratory for quality assurance of malaria 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. Additionally, projects for assessing the safety and efficacy of newly introduced artemisinin-based combination therapy—artemether-lumefantrine against uncomplicated malaria, studies on the effect of residual antimalarials in malaria patients and its effect on spread of drug resistant parasites, studies on effective and safe interventions for prevention of malaria in pregnancy and its burden were also undertaken.

Several collaborative projects were undertaken with the support and grants from national and international agencies like National Vector Borne Disease Control Programme, AIIMS, Department of Science and Technology, Department of Biotechnology, Council for Scientific and Industrial Research, WHO Global Malaria Programme, Global Fund for AIDS, TB and Malaria, ParaSight Ltd., London School of Hygiene and Tropical Medicine, Liverpool School of Tropical Medicine, Medicines for Malaria Venture, *etc*.

Towards our constant endeavour to disseminate the outcomes of research in scientific community, the scientists of NIMR published > 50 research papers in reputed journals/ books. They also attended various national and international conferences, workshops, delivered lectures and received awards/fellowships for their valuable contribution in areas of their expertise.

The activities related to human resource development were continued by the Institute through imparting training to various health personnel, VBD consultants, district programme officers, laboratory technicians, research scholars, M.Sc. students *etc*. The institute's scientific publications, *Journal of Vector Borne Diseases* along with *Malaria Patrika* and *Plasmodium* Newsletter were regularly published for disseminating biomedical information generated through research and recent findings about vector borne diseases.

Facilities like malaria parasite bank, insectary and animal house at the institute provided basic support for research in different fields. Other activities included celebration of *Hindi Pakhwada* to encourage the progressive use of Hindi in official work, Information Education and Communication programmes, cleanliness and hygiene related activities under *Swachh Bharat Abhiyan* and observance of World Malaria Day.

I take this opportunity to thank all the scientists and staff for their valuable support in all the activities. I sincerely acknowledge the help and guidance of the Secretary, Department of Health Research, Government of India and Director General of the Indian Council of Medical Research and hope for her continuous patronage in future. I also thank the scientists and Publication Division of NIMR for their support in bringing out this report.

Neena Valecha
Director

Executive Summary

Vector Biology & Control

- Entomological surveys carried out to determine the bionomics of *Anopheles* spp. and their sibling species for establishing their role in malaria transmission in Jharkhand state (Ranchi, Giridih and Latehar districts), India revealed that *Anopheles culicifacies* was the most dominant vector species followed by *An. fluviatilis* and *An. stephensi*. The pre-monsoon survey indicated increase in the density of *An. culicifacies* against post-monsoon survey, establishing it the most dominant vector species collected. Density of indoor collection was higher than outdoor in all the districts except Latehar. The sibling species identification through polytene chromosome showed presence of B, C, B/C forms, whereas by molecular method, species complex A and B were also identified in Giridih District.
- A study on the distribution and biological attributes of malaria vectors in malarious villages of District Mewat, Haryana showed prevalence of *An. culicifacies* and *An. stephensi* vectors. Analysis of *An. culicifacies* sibling species composition revealed presence of species A [most prevalent (>95%)], B and C in all the study villages while ecological variants of *An. stephensi*, showed prevalence of "Type form".
- A study aimed for identification of sibling species of *An. fluviatilis* on the basis of variations in their palpal ornamentation indicated the presence of classical subapical pale band in majority of species T (85%) while species U tended to have broader subapical pale band similar to *An. minimus*.
- Proteogenomic analysis of salivary gland of *An. culicifacies* using high throughput mass spectrometry indicated presence of 117 proteins in different subcellular organs being highest in the cytoplasm and mitochondria. Their putative functional annotation showed that the largest proportion was for the proteins of carbohydrate metabolism pathway followed by cytoskeleton constituents, transporters *etc*. Molecular function was also ascribed to the proteins where almost 52% of the proteins were found to be binding proteins, 14% were oxidoreductases followed by transferases and hydratases.
- A study centred on molecular description of haemocyte transcriptome in *Anopheles stephensi* mosquito to know their basic molecular complexity, by analysis of a total of 13105858 Illumina sequencing reads, revealed that that only 66% of 3025 contigs were significantly homologous to the protein databases with unexpectedly abundant transcripts encoding hypothetical proteins with unknown functions.
- A study for molecular characterization of novel haemocyte transcript encoding Ninjurin based on comprehensive molecular and functional genomics approach, identified and annotated two (large and small) full transcripts encoding putative Ninjurin like proteins *AsNinj*L and *ASNinj*S. Further the possible role of mosquito ninjurin (*AsNinjL*) in haemocyte mediated cellular immune response was also characterized and predicted. Molecular modeling analyses provided crucial information about the key residues of ninjurin proteins, for further *in vitro* and *in vivo* functional analysis.
- Molecular analysis of Holotricin like antibacterial protein led to an identification

of a putative transcript, encoding 124 amino acid long full length protein, which yielded 54% identity ($2e^{-025}$) to Holotricin-3 from *Holotrichia dimophalia* (BAA02889.1). Phylogenetic analysis of Holotricin, showed poor match to almost all the insects (35-40% identity/$1e^{-02}$), except to *An. stephensi*, yielding >98% identity to the sequence assembly database.

- A study on comparative assessment of the salivary microbiome of the laboratory-reared *An. culicifacies* and *An. stephensi* mosquitoes revealed that salivary microbiome of *An. culicifacies* is unusually more diverse than *An. stephensi* (with limited overlapping community), predominated by uniquely associated distinct microbial population, e.g. *An. culicifacies* is dominated by Proteobacteria (~42%), while *An. stephensi* harbors Actinobacteria (~70%) which might be due to differential feeding adaptation and engagements such as food acquisition, ingestion and digestion processes, a knowledge which may guide future investigation to better understand the feeding associated molecular relationships and design vector management strategies.
- Molecular population genetics of the NADPH cytochrome P450 reductase (CPR) gene (widely known to confer insecticide resistance) in *An. minimus* collected from 10 different locations (eight Indian, one Thai and one Vietnamese) indicated that the *An. minimus* mosquitoes sampled in the two Southeast Asian localities contain several genetic characteristics of being parts of the ancestral population with low genetic diversity and no evidence of natural selection in the gene.
- A study conducted to know the impact of thermal conditions on survival of mosquito vectors revealed that earthen pots are most productive in terms of developmental period of larva to pupa, *i.e.* 6.5 days as compared to 7.2 days in plastic containers and 7.5 days in iron containers.
- The preliminary findings of a study conducted for determining the impact of rainfall on development and survival of anopheline larvae, undertaken in selected sites of District Baghpat, Uttar Pradesh and northeast district Delhi indicated that, the larval density of *An. culicifacies* reduced drastically with 40 mm rainfall.
- An analysis of study meant for control of dengue and chikungunya by preventing *Aedes* breeding in key containers in pre-monsoon season in west endemic zone of Delhi revealed that proper intervention in non-transmission season reduces *Aedes* breeding container and subsequently breeding and dengue cases in transmission season. Municipal Corporations of Delhi accepted the finding of the study and the Hon'ble Health Minister directed the MHO-MCD that the domestic breeding checkers (DBCs) should continue breeding survey throughout the year rather than 8 months, *i.e.* April to November. Indian Council of Medical Research, New Delhi appreciated the study and decided to circulate the findings of the study to NVBDCP and other ICMR institutes working on dengue and chikungunya.
- Phase-III studies for evaluation of NetProtect LLIN (impregnated with deltamethrin) against malaria vectors in the states of Haryana, Uttar Pradesh and Jharkhand were continued for the second year of the trial as per common protocol. Studies on human safety of nets showed that the nets were found to be safe and nothing adverse was reported that may be attributed due to the use of these nets by the community.
- A WHO project for assessing 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 revealed that the major vector species, *An. culicifacies* was mostly resistant to DDT and malathion, but variably susceptible to deltamethrin (pyrethroid) and bendiocarb (carbamate) with minimal/transient adverse effects to LLINs compliance. Studies on behavior of *An. culicifacies*, sibling species composition, biochemical and molecular mechanisms of resistance are under process.
- Studies for development of methods/strategies

for the management of pyrethroid resistance in malaria vectors in India using insecticides with novel mode of action has helped in designing criteria based on common biotransformation route towards selection of insecticides for use in rotation/alteration. The study also helped to identify insecticides from different class that can be used for insecticide resistance management for vector control in consideration, its cross-resistance with insecticides from the same/different class, mode of action. Topical assay to assess the intrinsic chemical toxicity for chlorfenapyr, DDT, deltamethrin, malathion and permethrin insecticides showed that pyrethroid resistant strain of *An. stephensi* had lesser LD_{50} and LD_{90} values to chlorfenapyr than the susceptible counterpart indicating an increase of intrinsic toxicity to chlorfenapyr in pyrethroid resistance strain and thus may be used for management of pyrethroid resistance.

- Studies conducted to assess the irritability due to chlorfenapyr, DDT, malathion, deltamethrin and permethrin; and intrinsic toxicity of chlorfenapyr in multiple insecticide-susceptible and -resistant laboratory strains of *Anopheles stephensi* showed that chlorfenapyr molecule exhibited least irritant effect against susceptible and resistant strains among all the insecticides tested allowing more landing time to the vector species on the impregnated surfaces to pick up lethal dose. Thus, chlorfenapyr could be an ideal insecticide for management of multiple-insecticide-resistance including pyrethroids.
- Furthering the study on insecticide susceptibility of *Aedes aegypti* in Delhi (which reported presence F1534C mutation during the last year) led to an identification of a novel knockdown resistance (*kdr*) mutation- T1520I in the population. Genotyping of F1534 and T1520 alleles revealed a high frequency of the F1534C mutation (0.79) and a very low frequency of the T1520I mutation (0.13). It was observed that T1520I mutation was found in individuals having the 1534C allele only, but never with wild type F1534, inferring that 1520I is linked to 1534C.
- A study in which association of F1534C-*kdr* mutation with insecticide resistance in *Aedes aegypti* was tested using dominant, recessive and additive models showed that F1534C-*kdr* conferred greater protection against DDT with all models and highest protection was shown using the recessive model; while lower protection was shown against deltamethrin when fitted with recessive or additive models. However, F1534C-*kdr* did not show significant protection against permethrin.

Parasite Biology

- To understand the origin and spread of virulent and drug-resistant forms of the malaria pathogen the genetic diversity and evolutionary history of Indian isolates of *Plasmodium falciparum* was estimated from mitochondrial genome sequence analyses which presented high genetic diversity with several characteristics of ancestral populations sharing many of the genetic features with African and to some extent Papua New Guinean (PNG) isolates. It was observed that one of the four single nucleotide polymorphisms (SNPs) that differentiate *P. falciparum* from *P. falciparum*-like isolates (infecting non-human primates in Africa) was segregating in five Indian *P. falciparum* isolates (which were named as *PfIndia*), inferring the probable host-switch events of the *P. falciparum* from Indian non-human primates.
- A molecular study conducted in the Cameroonians malarial patients in order to confirm if they are also susceptible to *P. vivax* infection showed molecular evidence of *P. vivax* mono and mixed malaria parasite infections in Duffy-negative native Cameroonians adding our knowledge to the growing evidences of *P. vivax* infection in Duffy-negative Africans.
- To study the complexity of infection in *P. vivax* populations an amplicon sequencing protocol on the Ion Torrent PGM platform was established for targeted re-sequencing of multiple genomic regions in field isolates which helped to discover mixed genotype populations within a patient facilitating understanding of dynamics of malaria pathogenicity, complexity of infection and drug resistance.

- Molecular characterization studies of novel plasmodium proteins [4-Diphosphocytidyl-2C-methyl-D-erythritol kinase (IspE) and Phosphoethanolamine methyltransferase (PMT)] which are essential for parasite growth and development showed that they have potential to act as an excellent antimalarial drug target candidates. Further studies are in progress.
- Structural and molecular study on interactions of macromolecular substrate (haemoglobin) and inhibitor with malarial cysteine proteases (Falcipains) suggested that haemoglobin interacts with Falcipain-2 (FP2) via specific interactions and Val^{187} and Glu^{185}, present at the C-terminus of FP2, are essential for haemoglobin binding; and multimeric units(10 mers) of Falstatin (macromolecular inhibitor) interacts with 10 molecules of FP2 in a 1:1 stoichiometric ratio instead of using only a BC loop as reported earlier.
- A study was conducted to determine the subpatent population of *P. falciparum* gametocytes in their freshly cultured clinical isolates and assessment of their ability to produce gametocytes in natural infections, and correlation of the expression of *Pfs25* gene in the isolates.
- It was observed that isolates which produce mature gametocytes *in vitro* also showed an increase in the *Pfs25* gene expression compared to the reference strain which suggested that a correlation exists between *Pfs25* gene expression and the gametocyte (mature) production ability by the isolate. The results indicated that *Pfs25* gene has a role to play in the process of gametocytogenesis in field isolates and disease transmission.
- Immune response studies on macrophage-T cells interaction in mouse model of malaria during lethal malaria parasite infection (*P. berghei*) and non-lethal parasite (*P. chabaudi*) in context with pro-inflammatory and anti-inflammatory cytokine revealed that IL-12, a pro-inflammatory cytokine produced mainly by professional antigen presenting cells (APCs), decreased initially in *P. berghei* infection while its production increased in case of *P. chabaudi*. Similarly the production of IL-6 in *P. chabaudi* infection increased till Day 7 post-infection and decreased thereafter in both *P. berghei* and *P. chabaudi*. However, much higher IL-6 was induced in *P. chabaudi* than that of *P. berghei*. Further studies are in progress.
- A study conducted to identify the molecular marker to understand the genotypes of *P. vivax* and their relation with relapse and new infection revealed that minisatellite markers from chromosome number 1 and chromosome number 9 are highly polymorphic in nature suggesting to imply for the relapse and new-infection.

Epidemiology

- An international and multi-institutional Indo-Danish collaborative project was initiated for establishing immunological correlates of protection against malaria vaccine candidates in high and low transmission malaria endemic regions of Jharkhand and Haryana, India. Compilation of the demographic details of the study area was completed, and the age and exposure-related immuno-epidemiological profiling of IgG reactivity against malaria vaccine candidates was finalized. Malaria prevalence surveys showed that the parasite rate in these areas was 2.8 and 46.6%, respectively. Further studies on the malaria transmission dynamics are in progress.
- 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, a Comprehensive Case Management Pilot programme (CCMP) was implemented collaboratively by the Government of Odisha, National Institute of Malaria Research and Medicines for Malaria Venture. It has led to significant increase in access to diagnosis and treatment in all the intervention areas and has shown expected results in low endemic blocks which further helped in controlling outbreak and preventing complications in other blocks. Assessment of impact in different transmission settings is underway.
- Entomological, parasitological and microbiological surveys undertaken to assess the health impact of Narmada Basin Dams and

Sardar Sarovar Project in Madhya Pradesh and Rajasthan, respectively identified the major mosquito breeding habitats and microbes present in the drinking water samples. Subsequently detailed recommendations and mitigating measures, *i.e.* improvement in drainage system, cleanliness and awareness, de-weeding, introduction of larvivorous fishes, channelization of pools in main river and larvicidal spray were suggested to health authorities to control the breeding.

- A spatio-epidemiological study of dengue in Delhi was undertaken to investigate and evaluate the epidemiology of dengue infection and to estimate the rate of asymptomatic and symptomatic dengue infection in Delhi. In all the localities surveyed during transmission season, solid waste was observed to be most preferred breeding site, whereas overhead and curing tanks were found to be the most preferred breeding containers during non-transmission season. Plastic containers in low income group, solid waste and plastic containers in medium income group, and solid waste and curing tanks in high income group were found as the most preferred breeding containers for *Ae. aegypti*.
- A study initiated for isolation of dengue virus from *Ae. aegypti* in Delhi on the request of Municipal Corporation of Delhi, New Delhi Municipal Corporation and Delhi Administration, showed that out of 18 study areas, 11 localities were positive for dengue virus infection with low income group showing highest mosquito infectivity followed by medium and high income groups. No vertical transmission of dengue virus was detected. Further, studies in non-transmission season would help in making dengue control strategies in Delhi.
- A multidisciplinary, multicentric and multi-institutional study for generation of evidence of change in climatic conditions on anopheline vectors and malaria, so as to suggest adaptation measures for addressing the adverse impact of climate change was undertaken in the northeastern states of India. The overall findings revealed that climatic conditions are changing as compared to 1960 and 1990 and hilly areas are showing evidence of malaria transmission even in the months of November and January when outdoor temperature is not conducive. Current adaptation measures in practice at the study sites were assessed and scope of capacity building and strengthening of health system were identified for addressing the adverse impact of climate change.
- A study was initiated to generate risk maps of malaria in India from the viewpoint of malaria prevalence, climatic determinants, anopheline vector's distribution and ecological risk. Maps of hot-spots of malaria based on temperature and malaria endemicity provided insight that linearly progressing temperature is not likely to cause increase in high intensity of malaria. The cut-off range of rainfall determined for causing outbreak varied from 100-500 mm with varying lag period, *i.e.* month to month to three months lag. The ecological risk at village level determined one month before malaria peak in three study sites using supervised classification identified many high and few low malaria potential risk sites. Work is in progress.
- A study was undertaken for malaria outbreak investigation in Tripura. The findings of investigation of malaria outbreak were compiled and analyzed in consultation with national programme. Additionally the impact of satellite derived temperature condition index (TCI) and vegetation condition index (VCI) in exacerbating the outbreak was also analyzed. The role of TCI in the month of April was found crucial in outbreak.

Clinical Research

- A project was approved for establishment of NIMR as a WHO-recognized laboratory for quality assurance of malaria 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. Till date, 41 panels have been prepared including 25 *P. vivax* and 16 *P. falciparum* panels as per the current SOPs of WHO. The project has helped to build and incorporate an improved Quality Assurance programme of malaria RDTs. Lot-testing of 10 RDT lots was conducted using

the WHO SOPs. The RDTs were received through the national programme and regulatory authorities of India for lot-testing.

- To assess the safety and efficacy of newly introduced Artemisinin-based combination therapy— Artemether Lumefantrine (ACT-AL) against uncomplicated *P. falciparum* malaria, studies were initiated in April 2014 at three sentinel sites of northeast states spread across international borders of the country which showed that the efficacy of ACT-AL ranged between 88.4 -100% at the sites.
- Mutation analysis in *pfmdr1*gene done in the samples revealed that the majority of the samples were wild type (57.5%) followed by mutant (26.8%) and mix type (13%) pattern. The 76T mutation in *Pfcrt* gene was also observed in higher frequency being highest in Changlang district in Arunachal Pradesh.
- A study was conducted in collaboration with the London School of Hygiene and Tropical Medicine for effective and safe interventions for prevention of malaria in pregnancy and its burden in three districts of Jharkhand state. The results of the interim analysis of data of women enrolled and delivered showed that intermittent screening and treatment (IST) is beneficial in comparison to passive case detection (PCD). It was proposed that routine and improved antinatal care (ANC) services may increase ANC coverage and improve disease surveillance, thereby reducing the burden of malaria in pregnancy.
- A study was conducted to explore the biochemical and molecular aspects of Glucose-6-phosphate dehydrogenase (G6PD) deficiency and pattern of excessive haemolysis in malaria positive G6PD deficient patients treated with few antimalarial drugs like primaquine. Mutational analysis of the samples from Mizoram and Uttar Pradesh showed wild type pattern for G6PD mediterranean variant and G6PD Odisha variant. Haemolysis at normal dose with primaquine was observed in very few samples but percent haemolysis increased with increasing dose; however more observations are required to confirm the dose dependent increase.
- A study was conducted to assess artemisinin resistance in uncomplicated falciparum malaria at malaria endemic sites (Odisha and Arunachal Pradesh) of India. The results indicate that real time PCR can be deployed as an additional and useful tool in artemisinin resistant studies and that the drug should be preserved effectively for delaying resistance and combating malaria.
- Studies on the effect of residual antimalarials in malaria patients and its effect on spread of drug resistant parasites in high malaria endemic districts in India showed that patients with residual levels of sulphadoxine and chloroquine on Day 0, showed higher frequency of mutation in *pfdhps* and *pfcrt* genes as compared to patients without residual levels on Day 0, suggesting that the residual levels of antimalarials encourage the emergence and spread of drug resistant parasites.

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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 state, India

Extensive entomological surveys were carried out in all the primary health centres (PHCs) of three districts of Jharkhand, namely Ranchi, Giridih and Latehar during 2014–15. Villages falling in different ecological settings like foothill, plain, urban, forest, riverine, stream, dam *etc*. were selected for the study. During pre-monsoon (February–March 2015) both adult and immature mosquitoes were collected using hand catch, total catch, light- trap, evening collection, landing collection, larval collection from indoors/outdoors and human dwellings/cattlesheds by standard WHO techniques.

In post-monsoon (September–October 2014) survey among the *Anopheles* spp. in the three districts, *An. culicifacies* (24.4%) was found to be the most dominant vector species followed by *An. fluviatilis* (0.06%) and *An. stephensi* (0.02%). Among other species *An. vagus* (24.5%), *An. annularis* (15.8%), *An. pallidus* (6.2%), *An. nigerrimus* (5.8%), *An. barbirostris* (0.11%), *An. jeyporiensis* (0.01%), and *An. splendidus* (0.07%) were recorded.

Species collected from cattlesheds were found significantly higher than human dwellings in all the villages of three districts. Light-trap collection showed that the number of species collected indoor were higher than outdoor in all the districts, however, higher density of outdoor collection was found in Latehar district. The pre-monsoon survey indicated increase in the density of *An. culicifacies* (36.8%) against post-monsoon survey, establishing it the most dominant vector species collected. Similarly, higher density was observed for *An. fluviatilis* (16.7%). Among other species, *An. annularis* (30.14%), *An. splendidus* (6.9%), *An. pallidus* (4.4%), *An. jeyporiensis* (2.2%), *An. nigerrimus* (1.7%), *An. vagus* (0.25%) and *An. barbirostris* (0.091%) were recorded.

Biting time of *An. culicifacies* was recorded from 2000 to 0200 hrs, whereas *An. fluviatilis* preferred to bite from 2100 to 0100 hrs in selected villages. Larvae of *An. fluviatilis* and *An. culicifacies* were collected from streams, river pits and riverbed pools, whereas larvae of *An. annularis* were recorded from ponds and dam margins in all the three districts.

The sibling species identification through Polytene Chromosome showed presence of B, C, B/C forms, whereas by molecular method, species complex A and B were also identified in Giridih district of Jharkhand. Analysis of data for the survey carried out in February–March 2015 in all the three districts is under progress.

1.1.2 Entomological investigation related to malaria vectors in epidemic prone Nuh CHC of District Mewat, Haryana

A study was initiated on the distribution and biological attributes of malaria vectors in malarious villages under PHC's Ujina and Nuh. Field surveys revealed rain-fed perennial ponds, low-lying areas filled with seepage water and cemented water storage tanks as major breeding habitats in and around these villages (Figs. 1 a-e).

As a result both *An. culicifacies* and *An. stephensi* were found prevalent in the study villages. Both the vectors were found almost in equal proportion, however, in some villages *An. stephensi* outnumbered *An. culicifacies* (Fig. 2). Analysis of *An. culicifacies* sibling species composition using cytotaxonomy and PCR assay revealed prevalence of species A, B and C. Species

Figs. 1(a-e). Breeding habitats of vector anophelines in the study area; (a) Perennial pond; (b) Low-lying area with seepage water; (c–e) Cemented water storage tanks.

A was predominant in all the study villages constituting >95% of *An. culicifacies* population and was found polymorphic for i^1 inversion. For

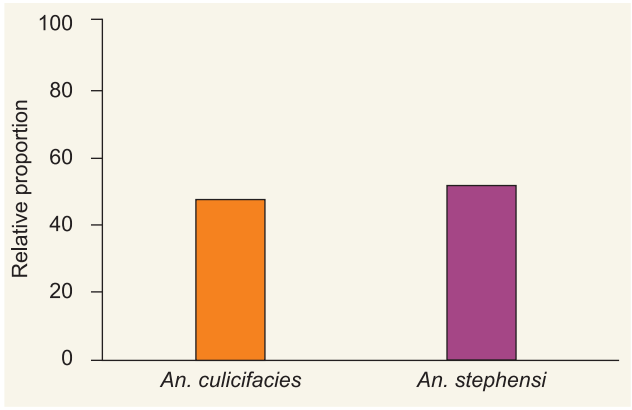


Fig. 2: Prevalent malaria vectors in the study villages.

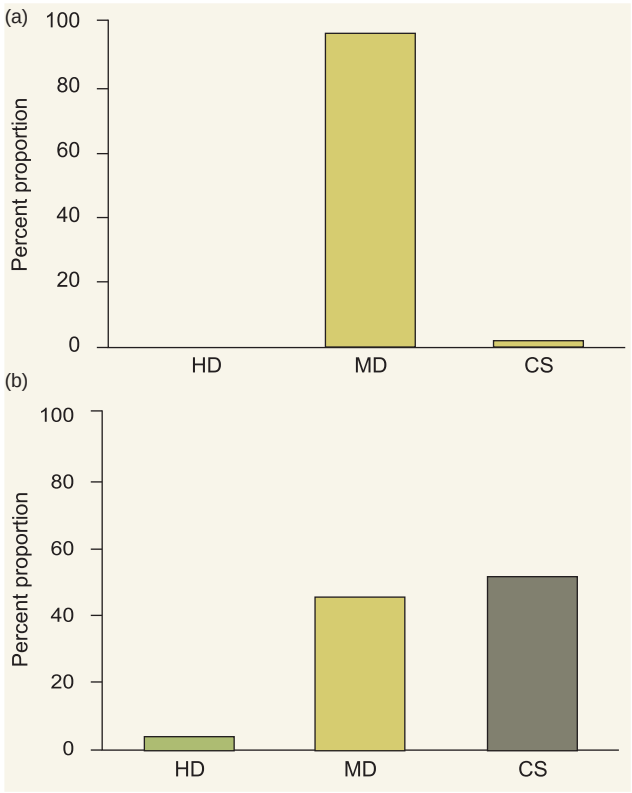


Fig. 3: Preferred resting sites of malaria vectors—(a) *An. stephensi*, and (b) *An. culicifacies*. (HD-Human dwellings; MD-Mixed dwellings; CS-Cattlesheds).

ecological variants of *An. stephensi,* single female cultures were established in the laboratory which showed prevalence of "Type form" on the basis of ridge number on the egg float.

The preferred resting sites for both the vectors were cattlesheds/mixed dwellings (Fig. 3) and blood meal source analysis using counter current immunoelectrophoresis showed that these vectors are primarily zoophagic. Longitudinal study is underway to know the seasonal variations in the prevalence of malaria vector species, their transmission potential and response to insecticides used in public health.

1.1.3 Variations in Palpal ornamentation of *Anopheles fluviatilis* species T and U (Diptera: Culicidae)

Anopheles fluviatilis sensu lato James is a highly efficient malaria vector in Indian subcontinent and Iran comprising of at least four sibling species. An important morphological characteristic feature for the differentiation of this species complex from the *Minimus* Complex is the ratio of length of subapical pale band to dark band intervening apical and subapical pale bands, present on the maxillary palpi of female mosquito. This study report variation in the subapical pale band in *An. fluviatilis*, specially in species U, to the extent that palpi of some specimens mimics members of *Minimus* Complex, inferring that palpal ornamentation may not be reliable characteristics for the identification of *An. fluviatilis*.

A total of 53 samples were identified for sibling species based on DNA sequences of D3 domain of 28S rDNA (Singh *et al* 2004) of which 44 samples were randomly sampled from F_1 generation of field collected mosquitoes and nine samples were selectively pinned from the field which were suspected to be *An. minimus* based on palpal characteristics. Subapical pale band was classified into three categories based on ratio of its width to dark band intervening apical and subapical pale bands, *i.e.* (i) 'classical' with ratio <1/3, (ii) 'intermediate' with ratio between 1/3 and 3/4, and (iii) 'broader' with ratio >3/4. The distribution of

Table 1. Distribution of ratio of subapical pale band to dark band intervening apical and subapical pale bands in members of *An. fluviatilis* species

		Species T	Species U	Total
Randomly sampled (F_1)	Classical	17 (85)	10 (42)	27
	Intermediate	3 (15)	9 (37)	12
	Broader	0	5 (21)	5
	Total	20	24	44
Selectively sampled (Wild caught)	Broader	0	9 (100)	9
	Grand total			53

Figures in parentheses indicate percentages.

these three categories of subapical pale bands in different sibling species has been shown in Table 1. Species V which was recorded in an earlier study (Nanda *et al* 2013) was absent in this collection. It was observed that classical subapical pale band was present in majority of species T (85%) and 'broader' category was absent. In contrast species U showed wide variation in subapical pale band with 'broader' category present in 21% of individuals (Fig. 4). All the nine pinned specimens (field collected) with 'broader' subapical pale band were found to be species U. Thus, species U tended to have broader subapical pale band as compared to species T. A photograph of palpal ornamentation in species U with 'broader' subapical pale band has been displayed as Fig. 5.

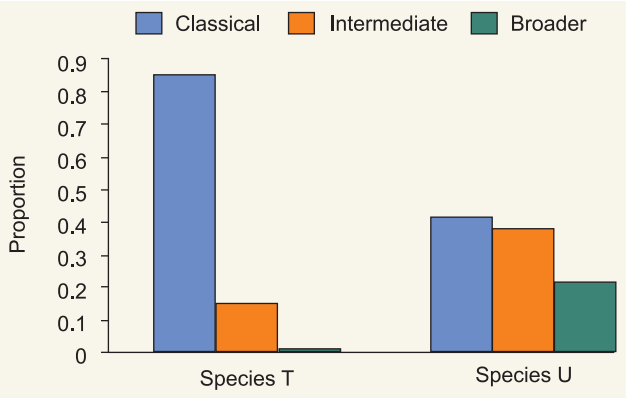


Fig. 4: Proportion of mosquitoes with three different classified categories of subapical pale band in *An. fluviatilis* species T and U.

Fig. 5: Palpal ornamentation in *An. fluviatilis* species U with broader subapical pale band similar to *An. minimus*.

1.1.4 Proteogenomic analysis of midgut and salivary gland of *Anopheles culicifacies* using high throughput mass-spectrometry

Mosquito midgut is an important target for the host parasite interaction studies as it plays a major role in parasite growth and maturation and vector susceptibility. Proteomic approaches coupled with bioinformatics analysis have been used to study the expression of functional proteins/enzymes of *An. culicifacies* susceptible and refractory species midgut in order to understand the mechanism of refractoriness that may help in contributing to unravel the host pathogen interactions.

The study involved proteomics approaches, namely in-solution and in-gel digestion strategies, followed by analysis through LC-MS/MS. Further, bioinformatics analysis was carried out to find out the functional annotated proteins, biological process, molecular function and their sub-cellular location using Gene ontology, SMART analysis, CELLO *etc*. In-solution and in-gel approach coupled with LC-MS/MS identified a total of 91 proteins in susceptible species A and 69 proteins in refractory species B. A total peak spectrum analyzed by m/z values of both susceptible species A and refractory species B is represented in (Fig. 6).

Comparative analysis between susceptible species A and refractory species B of *An. culicifacies* indicated that mainly the proteins involved in proteolysis mechanism, catalytic activity, peptidases activity and immune related proteins were dominating in refractory as compared to susceptible species (Fig. 7). The data/results also reflect significant increase in number of these proteins in midgut of refractory *An. culicifacies* species B indicating that these may be responsible for inhibiting the parasite growth and linked to the melanization of oocysts or parasite lysis mechanisms in natural populations of refractory mosquitoes.

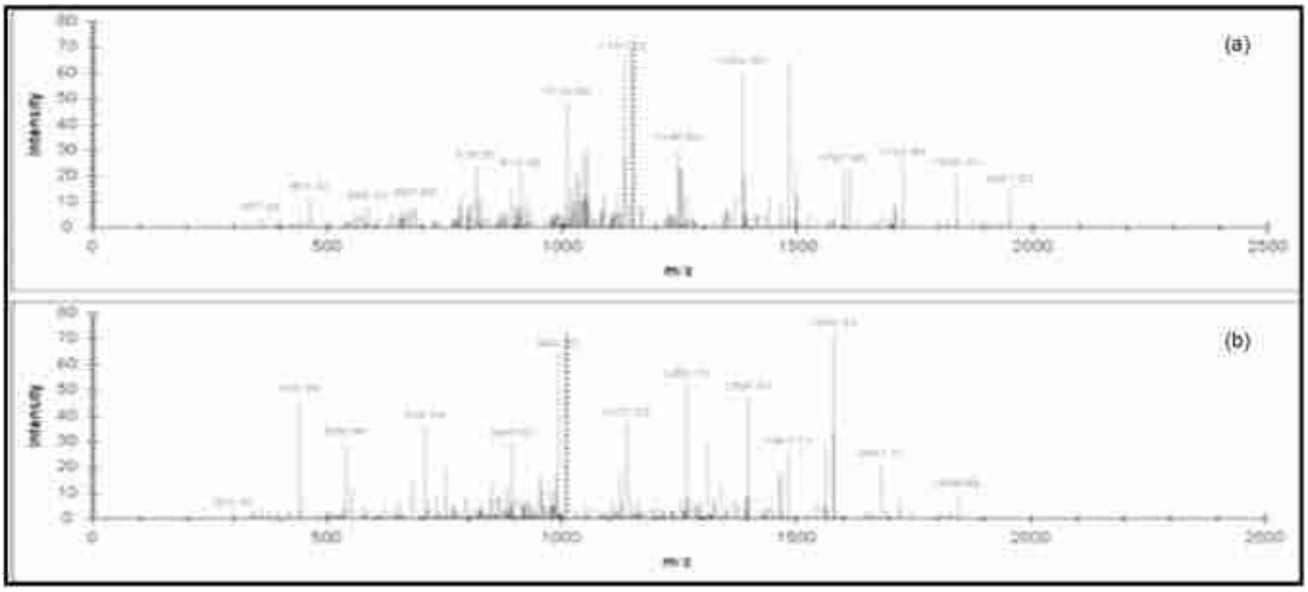


Fig. 6: Representation of total peak spectrum analyzed by m/z values by mass spectrometry: (a) Peak spectrum of susceptible *An. culicifacies* species A midgut proteins; and (b) Peak spectrum of refractory *An. culicifacies* species B midgut proteins.

The progress of these studies at protein level suggests that the identified annotated putative proteins/enzymes may help to explore natural vector-parasite systems and reveal valuable insights into the mechanism of refractoriness which in turn would be useful for bringing out novel strategies for malaria control.

Salivary proteins are directly involved in human-vector contact during biting and, therefore, play a key role in pathogen transmission. Hence, the proteomic study of salivary gland proteins is the first essential step towards understanding the refractory mechanisms and host-parasite relationships. In this post-genomic era, mass-spectroscopy has emerged as a powerful tool for high-throughput analyses of proteomes. The baseline data of salivary gland proteins generated from this study will be highly relevant and serve as the basis of future research work to understand the feeding mechanisms.

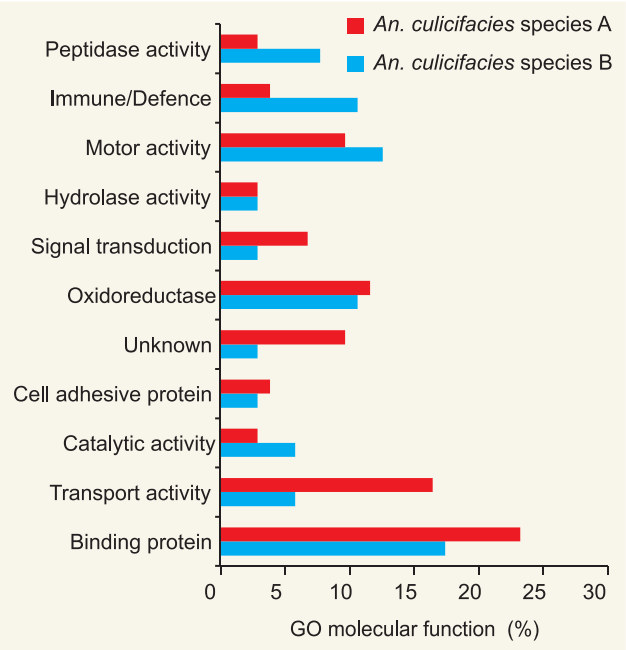


Fig. 7: Depiction of comparative analysis of molecular function identified by gene ontology (GO) between *An. culicifacies* susceptible species A and refractory species B.

The proteome of salivary gland of sensitive *An. culicifacies* species A was characterized using in-solution and in-gel digestion strategies followed by LC-MS/MS and SEQUEST algorithm. In total 117 proteins were identified in the salivary gland dataset out of which 81 proteins were depicted via gel-free approach and 36 *via* in-gel approach and 15 proteins were found to be same in both the profiles. Putative functional annotation was carried out of all the listed proteins by exploiting gene ontology tool. Their subcellular localization was also depicted using GO and CELLO algorithms and proteins were found to be localized at cytoskeleton (9), cytoplasm (29), mitochondria (23), peroxisome (2), unknown (8), nucleus (14), extracellular (9), intracellular (3), endoplasmic reticulum (3), membrane (1), Golgi (1) as shown in Fig. 8(a). The biological process was also assigned for most of the proteins. The largest proportion was for the proteins of carbohydrate metabolism pathway (18%) followed by cytoskeleton constituents (11%),

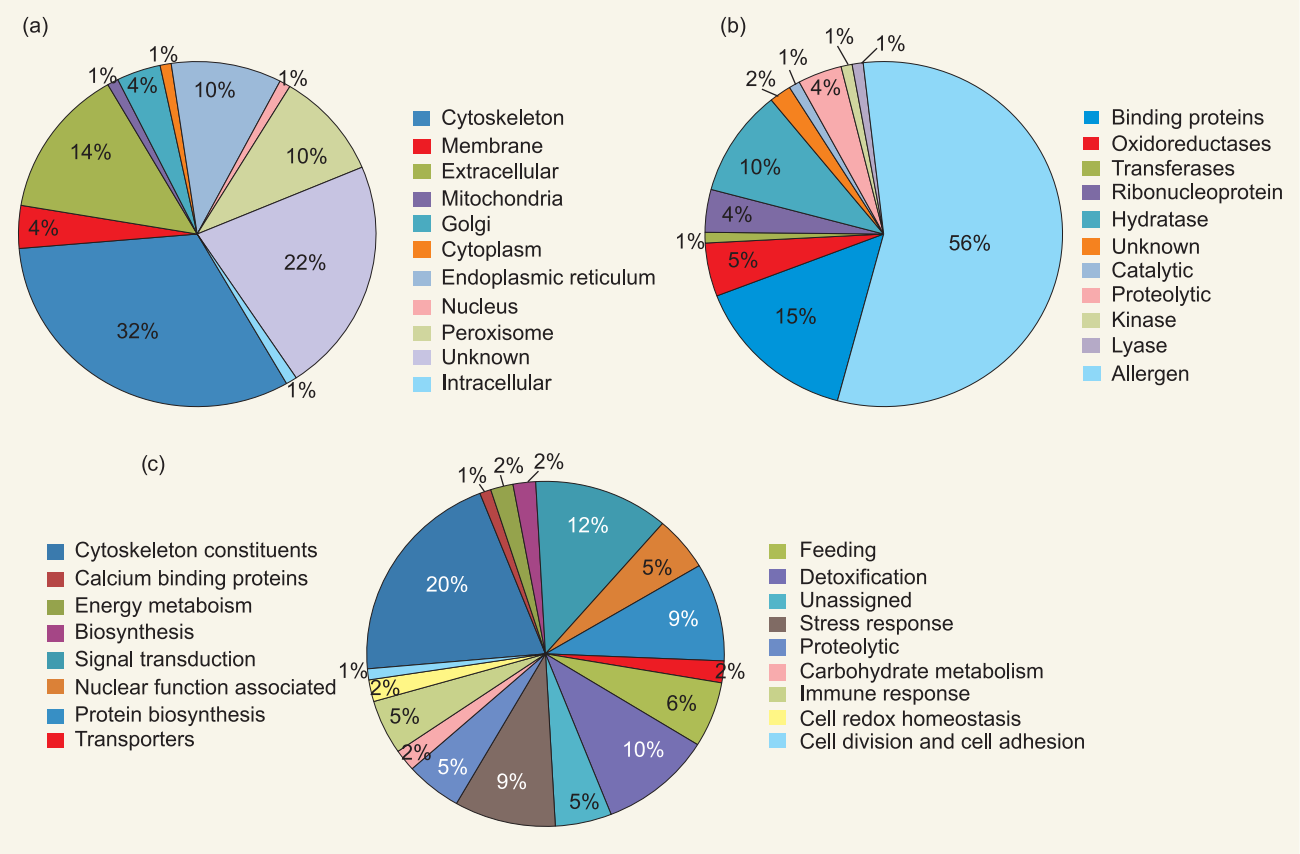


Fig. 8: Depiction of identified salivary proteins of *An. culicifacies* species A using gene ontology tool—(a) Intracellular localization of proteins identified by Q-TOF-MS/MS; (b) Functional (putative) classification of identified known and novel proteins; and (c) GO process.

transporters (9%), nuclear function associated proteins (9%), energy metabolism (8%), feeding (4%), detoxification (4%) *etc*. as detailed in Fig. 8 (b). Molecular function was also ascribed to the proteins where almost 52% of the proteins were found to be binding proteins, 14% were oxidoreductases followed by transferases and hydratases (Fig. 8c).

For *An. culicifacies* species B the salivary gland extract was digested using in-solution and in-gel approach followed by LC-MS/MS and identification of proteins using SEQUEST algorithm. A total of 20 proteins were identified from in-gel approach corresponding to total 23 digested bands (Fig. 9).

Further analysis of biological process, molecular function and their subcellular localization is in process using gene ontology and other bioinformatics approaches. The results can be further extended to elucidate the possible role of identified proteins in salivary gland-parasite interactions during pBM (post blood meal) and will provide important insight towards the development of malaria control strategies.

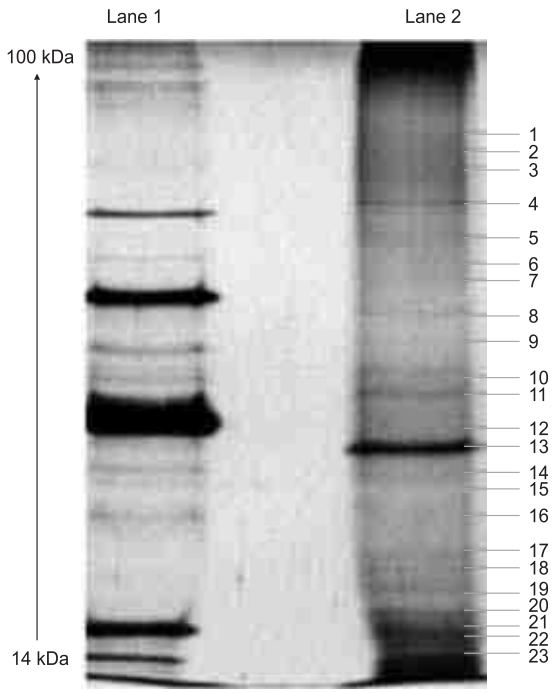


Fig. 9: Salivary gland protein profiling of *An. culicifacies* species B. Silver stained SDS PAGE gel of the midgut extract is shown in Lane 2 and Protein markers with range 14 to 100 kDa shown in Lane 1(M).

1.1.5 Molecular description of haemocyte transcriptome in *Anopheles stephensi* mosquito

Haemocytes are tiny circulating blood cells of the insects known to play multiple roles in physiological and cellular immune responses. Unlike *Drosophila* haemocyte, the biology of adult female mosquito haemocyte is believed to be more complex, especially due to its unique nature of blood feeding, digestion and host suitability for microbial pathogens. Therefore, to know basic molecular complexity of naive mosquito (*An. stephensi*) haemocyte encoded proteins, a total of 13105858 Illumina sequencing reads were analyzed, that could be assembled into 3025 contigs. Only 66% contigs yielded significant homology to the protein databases. Unexpected observation of abundant transcripts encoding hypothetical proteins with unknown functions reflects our limited knowledge over molecular nature and complexity of the mosquito haemocyte functions (Table 2). Ongoing identification of unique transcripts that are involved in immunity, chemo sensing, cell-cell communication, nitrogen fixation/metabolism *etc.* may provide initial evidence that mosquito haemocytes carry unique ability to meet and manage cell-specific diverse functions of the mosquito blood.

Table 2. Molecular catalogue of genes abundantly expressing in the haemocytes of *Anopheles stephensi*. The genes are shortlisted from 244 total genes selected (>5000 read count), with non-constitutive functions

S.No.	Assembled contig	Length	Functional prediction/Domain prediction	Best match to *An. gambiae*	Read count
1.	AsHcSf-contig_2	285	Hypothetical protein HELRODRAFT_178864	–	91091
2.	AsHcSf-contig_4	346	Hypothetical 4.2 kDa secreted peptide	–	43132
3.	AsHcSf-contig_5	283	No significant similarity found	–	12961
4.	AsHcSf-contig_9	591	No significant similarity found	–	24716
5.	AsHcSf-contig_11	1311	No putative conserved domains have been detected	–	69608
6.	AsHcSf-contig_29	1827	No putative conserved domains have been detected	–	54953
7.	AsHcSf-contig_37	497	No putative conserved domains have been detected	AGAP004400	36793
8.	AsHcSf-contig_46	1283	TCTP family	AGAP002667	28886
9.	AsHcSf-contig_50	352	ATP synthase regulation; hypothetical protein ZHAS_00014968	–	21869
10.	AsHcSf-contig_57	412	No significant similarity found	–	14307
11.	AsHcSf-contig_64	636	No putative conserved domains	AGAP000669	21111
12.	AsHcSf-contig_66	745	IscU like family; NifU	AGAP005813	24344
13.	AsHcSf-contig_71	746	Apolipophorin-III and similar insect proteins	AGAP013365-	30243
14.	AsHcSf-contig_74	932	No significant similarity found	–	12652
15.	AsHcSf-contig_82	1594	Myofilin; no putative conserved domains	AGAP004161	28236
16.	AsHcSf-contig_85	632	Salivary anti-thrombin anopheline; ANOPHELIN	AGAP008004	20165
17.	AsHcSf-contig_89	316	Hypothetical protein EGM_09670, partial [Macaca fascicularis]; Unknown	–	5959
18.	AsHcSf-contig_95	325	No significant similarity found	–	6212
19.	AsHcSf-contig_97	455	No putative conserved domains	AGAP012418	11451
20.	AsHcSf-contig_99	2316	ATP synthase alpha/beta family	AGAP005134	29843
21.	AsHcSf-contig_102	716	Putative cytochrome c oxidase subunit	AGAP009526	11578
22.	AsHcSf-contig_110	262	No putative conserved domains	–	5449
23.	AsHcSf-contig_118	625	No putative conserved domains	AGAP003777	15422
24.	AsHcSf-contig_119	1120	No putative conserved domains	–	18693
25.	AsHcSf-contig_124	1903	No putative conserved domains	AGAP003453	8532
26.	AsHcSf-contig_127	2044	Poly adenylate binding protein (5% query cover)	AGAP011092	16271
27.	AsHcSf-contig_131	1123	WD40 domain/Protein kinase c	AGAP010173	21366
28.	AsHcSf-contig_135	489	No significant similarity found	–	7222
29.	AsHcSf-contig_140	1176	No putative conserved domains	–	9102
30.	AsHcSf-contig_144	400	No putative conserved domains	–	6588

(contd...)

(contd.. Table 2)

S.No.	Assembled contig	Length	Functional prediction/Domain prediction	Best match to *An. gambiae*	Read count
31.	AsHcSf-contig_145	381	No putative conserved domains	–	6912
32.	AsHcSf-contig_146	265	No putative conserved domains	–	6020
33.	AsHcSf-contig_150	1003	Peptide methionine sulfoxide reductase	AGAP012395	11499
34.	AsHcSf-contig_154	694	No putative conserved domains	AGAP007208	7742
35.	AsHcSf-contig_155	503	No putative conserved domains	AGAP005888-	7453
36.	AsHcSf-contig_158	736	No putative conserved domains	AGAP003778	7551
37.	AsHcSf-contig_160	1295	Death-associated protein	AGAP011832	7243
38.	AsHcSf-contig_165	1171	Calcium-binding EGF-like domain	AGAP004936	12137
39.	AsHcSf-contig_168	1028	No putative conserved domains	AGAP011317	8076
40.	AsHcSf-contig_170	1190	No putative conserved domains; D7 protein	AGAP006278	14746
41.	AsHcSf-contig_171	691	No putative conserved domains	AGAP003776	7103
42.	AsHcSf-contig_172	574	Insect cuticle protein	AGAP006001	6056
43.	AsHcSf-contig_173	934	No putative conserved domains; Uncharacterized; salivary protein	AGAP007851	5737
44.	AsHcSf-contig_175	1014	Protein of unknown function (DUF1397	AGAP006275	9454
45.	AsHcSf-contig_179	857	EF-hand, calcium binding motif	AGAP006181	9453
46.	AsHcSf-contig_183	1562	Ornithine decarboxylase antizyme	AGAP010131	6569
47.	AsHcSf-contig_184	1183	Ferritin heavy chain-like protein	AGAP002465	12898
48.	AsHcSf-contig_187	1015	Hypothetical protein	AGAP008011	5549
49.	AsHcSf-contig_190	711	Hypothetical protein	AGAP013028	6447
50.	AsHcSf-contig_191	516	Hypothetical protein	AGAP013060-	6199
51.	AsHcSf-contig_192	758	Cathepsin	AGAP011828	6139
52.	AsHcSf-contig_193	621	Hypothetical protein	AGAP003620	8782
53.	AsHcSf-contig_194	2381	Prophenol oxidase	AGAP004977	15824
54.	AsHcSf-contig_200	1193	Fibrinogen	AGAP011197	10059
55.	AsHcSf-contig_214	1250	Thioredoxin-dependent peroxidase	AGAP011054	7922
56.	AsHcSf-contig_216	668	Learning-associated protein	AGAP005685	6254
57.	AsHcSf-contig_218	1307	Gelsolin ; Drosophile melanogaster	AGAP011369	9985
58.	AsHcSf-contig_228	1337	Fatty acid synthase	AGAP009176	7291
59.	AsHcSf-contig_237	737	No putative conserved domains	AAEL010634	6531
60.	AsHcSf-contig_253	1413	No significant similarity found	–	5510
61.	AsHcSf-contig_255	1389	Deoxyribonuclease I	AGAP011696	6366
62.	AsHcSf-contig_256	1936	Conserved hypothetical protein	AGAP013481	8133
63.	AsHcSf-contig_267	1276	Amino acid transporter protein/ hypothetical	AGAP000586	5335
64.	AsHcSf-contig_280	1452	Basic leucine zipper (bZIP) domain of Cyclic AMP-responsive element-binding protein-like	AGAP008762	5451
65.	AsHcSf-contig_286	1522	Nimrod	AGAP009762	7492
66.	AsHcSf-contig_294	1311	7 transmembrane receptor (rhodopsin family)	AGAP013149	9308
67.	AsHcSf-contig_329	1503	No significant similarity found	–	5447
68.	AsHcSf-contig_330	1292	Ferritin light chain-like protein	AGAP002464	6784

1.1.6 Molecular characterization of novel haemocyte transcript encoding Ninjurin

Ninjurin, previously identified as a two-pass transmembrane protein is induced upon nerve injury in vertebrates. Recent studies demonstrate that ninjurins are cell adhesion molecules, capable of regulating many cellular functions, *viz*. embryogenesis, injury, inflammation, signals *etc*. However, their structural and functional properties controlling these cellular responses, especially innate immune responses have not been investigated in detail.

Through comprehensive molecular and functional genomics approach, this study identified and annotated two (large and small) full transcripts encoding putative Ninjurin like proteins *AsNinj*L and *AsNinjS* (Fig. 10). Further, the possible role of mosquito ninjurin (*AsNinjL*) in haemocyte-mediated

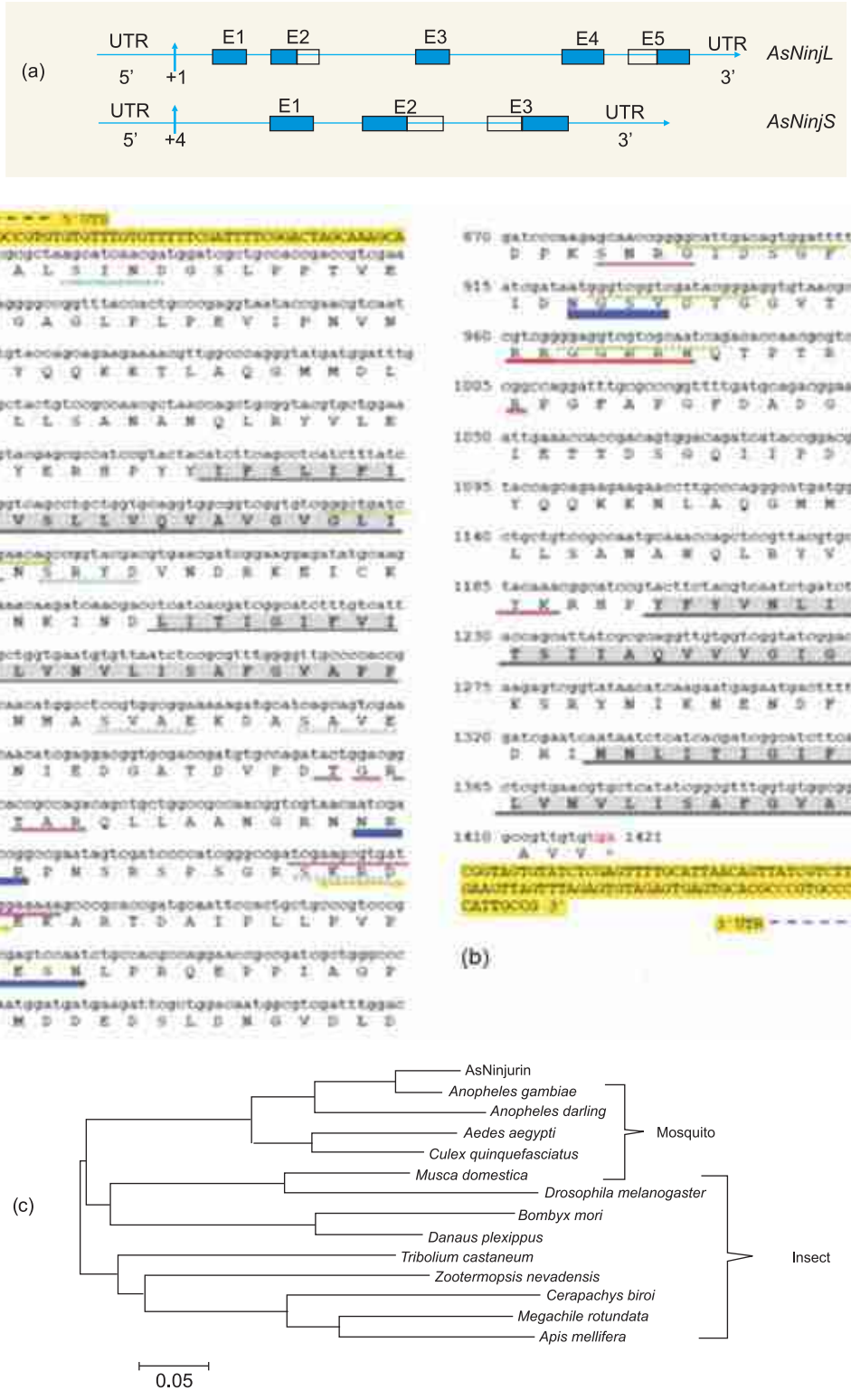


Fig. 10: Genomic and molecular gene organization of mosquito Ninjurin: (a) Schematic representation of the genomic architecture of the mosquito *Anopheles stephensi* ninjurins. Grey regions indicates the predicted transmembrane of the ninjurin domain, and +1 mark the transcription initiation site; (b) Gene organization and molecular features of complete CDS of *AsNinjL*: The gene contains 1854 bp, encoding 423 AA long peptides with two ninjurin domains, whereas *AsNinjS* contains only one ninjurin domain. Both 5' and 3'-UTR regions are shown in bold and capital letters. The complete CDS region of 423 amino acids starts from ATG/methionine/ green color, ending with TGA/Red/*. The different predicted motifs, *viz*. N-Glycosylation site (dark blue); PKC (pink); CKP (sky blue); cAMP phosphorylation site (dark yellow arrow); Amidation site (dark red); Mrystoylation site (green dotted) are underlined. Other additional structural features like transmembrane domains underlined by dark grey lines; and (c) Phylogenetic relation of mosquito and insect ninjurins.

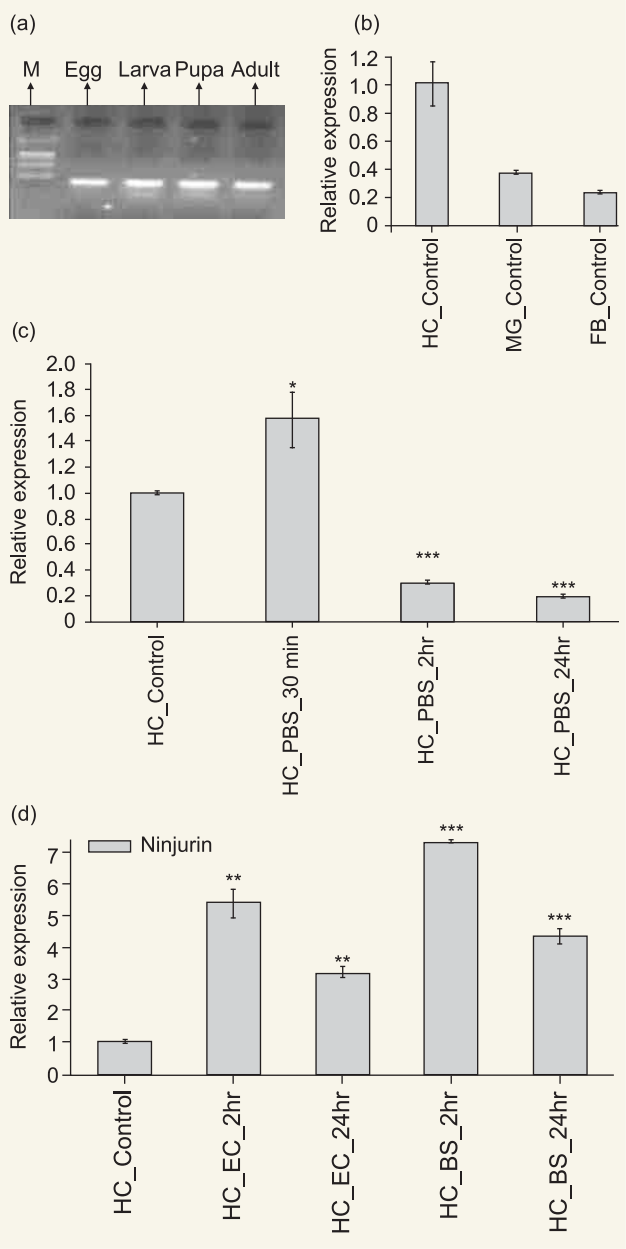


Fig. 11: Transcriptional behaviour of mosquito ninjurin protein: (a) *AsNinj* expression during the development of mosquito. Developmental stages include egg, larva, pupa, adult female; M = 500 bp Ladder; (b) Tissue specific relative expression of *AsNinj*; HC : Haemocyte; MG : Midgut; FB : Fat body; (c) Injury specific response of (PBS: phosphate buffer saline); and (d) Up regulation of *AsNinj* in response to microbial challenge.

cellular immune response was also characterized and predicted (Fig.11). Molecular modeling analyses provide crucial information about the key residues of ninjurin proteins, for further *in vitro* and *in vivo* functional analysis (Fig.12).

1.1.7 Molecular analysis of Holotricin-like antibacterial protein

Holotricin belongs to a small glycine rich

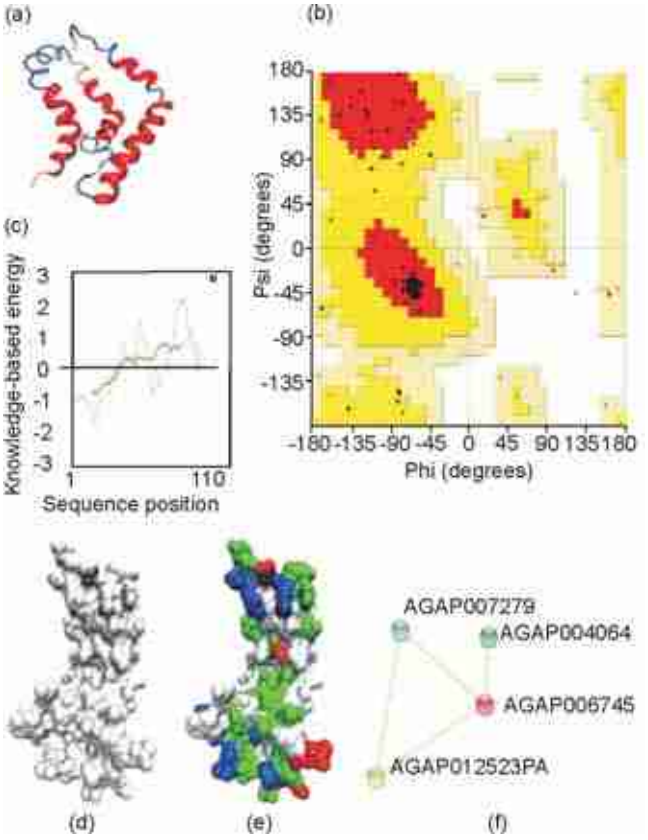


Fig.12: 3D- *In sillico* analysis of mosquito Ninjurin: (a) Secondary structure prediction of *AsNinjL*; (b) Ramachandran Plot analysis; (c) Prosa energy distribution of the molecular modeled mosquito Ninjurin; (d & e) Surface representation of the Ninjurin— two front views; (d) One side view; (e) Polar, negatively and positively charged atoms shown in green, red and blue, respectively; and (f) STRING-based network prediction analysis of *AsNinjL*.

antibacterial peptide, first identified from insect *Holotrichia dimophalia* larval haemolymph, inducible in response to *Escherichia coli* infection. A putative homolog identified from mosquito *Ae. aegypti* haemocyte is significantly up-regulated in response to dengue virus infection. Interestingly, in this study initial BLASTX analysis of haemocyte transcriptome data against non-redundant database, identified a putative transcript, encoding 124 amino acid long full length protein, which yielded 54% identity ($2e^{-025}$) to Holotricin-3 from *Holotrichia dimophalia* (BAA02889.1).

In an attempt to know the molecular nature and origin, this transcript was extensively searched against multiple insect (genome, scaffold, transcripts, ESTs, and assembled contigs) databases available at www.vectorbase.org (Figs. 13 a & b). Holotricin, showed poor match to almost all the insects (35-40% identity/$1e^{-02}$), except to *An. stephensi*, yielding >98% identity to the

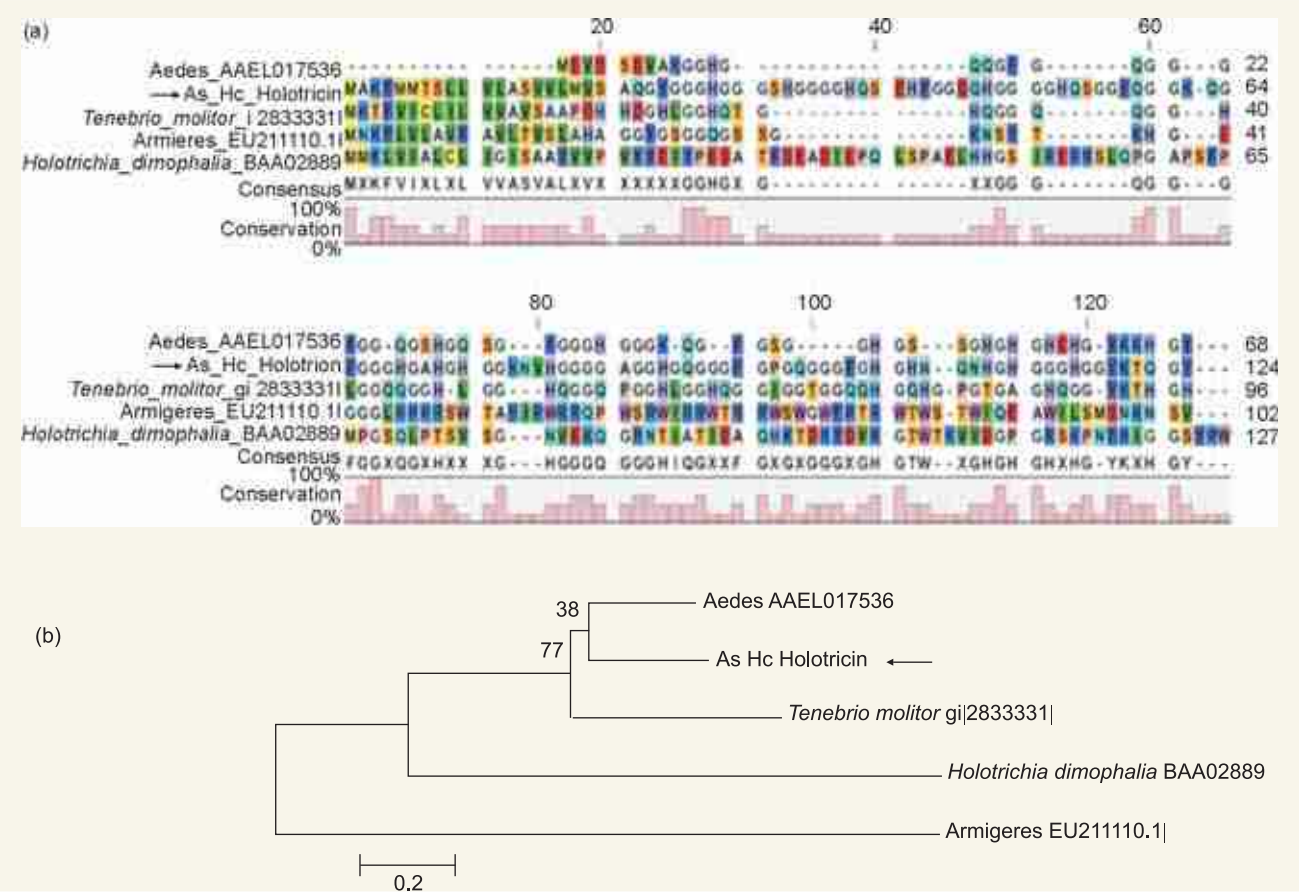


Fig. 13: Molecular analysis of haemocyte Holotricin— (a) Multiple sequence alignment; and (b) Phylogenetic analysis of Holotricin.

sequence assembly database. A future investigation is expected to validate the functional relationship of this putative transcript in *An. stephensi* mosquito.

1.1.8 Salivary microbiome comparison between two anopheline mosquito species

Mutualisms and symbiotic association between microbes and insects are ubiquitous and facilitate exploitation of much diverse ecology. Mosquito microbial flora has also been shown to regulate the immunity against parasite development. Whether, this microbial association also influences feeding and adaptation to diverse ecologies, remains unknown. Surprisingly, *An. stephensi* and *An. culicifacies* is dominant malarial vector in India, but both have different ecological adaptive preferences. For example *An. stephensi* transmit urban malaria, while *An. culicifacies* is major malaria vector in rural India. Available draft genome sequence comparison suggests that the genomic structure of both the mosquito not only differs in size, but also significantly varies in the number of encoded genes/proteins. Thus, how this genomic difference affects the mosquito associated biology of malaria transmission is yet to be explored.

To further clarify the complexity of ecological adaptive preferences, this study investigated the microbial community structure and diversity associated with different tissues facilitating mosquito feeding and digestion. The recent metagenomic study indicated that the overlapped microbial community was dominated by salivary gland than gut, in the laboratory-reared mosquito *An. culicifacies*. Further, the study was extended to map and compare the salivary microbiome of the laboratory-reared *An. culicifacies* and *An. stephensi* mosquitoes. A comprehensive metagenomic data analysis, revealed that salivary microbiome of *An. culicifacies* is unusually more diverse than *An. stephensi* (Fig.14).

A preliminary analysis revealed that both the mosquitoes have limited overlapping community, and predominated by uniquely associated distinct microbial population, e.g. *An. culicifacies* is dominated by Proteobacteria (~42%), while *An.*

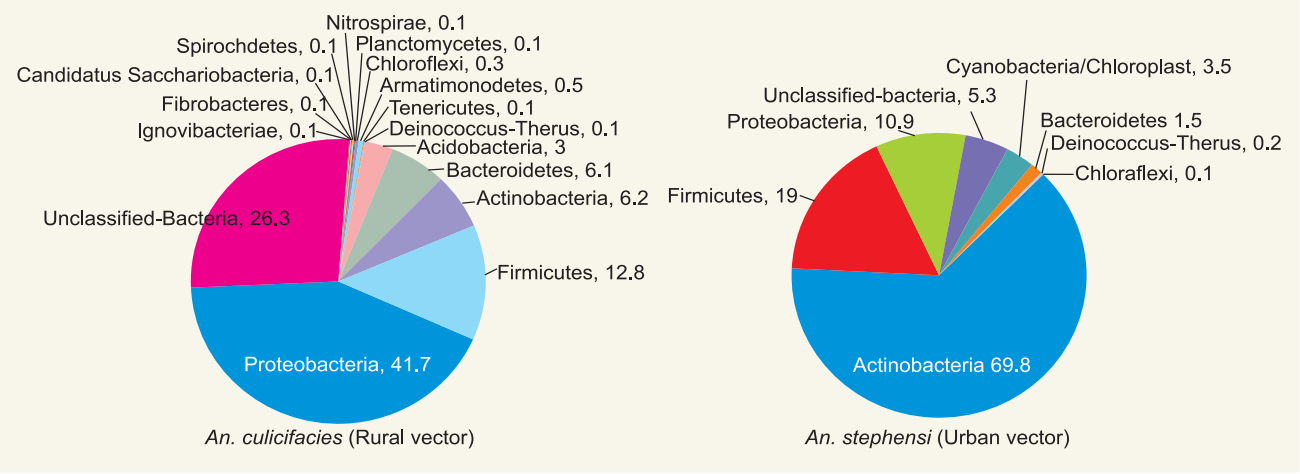


Fig. 14: Salivary gland associated microbial flora diversity in *An. culicifacies* and *An. stephensi*: Taxonomic assignment and relative percentage of the microbial community-associated with the salivary glands shown.

stephensi harbours Actinobacteria (~70%). Probably this may be due to differential feeding adaptation and engagements such as food acquisition, ingestion and digestion processes, a knowledge which may guide our future investigation to better understand the feeding associated molecular relationships and design vector management strategies.

1.1.9 Molecular population genetics of the NADPH cytochrome P450 reductase (CPR) gene in *Anopheles minimus*

Development of insecticide resistance (IR) in mosquito vectors is a primary hurdle to malaria control programme. Since, IR has genetic basis, and genes constantly evolve with response to environment for adaptation to organisms, it is important to know evolutionary pattern of genes conferring IR in malaria vectors. The mosquito *An. minimus* is a major malaria vector of the southeast Asia and India and is susceptible to all the insecticides, and thus of interest to know if natural selection has shaped variations in the gene conferring IR. If not, the DNA fragment of such a gene could be used to infer population structure and demography of this species of malaria vector. Hence, this study was initiated, wherein a 569 bp DNA segment of the NADPH cytochrome P450 reductase (CPR) gene (Fig. 15) was sequenced (widely known to confer IR) in 123 individuals of *An. minimus* collected in 10 different locations (eight Indian, one Thai and one Vietnamese) (Fig. 16). Two Indian population samples were completely monomorphic in the CPR gene. In general, low genetic diversity was found with no evidence of natural selection in this gene. The data were, therefore, analyzed to infer population structure and demography of this species. The 10 populations could be genetically differentiated into four different groups; the samples from Thailand and Vietnam contained high nucleotide diversity (Fig. 17). All the 10 populations conform to demographic equilibrium model with signature of past population expansion in four populations as analyzed with the Baysian skyline plots (Fig. 18). The results in general indicate that *An. minimus* mosquitoes sampled in the two southeast Asian localities contain several genetic characteristics of being parts of the ancestral population.

1.1.10 Impact of thermal conditions on survival of mosquito vectors

The role of material for rearing container in development of larvae was studied at 22, 26, 28, 30, 34 and 38°C temperature. Overall, earthen pots were found most productive in terms of developmental period of larva to pupa, *i.e.* 6.5 days as compared to 7.2 days in plastic containers and 7.5 days in iron containers.

1.1.11 Impact of rainfall on development and survival of malaria vectors

To study the impact of rainfall on breeding habitats of anopheline larvae, fortnightly field surveys before and after rainfall were undertaken at selected sites of District Baghpat (Uttar Pradesh) and northeast district of Delhi. The preliminary findings indicate that with 40 mm rainfall the larval density of *An. culicifacies* reduced drastically. Study is in progress.

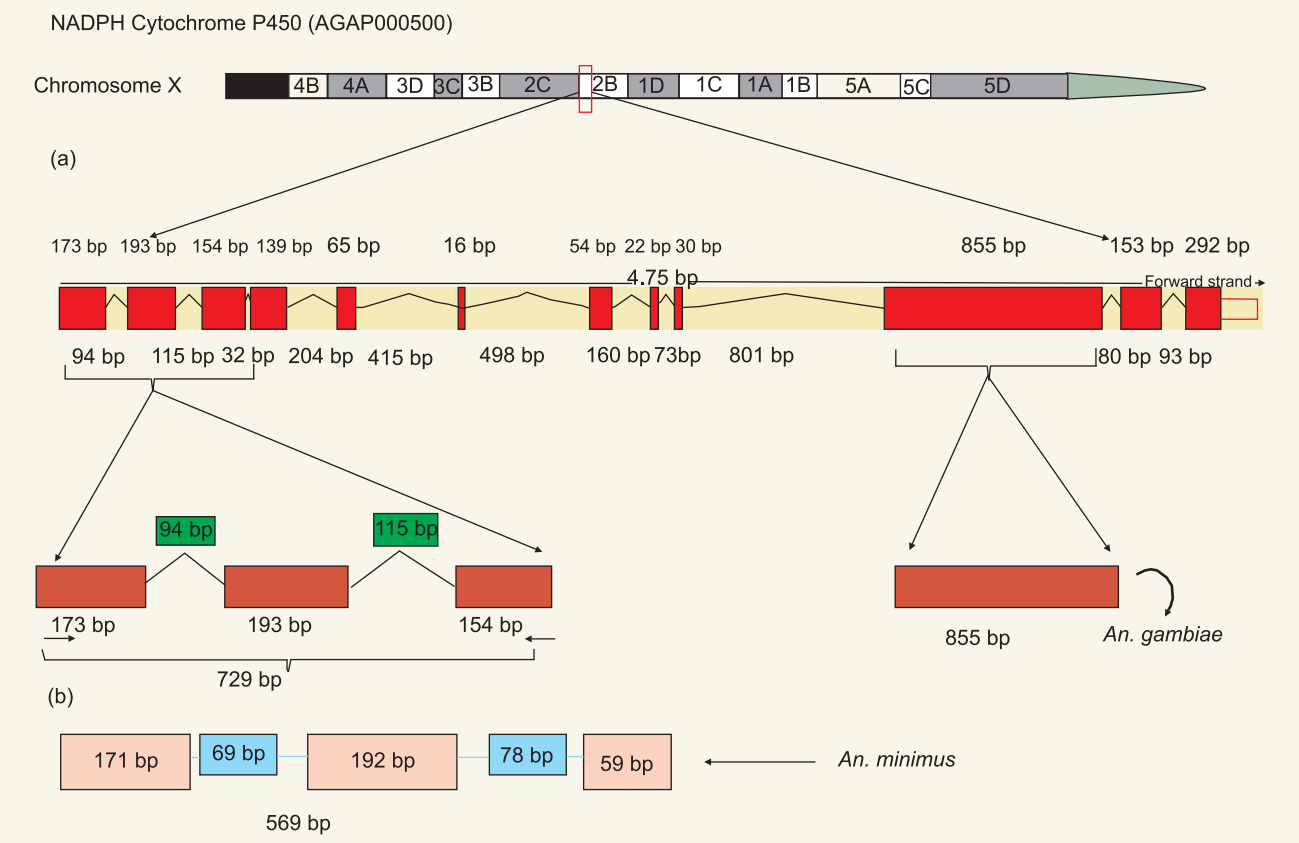


Fig. 15: Detail of NADPH cytochrome P450 reductase (CPR) gene segment: (a) Location and characteristic details of the CPR gene in the X-chromosome of *An. gambiae*; and (b) Portion of the gene homologous to the sequenced portion of *An. minimus*.

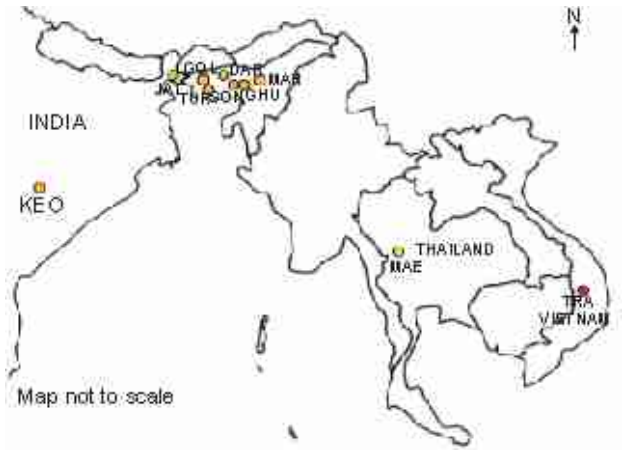


Fig. 16: Details of sample collection sites and schematic map indicating location of the sample collection sites of *An. minimus* from India, Thailand and Vietnam. The name of the population samples have been abbreviated as follows: DAR–Darrang, JAL–Jalpaiguri, GOL–Goalpara, MAR–Marigaon, SON–Sonapur, GHU–Ghuli, TUR–Tura, KEO–Keonjhar, MAE-Mae Sot, TRA-Tra My (Quang NamProvince).

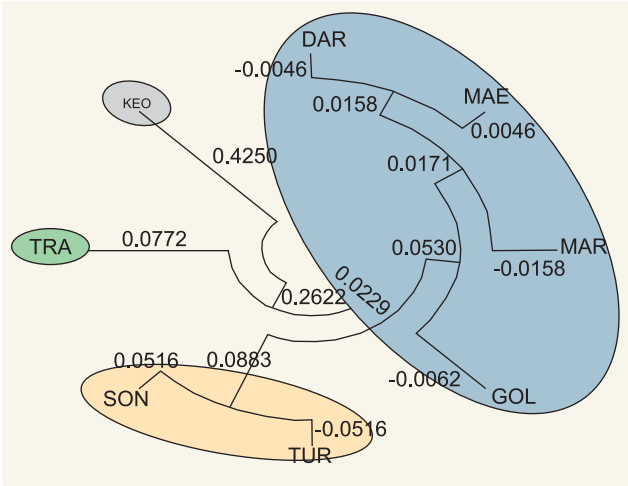


Fig. 17: Neighbour-joining (NJ) population phylogenetic tree based on the CPR gene fragment of *An. minimus*. Blue colour represents one clade which consists of the population samples from Darrang, Marigaon, Goalpara and Mae Sot, yellow colour represents another clade which consists of samples from Sonapur, and Tura, light green colour represents entirely separate clade of one population sample from Tra My (Vietnam) and grey colour represents another separate clade of population sample from Keonjhar in the phylogenetic tree. Numbers on branches represent the branch length of the clades.

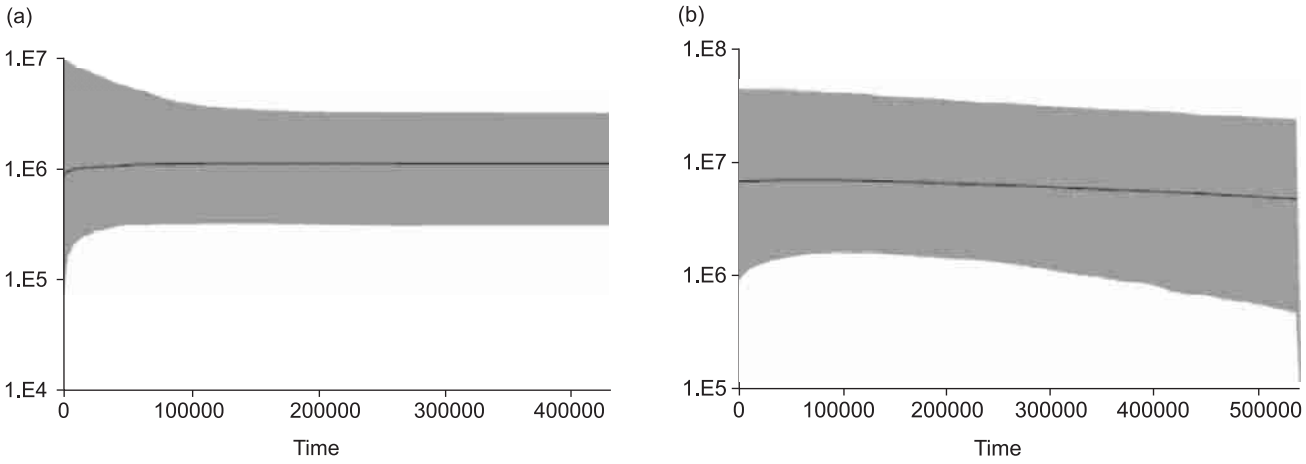


Fig. 18: Bayesian skyline plots in Indian and southeast Asian population samples: (a) Baysian skyline plot in Indian population of *An. minimus*; and (b) Baysian skyline plot in SE Asian population of *An. minimus*. The Y-axis represents the effective population size and X-axis represents time in 1000 years ago.

1.2 Vector Control

1.2.1 Control of dengue and chikungunya by controlling *Aedes* breeding in key containers in pre-monsoon season in one of the endemic zones of Delhi

This study was based on the hypothesis that 'control of the breeding of *Ae. aegypti* in key habitats during non-transmission season would detect breeding in secondary habitats during transmission season, thus help prevent dengue transmission'. The study was carried out in 20 high dengue case reporting localities of west zone in Delhi during July 2012 – May 2014. About 7000 houses covering approximately 35,000 populations of 20 localities were surveyed in collaboration with the Municipal Corporation of Delhi.

Before the commencement of surveys, meetings were organized with counsellors, resident welfare associations, schools, trade unions, local bodies/ personnel, *etc*. requesting them to permit NIMR and MCD team to visit their premises and to check their water storage containers on regular basis (Fig.19). During the entire study period, source reduction was carried out by the community and MCD workers. A total of 1408 unused containers containing water were emptied and were scrubbed with scrubber to kill the attached eggs. About 185 overhead tanks having broken lids were covered with cloth. One mg/l temephos granules was introduced in 1213 containers as per the WHO recommendations for drinking water (Fig. 20).

During 2014–15, analysis of study was undertaken which revealed that breeding takes place throughout the year. Overhead tanks and water storage containers were identified as key containers of the study areas because they support breeding in both transmission (June–November) and non-transmission (December–May) seasons. During transmission season vector breeding spread from key containers to secondary containers, *i.e.* cemented tanks, coolers, solid waste, mud pots; with maximum breeding in solid waste. There was not much change in the incidence of dengue cases in other localities of west zone, whereas as an outcome of intervention during non-transmission season no case was reported from the study area (except one NS1 case). This revealed that proper intervention in non-transmission season reduces

Fig. 19: Meetings with community before the commencement of study.

Fig. 20: *Aedes* source reduction (1 mg/l temephos granules application).

Ae. breeding containers and subsequently breeding and dengue cases in transmission season.

The Municipal Corporations of Delhi accepted the findings of the study and the Hon'ble Health Minister directed the MHO-MCD that the domestic breeding checkers (DBCs) should continue breeding survey throughout the year rather than eight months, *i.e.* April to November. The Indian Council of Medical Research, New Delhi appreciated the study and decided to circulate the findings of the study to the NVBDCP and other ICMR institutes working on dengue and chikungunya.

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 of the trial were continued for the second year as per the common protocol. During last one year, bioefficacy studies performed every month on community used nets under field conditions showed reduced mortality in *An. stephensi* in cone bioassays on NetProtect LLIN. However, it was within the prescribed limit of >80% mortality. Studies on human safety of nets showed that the nets were found to be safe and nothing adverse was reported that may be attributed due to the use of these nets by the community. The study is in progress and shall be concluded after the completion of three years of field evaluation.

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)

The field site at Kondagaon became completely functional by June 2013 with trained staff and needed infrastructure. Census was conducted in 80 of the 105 villages of CHC Keshkal comprising of about 0.76 lakh population in 4 PHCs and 25 subcentres and each village was given a single digit unique identification number as V1, V2, ...V80. The major vector species, *An. culicifacies* was found mostly resistant to DDT and malathion, but variably susceptible to deltamethrin (pyrethroid) and bendiocarb (carbamate). LLINs were distributed to all the inhabitants in the 80 villages based on earlier census data and enumerated sleeping units against informed consent with average distribution of 1.99 nets/household (1.67-2.31 at 95% CL]. Assessment of compliance on LLIN use was made immediately after distribution and was 61% and efforts were made to increase the compliance to >80% within a month. Adverse events were assessed within a month after distribution and were minimal and transient and as such did not affect the use rates. Based on the susceptibility status to insecticides and prevalence rates of malaria in the respective villages, randomization of villages was done for spray of bendiocarb 80 WP in 40 villages. In the study villages, cohorts of <12 yr children were recruited for the study. For epidemiological work, 125 ASHAs and 24 male MSWs were engaged, where regular surveillance was undertaken and drugs were provided to the positive cases detected by RDKs. Slides were also collected and armpit temperature was recorded. Studies on behaviour of *An. culicifacies*, sibling species composition, biochemical and molecular mechanisms of resistance were undertaken.

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

In silico study with 210 insecticides and

7 synergists has helped to design criteria based on common biotransformation route for selection of insecticides for use in rotation/alteration for management of pyrethroid resistance. This study has also helped to identify insecticides from different class that can be used for insecticide resistance management for vector control in consideration, its cross-resistance with insecticides from the same/different class, mode of action. Further identification of allosteric enzymes may help to refine the above study and its role for insecticide resistance management.

Topical assay to assess the intrinsic chemical toxicity for chlorfenapyr, DDT, deltamethrin, malathion and permethrin insecticides have been assessed and it was observed that pyrethroid resistant strain of *An. stephensi* has shown lesser $LD_{50}$and LD_{90} values to chlorfenapyr than the susceptible counterpart. This study showed an increase of intrinsic toxicity to chlorfenapyr in pyrethroid resistance strains. Hence, may be used for management of pyrethroid resistance.

Native PAGE studies showed additional esterase band in resistant *An. stephensi* population than in susceptible ones, while qualitative and quantitative differences were observed in expression of esterase in *An. culicifacies* susceptible laboratory strains and *An. culicifacies* field collected wild population. Disappearance of few esterase bands was observed when malathion was used for native PAGE inhibitor studies as compared to deltamethrin.

1.3.3 Chlorfenapyr: Irritant effect compared to other insecticides and its intrinsic toxicity in multiple-insecticide-susceptible and -resistant *Anopheles stephensi* (Diptera: Culicidae)

Studies were conducted to assess the irritability due to chlorfenapyr, DDT, malathion, deltamethrin and permethrin and intrinsic toxicity of chlorfenapyr in multiple-insecticide-susceptible and -resistant laboratory strains of *An. stephensi* following standard WHO methods. For effective management of vector resistance there is a need for new insecticide molecules with novel modes of action. For desired toxic effect of an insecticide, apart from other behavioural aspects, toxicity and chemical nature of the molecule are important that may cause irritability in the mosquito to the insecticide affecting the uptake. In this study, a pyrrole class insecticide, chlorfenapyr (a late acting insecticide) was tested for its irritability against multiple-insecticide-susceptible and -resistant strains of *An. stephensi* Liston 1901 (Diptera: Culicidae) (Table 3) followed by its toxicity studies (Table 4).

Chlorfenapyr molecule showed least irritant effect against susceptible and resistant strains among all the insecticides tested allowing more landing time to the vector species on the impregnated surfaces to pick-up lethal dose. Thus, chlorfenapyr could be an ideal insecticide for management of multiple-insecticide-resistance including pyrethroids.

Table 3. Irritant effect in multiple-insecticide-susceptible and –resistant *Anopheles stephensi* strains against different insecticide impregnated papers and respective controls

Control/ Insecticide (n)	FT_{50} (min)		FT_{95} (min)	
	Susceptible	Resistant	Susceptible	Resistant
OC C (20)	7.5	6.5	11.5	15
DDT 4% (50)	1.1	2.1	2.4	6.7
OP C(20)	4.2	2.1	13.4	12.2
Malathion 5% (50)	3.3	3.1	8.3	11.2
PY C (20)	9.9	11.6	22	15.9
Deltamethrin 0.05% (50)	0.2	2.7	1.5	10.6
Permethrin 0.75%(50)	0.1	1	0.7	4.1
Chlorfenapyr 5%(50)	9.8	8.9	25	19.2

(n)– Number of mosquitoes exposed; OC C–Organochlorine control; OP C–Organophosphate control; PY C–Pyrethroid control; FT_{50} and FT_{95}-time to take off for 50 and 95% of the exposed mosquitoes.

Table 4. Intrinsic toxicity of chlorfenapyr against insecticide-susceptible and -resistant *Anopheles stephensi* strains

Species/Resistant strains	Total hours	LD_{50} (ng/mg)	Lower–Upper limit at CI95%	LD_{95} (ng/mg)	Lower–Upper limit at CI 95%	Chi-square	*P*-value
An. stephensi (susceptible to DDT, MLN, DM)	24	0.827	0.313–1.299	5.425	2.855 – 48.493	3.649	0.456
	48	0.616	0.217–0.983	3.874	2.149 – 22.729	4.014	0.404
	72	0.629	0.165–1.095	3.741	1.922 – 43.210	5.665	0.226
An. stephensi (resistant to DDT, MLN, DM)	24	0.674	0.484–0.892	3.401	2.241 – 7.070	2.027	0.731
	48	0.683	0.535–0.819	2.134	1.654 – 3.293	1.029	0.905
	72	0.713	0.191–1.025	1.936	1.287 – 17.592	3.201	0.525

MLN–Malathion; DM–Deltamethrin; LD–Lethal doses that kill 50% (LD_{50}) and 95% (LD_{95}) of the exposed mosquitoes; CI–Confidence limit.

1.3.4 Identification of a novel knockdown resistance (*kdr*) mutation T1520I in *Aedes aegypti* and development of PCR-based assay for their detection

The last year studies have reported presence of a knockdown resistance (*kdr*) mutation F1534C in *Ae. aegypti* in Delhi population through PCR-based assay.

Furthering, several regions of the voltage-gated sodium channel (VGSC) were sequenced to explore existence of novel mutations. DNA sequencing of representative samples revealed the presence of at least one novel mutation T1520I in this population.

Three regions of VGSC were amplified and sequenced: (i) partial domain II (P to S6); (ii) partial domain III (S4-S6); and (iii) partial domain IV (S5-S6) using published primers. PCR products were amplified, purified using QIaquick PCR purification kit (Qiagen Inc.) and subjected to cycle sequencing reaction using BigDye Terminator v3.0.

Presence of two mutations, first due to point mutation T>C on second codon of F1534 residue leading to F→C (TTC>TGC) substitution and another due to C>T mutation on T1520 residue leading to T→I (ACC>ATC) substitution was recorded.

Development of PCR-RFLP for F1534 and T1520-kdr alleles

For development of single PCR-RFLP assays for detection of *kdr* alleles at two loci (F1534 and T1520) in domain III-S6, DNA sequences spanning 200 bp upstream to F1534 and 200 bp downstream to T1520 were checked for 1534C- and 1520I-specific restriction sites using an online tool available at *http://insilico.ehu.es/restriction/two_seq*. Two unique restriction enzymes *Ssi*I and *BsaB*I were selected which were specific to1534C (TTC>TGC) and 1520I (ACC>ATC) sequences, respectively. The intron region was excluded when designing PCR-RFLP due to the existence of *indel* in the intron upstream of T1520 as revealed by sequencing of cloned PCR product. Two primers flanking these two loci, *i.e.* AekdrF (5'-TGGGAAAGCAGCCGATTC-3') and AekdrR (5'-CCTCCGTCATGAACATTTCC-3') were designed with expected amplicon size of 171 bp. The expected sizes of cleaved product for the 1520I allele were 143 and 28 bp when digested with *BsaB*I, and 103 and 68 bp for 1534C when digested with *SSi*I. The diagnostic criterion for 1520I allele was taken as the presence of 143 bp band only (resolution of 28 bp cleaved product cannot be resolved on agarose gel), whereas presence of 103 and 68 bp bands were considered as diagnostic criteria for 1534C allele. Uncut product of 171bp was considered the wild allele.

For PCR-RFLP, amplification was carried out in 15 ml of reaction mixture containing 1 × buffer, 200 mM of each dNTP, 0.25 mM of primers AekdrF and AekdrR and 0.5 unit of *Taq* DNA polymerase. The PCR conditions were initial denaturation at 95°C for 3 min followed by 35 cycles each of denaturation at 95°C for 15 sec, 50°C for 15 sec and extension at 72°C for 30 sec and a final extension at 72°C for 7 min. The PCR product was subjected to two separate restriction digestion reactions, one with *BsaB*I and another with *SSi*I. Each restriction digestion reaction mixture (20 ml) contained 5 ml of PCR product, 2 units of restriction enzyme and 1× buffer, which was incubated for four hours or overnight at 65°C for *BsaB*I and 37°C for *SSi*I. The cleaved product was run on 2.5% agarose gel containing ethidium bromide and visualized with a gel documentation system (Figs. 21 and 22).

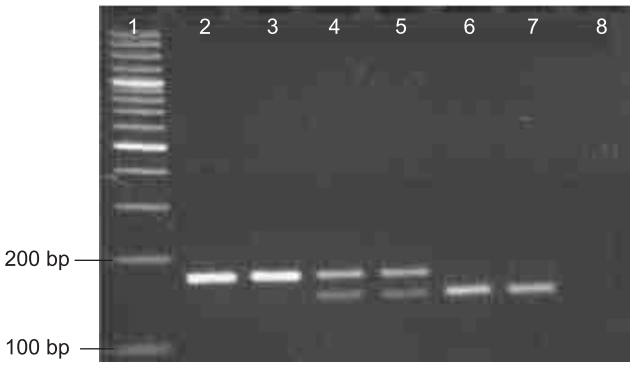


Fig. 21: Gel photograph showing PCR-RFLP assay for genotyping of T1520 alleles. Lane 1: 100 bp DNA ladder; lanes 2-3: TT; lanes 4-5: TI heterozygotes; lanes 6-7: II; and lane 8: negative control.

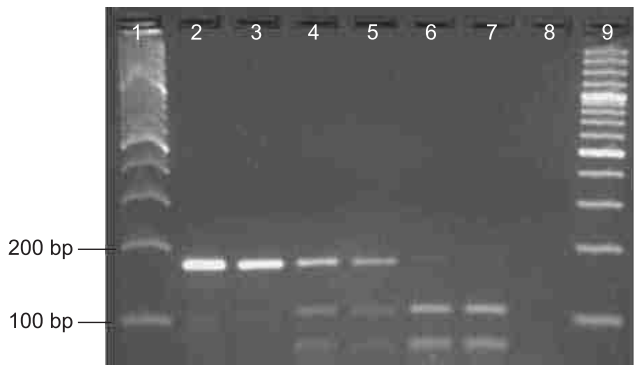


Fig. 22: Gel photograph showing PCR-RFLP assay for genotyping of F1534 alleles. Lanes 1 and 9: 100 bp DNA ladder; lanes 2-3: FF; lanes 4-5: FC heterozygotes; lanes 6-7: CC; and lane 8: negative control.

Genotyping of F1534 and T1520 alleles using new PCR-RFLP

Genotyping of F1534 and T1520 alleles were performed on 203 mosquitoes, which revealed a high frequency of the F1534C mutation (0.79) and a very low frequency of the T1520I mutation (0.13). Genotyping results showing association of T1520 and F1534 alleles are shown in Table 5. It was observed that T1520I mutation was found in individuals having the 1534C allele only, but never with wild type F1534. The data infer that 1520I is linked to 1534C. Linkage disequilibrium (LD) analysis revealed perfect disequilibrium (D′ = 1.0, $\chi^2 = 8.02$) though r^2 was low (0.04) due to a relatively low frequency of allele 1520I as compared to 1534C, where all the individuals with 1520I allele showed association with 1534C, but not all 1534C are associated with 1520I. The present data revealed the presence of three haplotypes with haplotype frequencies $f_{TF} = 0.21$, $f_{TC} = 0.66$ and $f_{IC} = 0.13$. However, f_{IF} was absent.

The result shows that F1534 genotypes show significant deviation from Hardy-Weinberg equilibrium (HWE) in all the populations ($p < 0.0001$) except in south Delhi-II. Initially it was thought that this might be due to discrepancy in allele-specific PCR genotyping, which often fails to prevent non-specific annealing during PCR extension. However, when genotyping using highly specific PCR-RFLP method was carried out, there was no change in HWE parameter for F1534 alleles. Surprisingly, T1520 genotypes in the same group of mosquitoes were in perfect HWE ($p = 0.99$). The possible explanation for such a deviation may be the presence of heterogeneous populations or gene duplication. Further, studies are required to resolve this conflict.

Table 5. Genotyping results of PCR-RFLP assays for F1534C and T1520I alleles and their association

		F1534 genotypes			
		FF	FC	CC	Total
T1520 genotypes	TT	28	22	105	155
	TI	0	8	37	45
	II	0	0	3	3
	Total	28	30	145	203

*p*HWE (Fisher's exact test): T1520 alleles = 0.991; F1534 alleles = 0.000.

Validation of new PCR-RFLP assays

Among the samples genotyped using the new PCR-RFLP, a portion of domain-III was sequenced for 20 samples (two sample of TT/CC, five samples of TI/FC, eleven samples of TI/CC and two samples of II/CC). Genotyping results matched with DNA sequencing results.

1.3.5 Association of F1534C-*kdr* mutation with insecticide resistance in *Aedes aegypti*

The association of the *kdr* mutations with resistance phenotype was tested using Fishers' Exact test and Odds Ratio (OR) estimation using dominant, recessive and additive models.

The proportions of dead and live mosquitoes after exposure to insecticides for each genotype are shown in Fig. 23. Odds Ratio estimates at 95% confidential intervals (CI) and Fisher's exact test using different models (dominant, recessive and additive) for dead and live mosquitoes in each treatment group are presented in Table 6. It was observed that F1534C-*kdr* conferred greater protection against DDT with all models and highest protection was shown using the recessive model

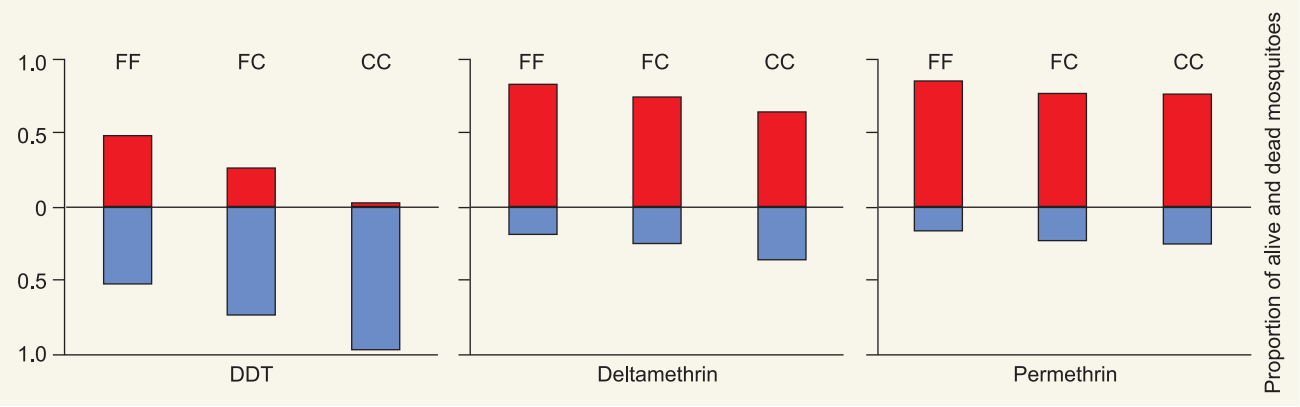


Fig. 23: Proportion of dead and alive mosquitoes in each genotype for F1534 alleles exposed to DDT 4%, deltamethrin 0.05 and 0.75% permethrin for one hour. (FF, FC, CC-Genotype).

Table 6. Association of F1534 alleles with insecticide resistance phenotypes

Insecticide		Genotype			Odds ratio (95% CI)			Fisher's exact test (*p*-value)		
		FF	FC	CC	Recessive model	Dominant model	Additive model	Recessive model	Dominant model	Additive model
DDT	Dead	38	26	4	16 (5.64–45.42)	5.72 (3.18–10.30)	5.81 (3.76–8.96)	<0.0001	<0.0001	<0.0001
	Alive	41	72	113						
Deltamethrin	Dead	51	55	46	2.0 (1.06–3.75)	2.1 (0.99–4.33)	1.85 (1.84–2.89)	<0.05	NS	<0.01
	Alive	11	19	26						
Permethrin	Dead	50	59	99	1.05 (0.58–1.89)	0.77 (0.37–1.61)	1.37 (0.89–2.14)	NS	NS	NS
	Alive	9	18	31						

NS—Non-significant.

(OR = 16.0, 95% CI: 5.6-45.4; $p = 0.000$). Lower protection was shown against deltamethrin when fitted with recessive (OR = 2.0, 95% CI: 1.06–3.75; $p < 0.05$) or additive (OR = 1.85, 95% CI: 1.84–2.89; $p < 0.01$) models. However, F1534C-*kdr* did not show significant protection against permethrin.

❑

Parasite Biology

2

2.1 New insights into the evolutionary history of *Plasmodium falciparum* from mitochondrial genome sequence analyses of Indian isolates

Estimating genetic diversity and inferring the evolutionary history of *Plasmodium falciparum* could be helpful in understanding origin and spread of virulent and drug-resistant forms of the malaria pathogen and, therefore, contribute to malaria control programme. Genetic diversity of the whole mitochondrial (*mt*) genome of *P. falciparum* sampled across the major distribution ranges had been reported, but no Indian *P. falciparum* isolate had been analyzed so far, even though India is highly endemic to *P. falciparum* malaria.

In this study, the whole *mt* genome of 44 Indian field isolates were sequenced and published data set of 96 genome sequences were utilized to present global genetic diversity and to revisit the evolutionary history of *P. falciparum*. Indian *P. falciparum* presents high genetic diversity with several characteristics of ancestral populations and shares many of the genetic features with African and to some extent Papua New Guinean (PNG) isolates (Fig. 1). Similar to African isolates, Indian *P. falciparum* populations have maintained high effective population size and undergone rapid expansion in the past with oldest time to the most recent common ancestor (TMRCA) (Fig. 2). Interestingly, one of the four single nucleotide polymorphisms (SNPs) that differentiate *P. falciparum* from *P. falciparum*-like isolates

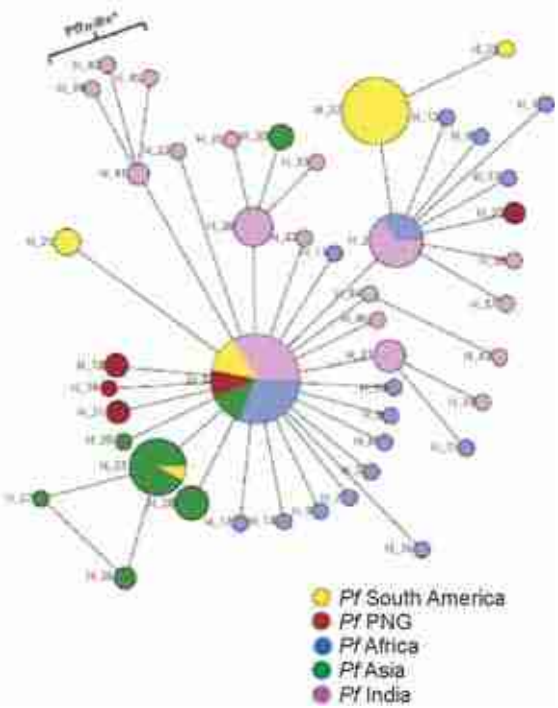


Fig. 1: Reconstructed haplotype network of global *Plasmodium falciparum* isolates with whole *mt* genome sequences. Indian haplotypes are indicated by pink colour, African by blue, Asian by green, South American by yellow and Papua New Guinean by red. It may be noted that PfIndia* form an entirely different clade with four separate haplotypes (H_39, H_40, H_41 and H_45). The size of circle is proportional to the haplotype frequency.

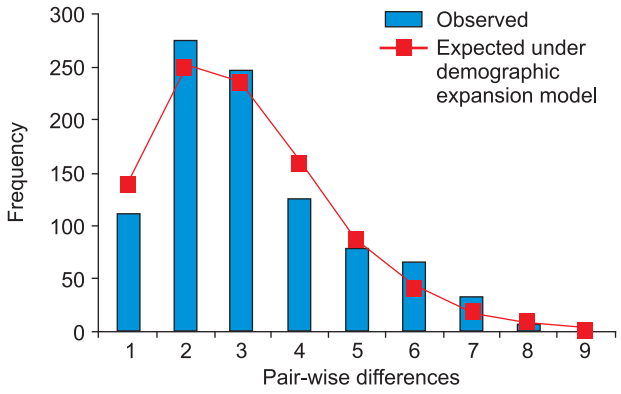


Fig. 2: Mismatch distribution of pair-wise number of differences in Indian *Plasmodium falciparum*. The bars represent observed frequency of the pair-wise differences among *mt* genome sequences and the line represents the expected curve for a population that has undergone a demographic expansion. Observed mismatch distribution was compared with expected under sudden demographic expansion model with a parametric bootstrap of 1000 replicates.

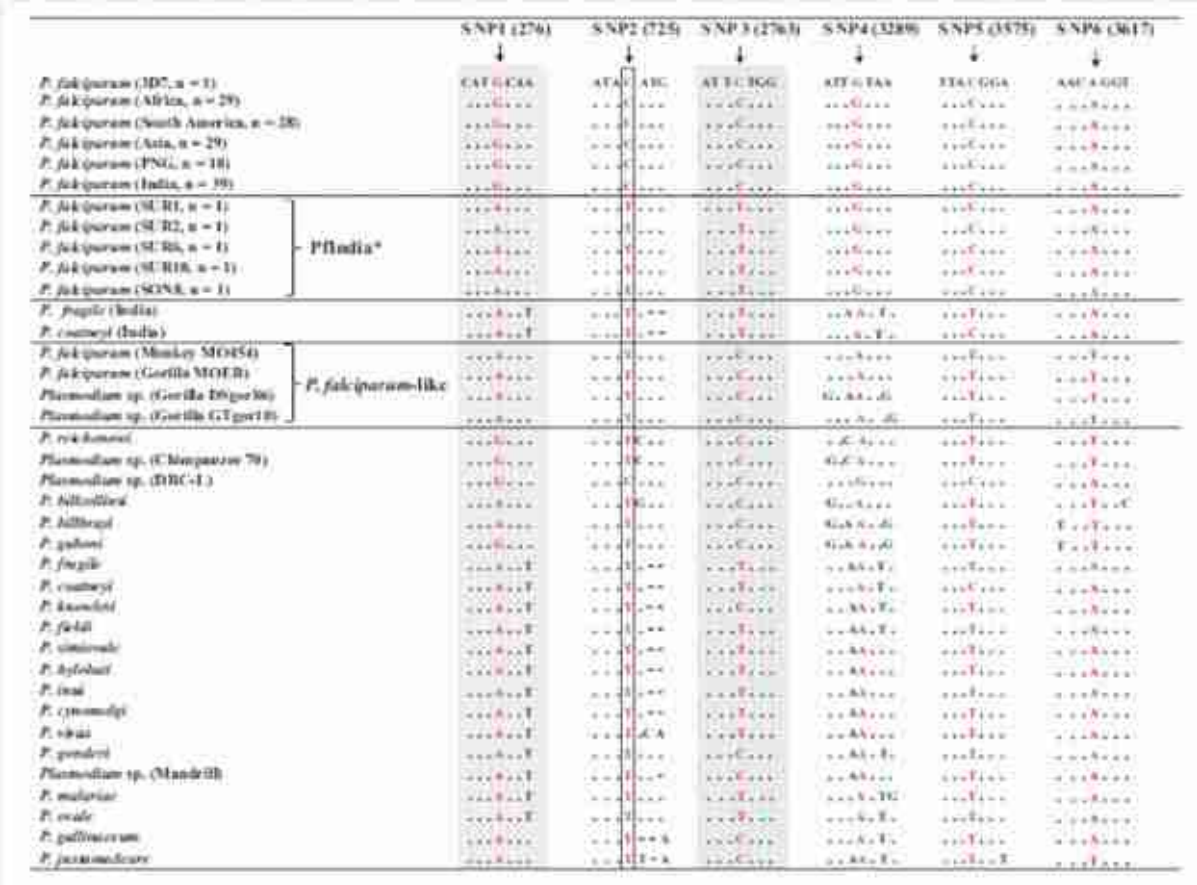


Fig. 3: DNA sequence alignment of parts of *mt* genome sequence of *Plasmodium* species infecting humans and non-human primates showing six SNPs. The four SNPs (725th, 3289th, 3575th and 3617th) considered as the diagnostic SNPs to distinguish between the *Plasmodium* species infecting humans from the non-human primates. The rest two SNPs (276th and 2763rd) were found exclusively in the five *Plasmodium falciparum* (PfIndia*) isolates and also in many different species of *Plasmodium* infecting non-human primates including Indian *P. coatneyi* and *P. fragile*.

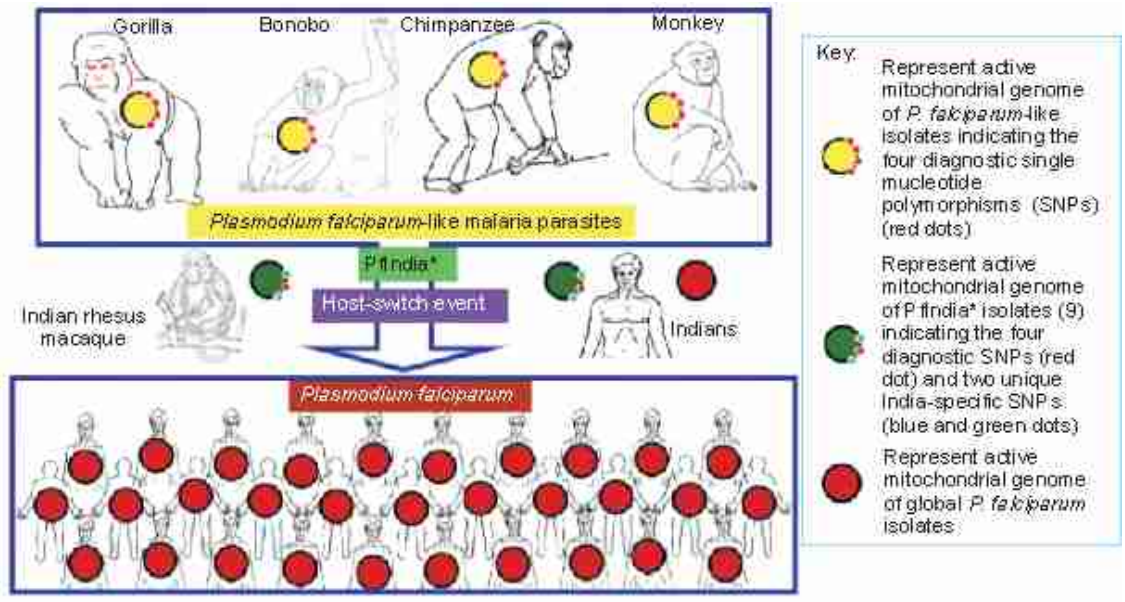


Fig. 4: A hypothetical evolutionary model of host-switch events from *Plasmodium falciparum*-like malaria parasites (infecting African non-human primates) to *P. falciparum* (infecting humans). Each different colour-filled circle represents a different mitochondrial (*mt*) genome haplotype of the malaria parasites and the small dots in each circle represent SNPs present in the *mt* genomes. Note that *P. coatneyi* and *P. fragile* infecting Indian rhesus macaques bear identical *mt* genome haplotypes to Indians and are designated PfIndia*.

(infecting non-human primates in Africa) was found to be segregating in five Indian *P. falciparum* isolates (Fig. 3). This SNP was in tight linkage with two other novel SNPs that were found exclusively in these five Indian isolates. These five isolates have been named as PfIndia*, inferring the probable host-switch events of the *P. falciparum* from Indian non-human primates (Fig. 4). While the results on the *mt* genome sequence analyses of Indian isolates on the whole add to the current understanding on the evolutionary history of *P. falciparum*, the host-switching events hypothesized to have occurred in India throw new insight into the evolutionary history of *P. falciparum*.

2.2 Molecular evidence of *Plasmodium vivax* mono and mixed malaria parasite infections in Duffy-negative native Cameroonians

The malaria parasite *P. vivax* is known to be majorly endemic to Asian and Latin American countries with no or very few reports of Africans infected with this parasite. Since, the human Duffy antigens act as receptors for *P. vivax* to invade human RBCs and Africans are generally Duffy-negative, non-endemicity of *P. vivax* in Africa has been attributed to this fact. However, recent reports describing *P. vivax* infections in Duffy-negative Africans from west and central parts of Africa have been surfaced including a recent report on *P. vivax* infection in native Cameroonians. In order to know if Cameroonians living in the southern regions are also susceptible to *P. vivax* infection, finger-prick blood samples from 485 malarial symptomatic patients in five locations were collected (Fig. 5) followed by PCR diagnostic assays with DNA sequencing of the 18S ribosomal RNA gene. Out of the 201 malaria positive cases detected, 193 were pure *P. falciparum*, six pure *P. vivax* and two mixed parasite infections (*P. falciparum* + *P. vivax*). The eight *P. vivax* infected samples (six single + two mixed) were further subjected to DNA sequencing of the *P. vivax* multidrug resistance 1 (*pvmdr1*) and the *P. vivax* circumsporozoite (*pvcsp*) genes. Alignment of the eight Cameroonian *pvmdr1* sequences with the reference sequence showed high sequence similarities, reconfirming *P. vivax* infection in all the eight patients. DNA sequencing of the *pvcsp* gene indicated all the eight *P. vivax* to be of VK247 type. Interestingly, DNA sequencing of a part of the human Duffy gene covering the promoter region in the eight *P. vivax*-infected Cameroonians to identify the T-33C mutation (Fig. 6) revealed all these patients as Duffy-negative. The results provide evidences of single *P. vivax* as well as mixed malaria parasite infection in native Cameroonians and add knowledge to the growing evidences of *P. vivax* infection in Duffy-negative Africans.

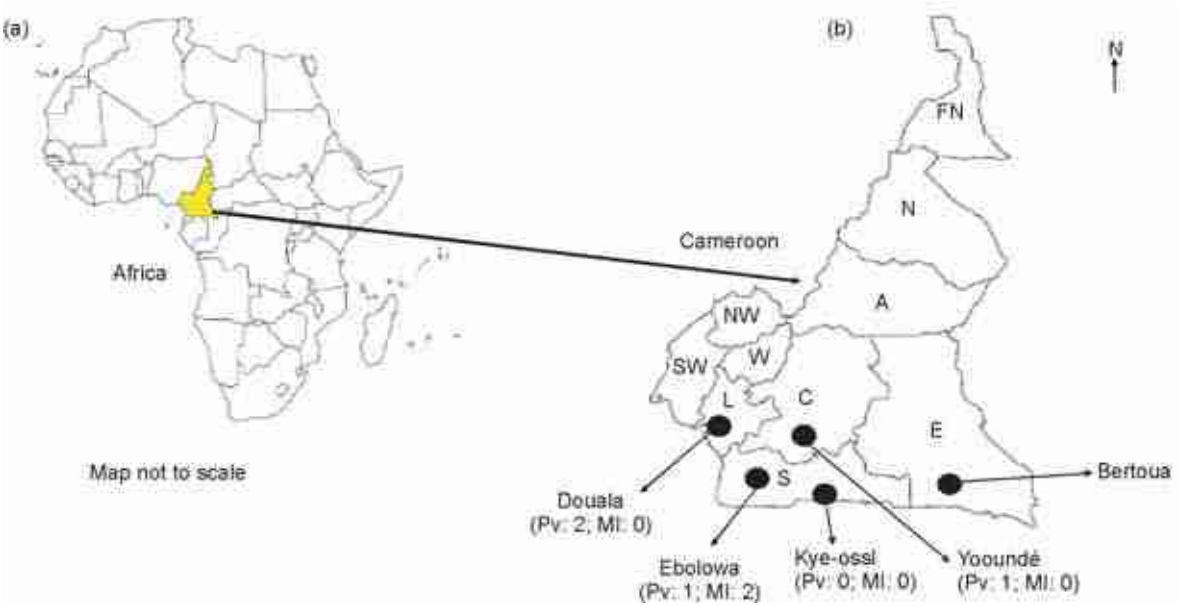


Fig. 5: Map of Africa: (a) Highlighting Cameroon; and (b) Map of Cameroon showing the sampling location sites; FN: Far North; N: North; A: Adamaoua; C: Centre; NW: Northwest; W: West; SW: Southwest; L: Littoral; S: South; E: East; Pv: *Plasmodium vivax*; MI: Mixed infection.

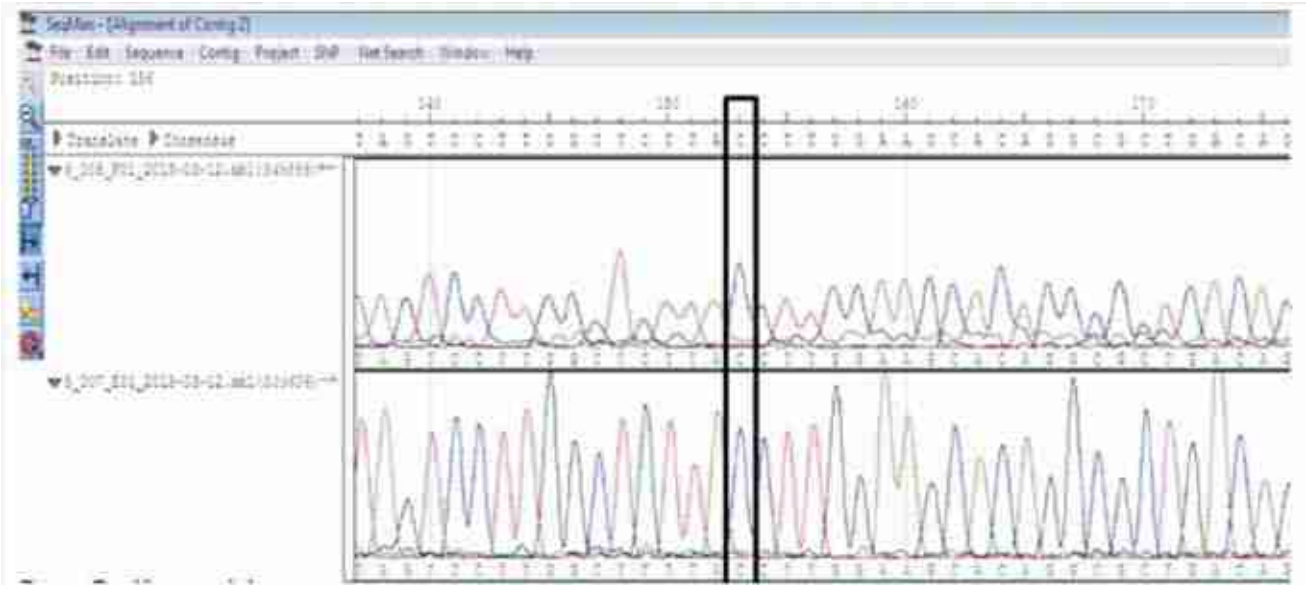


Fig. 6: DNA sequence alignment and associated chromatogram of the DNA sequence covering the promoter region of the Duffy gene in eight Cameroonians harbouring the *P. vivax* infection. The T-33C SNP is indicated in blue and the representative chromatogram showing a clear peak of "C" is shown below the alignment.

2.3 Optimization of ion Torrent to study complexity of infection in *Plasmodium vivax* populations

An increase or decrease in complexity of infection (*i.e.* infections comprising two or more distinct genetic types) can influence the malaria pathogenicity. Next-generation sequencing methods provide high resolution, scalability and sensitivity to examine genetic diversity of malaria parasite. An amplicon sequencing protocol on the Ion Torrent PGM platform have been established for targeted re-sequencing of multiple genomic regions in field isolates. This method to discover mixed genotype populations within a patient can facilitate understanding dynamics of malaria pathogenicity and drug resistance. Three vivax populations were selected (Chennai, Nadiad and Rourkela) for this study. Genetic diversity was evaluated in five genes *sera1, sera5, clag, msp3α* and *msp7* for feasibility of estimation of complexity of infection (COI). The feasibility of the selected loci for COI estimation was done using genomic DNA from the *P. vivax* reference strains Salvador I, Brazil I, India VII and North Korea (Table 1). Since, the data from this initial experiment seemed promising, sequencing of 13 *P. vivax* field isolates was executed (Table 2). In these initial sequencing runs, amplicon coverage, data quality and reproducibility were tested to understand the level of sequence diversity at these loci in the field isolates and determine the appropriate loci for COI estimation.

Multiple alleles were expected at these loci which in turn can distinguish between individual clones. Variants can be classified as homozygous or heterozygous based on whether all reads have a single allele at a particular nucleotide position or mixture of reads containing two or more alleles observed at that position. Diverse patterns of variant distribution at different loci were observed. The observed SNP distribution in reference strains and the 13 field isolates represents *clag* and *msp3α* as good candidates for COI estimation (Fig. 7).

Table 1. Observed sequence diversity in reference strains

Sample	*Sera1*	*Sera5*	*Clag*	*msp3α*	*msp7_1*	*msp7_2*
Salvador I	0	0	0	0	1	2
India VII	8	4	14	4	6	8
Brazil I	12	1	15	1	0	2
North Korea	8	4	14	13	1	3
No. of variable nucleotide positions	16	4	20	15	5	9

Table 2. Nucleotide variants in 13 *Plasmodium vivax* field isolates

	Sera1	*Sera5*	*Clag*	*msp3α*	*msp7_1*	*msp7_2*
No. of field isolates containing ≥1 variant	9/13	12/13	13/13	9/13	6/13	13/13
No. of variable nucleotide positions	25	21	23	25	10	14

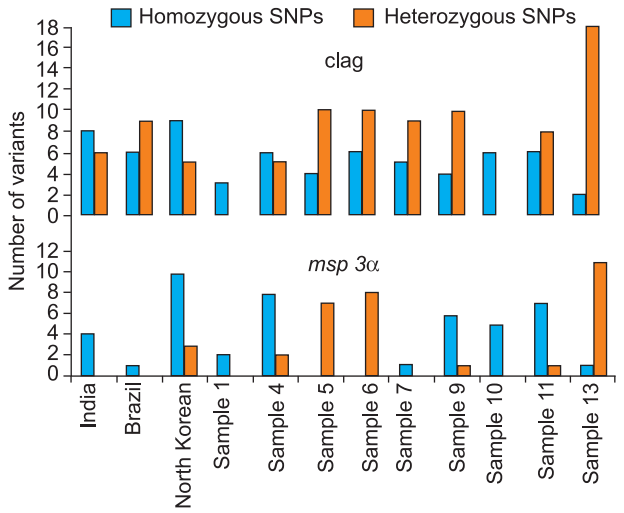


Fig. 7: SNP distribution in *clag and msp3α genes.*

2.4 Molecular characterization of novel *Plasmodium* proteins: An excellent antimalarial drug target candidates

2.4.1 4-diphosphocytidyl-2c-methyl-d-erythritol kinase (IspE)

Plasmodium proteins that are essential for parasite growth and development but absent in mammalian hosts are the excellent drug targets. 4-diphosphocytidyl-2c-methyl-d-erythritol kinase (IspE) of MEP pathway located in the apicoplast has the potential to act as an excellent drug target as it is actively expressed during the intraerythrocytic cycle of *Plasmodium* and at the same time absent in human beings. This study was aimed to characterize the *IspE* gene from *P. vivax (Pv*IspE) using molecular and bioinformatics approach. In continuation to the previous work, clinical parasite samples from different geographical regions of India were collected and the genetic polymorphism of *PvIspE* gene among Indian isolates was studied. *PvIspE* genes (1524bp) of all the samples were amplified and sequenced. Blast results show 99% homology among isolates of *P. vivax* collected from different geographical regions of India (Fig. 8).

Interestingly, *Pv*IspE sequence analysis showed a high degree of homology with all other sequences of *Plasmodium* species. Its evolutionary position with other organisms was also investigated by Phylogenetic analysis (Fig. 9). Also, 3-dimensional models of the *Pv*IspE protein were generated with detailed description of active site (Fig. 10).

Further, 3D structure of modeled *P. vivax* Indian IspE was aligned structurally with *P. falciparum* homologue domains, *P. vivax* Del-Bengaluru region and *Homo sapiens* (Fig. 11).

These results strengthen the applicability of *Pv*IspE as a potential antimalarial drug target and may set a firm base for the development of structure-based novel antimalarial compounds (inhibitors) against *Pv*IspE. Further, stage-specific activity assay of IspE and protein expression work is in progress.

2.4.2. Phosphoethanolamine methyltransferase (PMT)

Phosphatidylcholine (PC) synthesis is the most essential phospholipid synthesized through

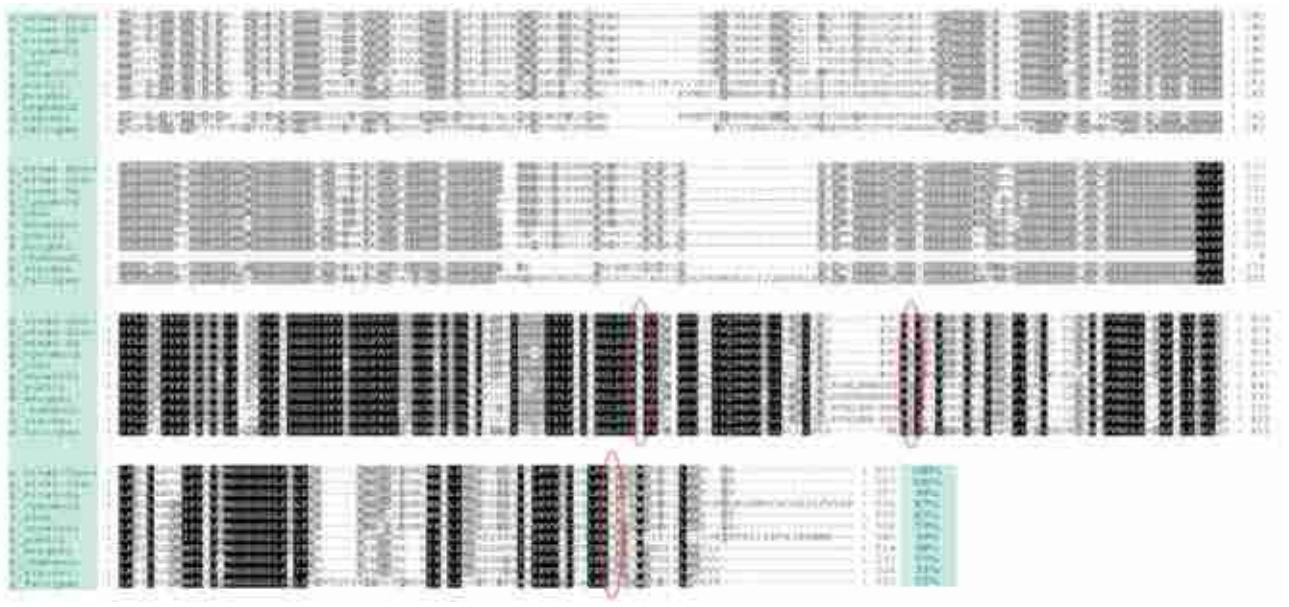


Fig. 8: Clustal alignment of amino acid sequence of *Pv*IspE Indian isolate with amino acid sequence of different *Plasmodium* IspE.

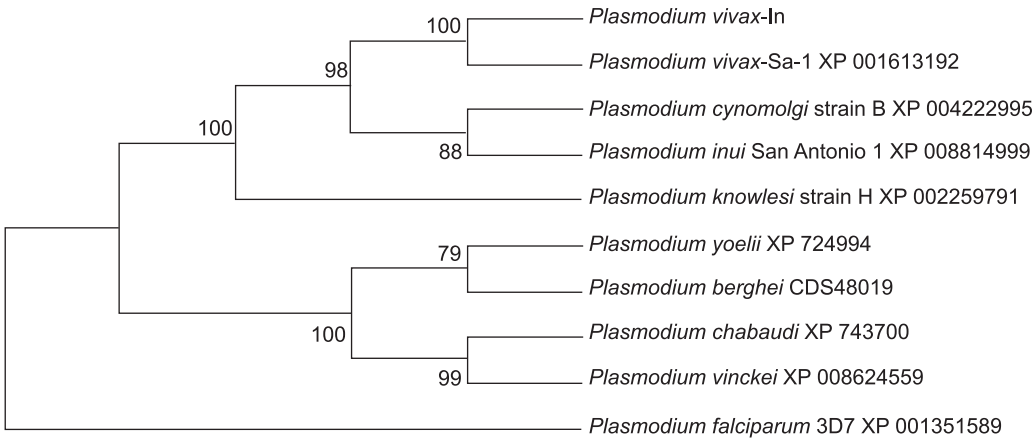


Fig. 9: Phylogenetic analysis of Indian *Pv*IspE with different *Plasmodium* IspE.

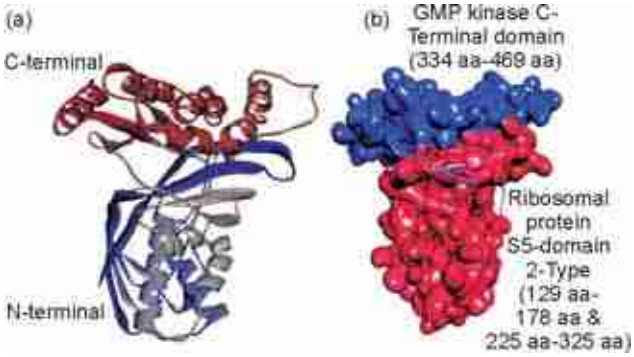


Fig. 10: (a) Homology model of Indian *Pv*IspE kinase in N-C terminal; and (b) Catalytical domain of *Pv*IspE enzyme showing two conserved catalytic domains.

serine-decarboxylase-phosphoethanolamine-methyltransferase (SDPM) pathway in *P. falciparum,* at very fast rate for the rapid multiplication of *P. falciparum* within human host. Phosphatidylcholine biosynthesis pathway is essential for survival in many organisms such as *Leishamnia major,* bacteria, and eukaryotes. Phosphoethanolamine *N*-methyltransferase is important enzyme for PC biosynthesis in *Caenorhabditis elegans*.

Absence of Phosphoethanolamine methyltransferase (PMT) *Plasmodium* in humans makes it good target for drug development. Phosphatidylcholine

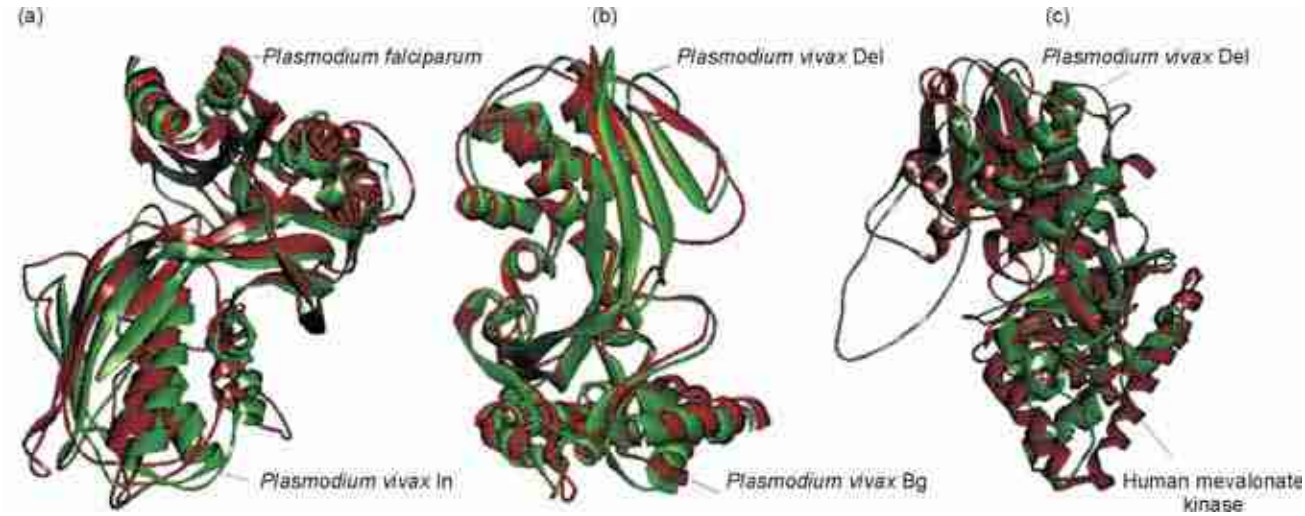


Fig. 11: (a) The 3D structure of modeled *P. vivax* Indian IspE was aligned structurally with *P. falciparum* homologue and both of them are very similar with TM-score = 0.6278. All the conserved domains are in close proximity. Red colour shows domain of *P. falciparum* and green colour shows domain of *P. vivax*; (b) The 3D structure of modeled *P. vivax*-Delhi was aligned structurally with *P. vivax*-Bengaluru and both of them are structurally similar and even there is good similarity in protein folds, with TM-score = 1.0000; and (c) The 3D structure of modeled *P. vivax* IspE was also aligned structurally with mevalonate kinase from *Homo sapiens*. Mevalonate kinase did not have structural similarity not even in protein folds with TM-score = 0.1406.

is the most abundant phospholipid in *Plasmodium* membranes. Parasite requires PC for growth, rapid multiplication at blood stages (rings, trophozoites, and schizonts) and for gametes development within the host. PMT has important role in the proliferation and survival of intraerythrocytic malaria parasites. Transcription of PMT enzyme at asexual replication within RBC and gametocyte development is important for multiplication (growth) as well as transmission of *Plasmodium* parasite.

Parasite samples from different geographical regions (Delhi, Jabalpur, Sonapur, Nadiad, Rourkela, Chennai, and Bengaluru) of India were collected in order to study the genetic polymorphism of *PMT* gene of both *P. falciparum* and *vivax* among Indian isolates. Two sets of primers were designed for amplification of *PMT* gene of 1382 base pairs. Gene amplification of *P. falciparum PMT* gene of Bengaluru region has been carried out by one set of primer and successfully amplified (743 bp) (Fig. 12). Further, other amplification of *PMT* gene of *P. falciparum,* sequencing and bioinformatics analysis are in process.

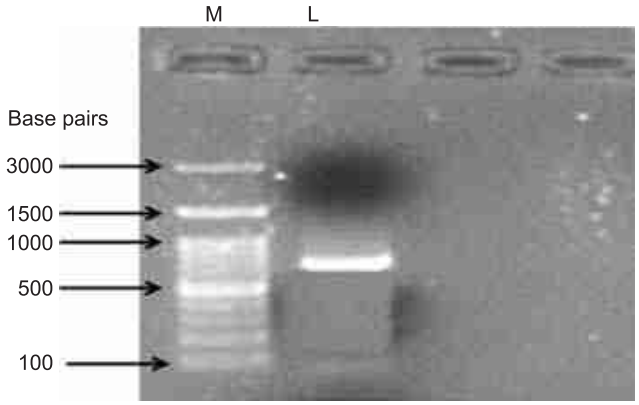


Fig.12: PCR amplified product of *PMT* gene of *P. falciparum*. Lane 1 (M) – Marker (100 bp DNA Ladder); and Lane 2 (L) – *PfPMT* gene amplified product (743 bp).

As the crystal structure of *Pf*PMT in complex with co-crystallized Phosphocholine is available in protein data bank (PDB), knowledge of binding pocket of Phosphocholine within the *Pf*PMT protein structure was used for designing 3-D coordinates for binding pocket in computational screening of compounds (Fig. 13).

Using this crystal structure, a total of 1481 approved compounds from Drug databank and 100,000 natural compounds from Zinc compound database have been screened on the basis of computational approaches like docking and Lipinski's rule of five, Veber rule using Discovery studio and Schrodinger softwares. Few compounds were identified to occupy the orientation alike Phosphocholine (endogenous ligand) within the binding pocket of *Pf*PMT enzyme. A brief summary of some compounds is given (Table 3). Procurement of identified compounds and testing of *in vitro* parasite culture are in progress.

Protein assay and stage-specific activity assay of PMT will be carried out to analyze the importance and extent of expression of target gene in all the stages of malaria parasite. Identified compounds will be tested for *in vitro* activity in parasite culture. Compounds with good inhibitory profile

Fig. 13: Binding pocket designed (orange colour) for docking studies based on interaction of Phosphocholine into the selected binding pocket (impotent interacting residues highlighted in black colour).

Table 3. Screening of compounds

	Generic_Name	LibDock score	Glide score
Drug Bank ID	Compounds from Drug Data Bank		
DB00116	Tetrahydrofolic acid	179.143	–9.13
DB01610	Dinoprost Tromethamine	162.029	–8.36
DB01132	Pioglitazone	159.079	–8.6
Zinc ID	IUPAC Name Compounds from Zinc compound database		
35485160	(3S)-N-[(1S)-1-[(4-methoxyphenyl)methylcarbamoyl]-3-methylsulfanyl-propyl]-1,2,3,4-tetrahydroisoquin	152.7	–8.4
4082322	1-[[1-(2-amino-3-phenyl-propanoyl)-4-piperidyl]carbonyl] pyrrolidine-2-carboxylic	125.43	–8.16
4074066	2-[[1-(2-amino-3-phenyl-propanoyl)-4-piperidyl] carbonylamino]-4-methyl-pentanoic	152.36	–7.43
4089741	2-[4-[(2-amino-3-methyl-pentanoyl)aminomethyl] cyclohexyl]carbonylamino-3-phenyl-propanoic	151.4	–7.62

may provide lead for further antimalarial drug development.

2.5 Interactions of macromolecular substrate and inhibitor with malarial cysteine proteases

Falcipain-2 (FP2) and Falcipain-3 (FP3) are major hemoglobinases of *P. falciparum*. Previous biochemical and structural studies have helped to explain the mechanism of inhibition of these two enzymes by small molecules. However, it is not well-known, how macromolecular substrate and inhibitor interacts with FP2 and FP3? A natural macromolecular substrate, hemoglobin binds to the C-terminus of FP2. This C-terminus domain is crucial for capturing hemoglobin and its deletion impairs the ability of FP2 to hydrolyze hemoglobin.

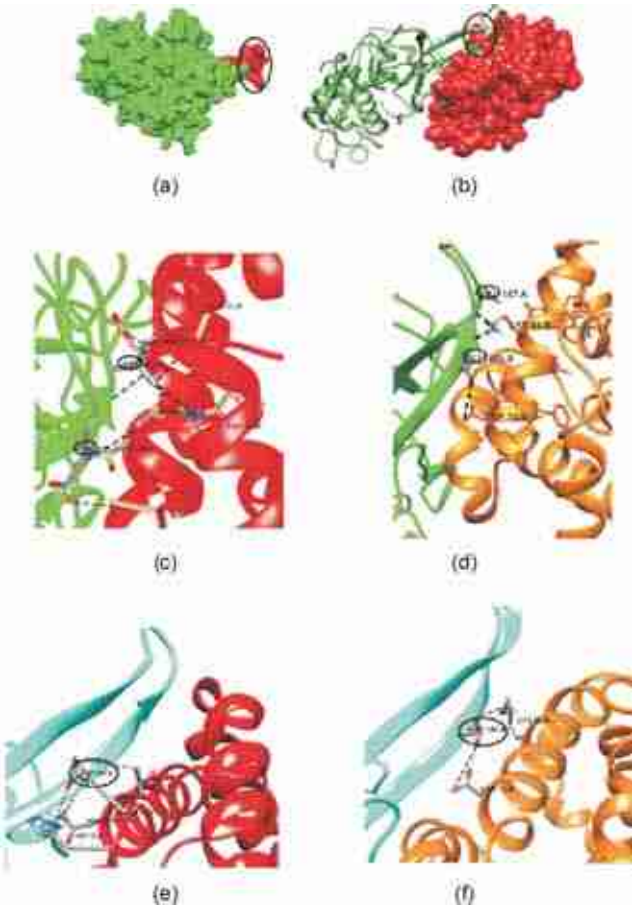


Fig. 14: Structural view of hemoglobin binding domain (C-terminus insertion) and its interaction: (a) FP2 showing hemoglobin binding domain (red); (b) A motif with an unusual 14 amino acid interacts with hemoglobin (monomer—red). The close view showing interactions of a motif of FP2 (green) with α chain (c—red) and β chain (d—orange) of hemoglobin. Similarly, interactions between C-terminus motif of FP3 (blue) with α chain (e—red); and β chain (f—orange) of hemoglobin has been shown.

Our structural (Fig.14) and mutagenesis studies suggest that hemoglobin interacts with FP2 via specific interactions and Val^{187} and Glu^{185}, present at the C-terminus of FP2, are essential for hemoglobin binding (Fig. 15). Since FP3 is also a major hemoglobinase and essential for parasite survival, its interactions with hemoglobin was further demonstrated. The results suggest that Asp^{194} of FP3 is functionally conserved and

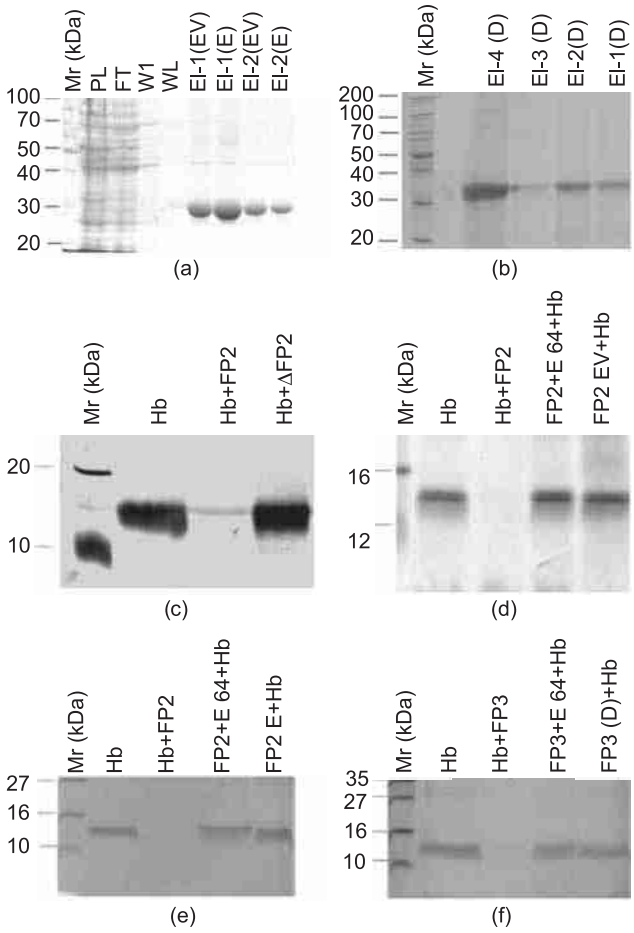


Fig. 15: Functional assays of mutants— Two mutants (E and EV) of FP2 were expressed, purified and refolded as described earlier: (a) Mutants were expressed in *E. coli* and purified by Ni-NTA chromatography using imidazole gradient. PL—Pre-load; FT—Flow-through; W1—Wash 1; WL—Wash last; and different—elutions of mutants were mentioned in the figure. The positions of molecular wt. markers (kDa) were indicated; (b) Similarly, a mutant of FP3; by changing Asp^{194} into Ala^{194} was expressed, purified by Ni NTA chromatography using imidazole gradient. Different elutions of mutants (EI-1 to EI-4) were mentioned in the figure. The positions of molecular wt. markers (kDa) were indicated; Different mutants ($^{\Delta}$FP2 (c); $^{\Delta EV}$FP2 (d); $^{\Delta E}$FP2 (e); $^{\Delta D}$FP3 (f); and wild enzymes (FP2 and FP3) were incubated with and without fluorogenic substrate in the presence of appropriate buffer. Hydrolysis of substrate was measured as fluorescent units (Fu). The error bars represent the standard error of two independent measurements, each performed in duplicate.

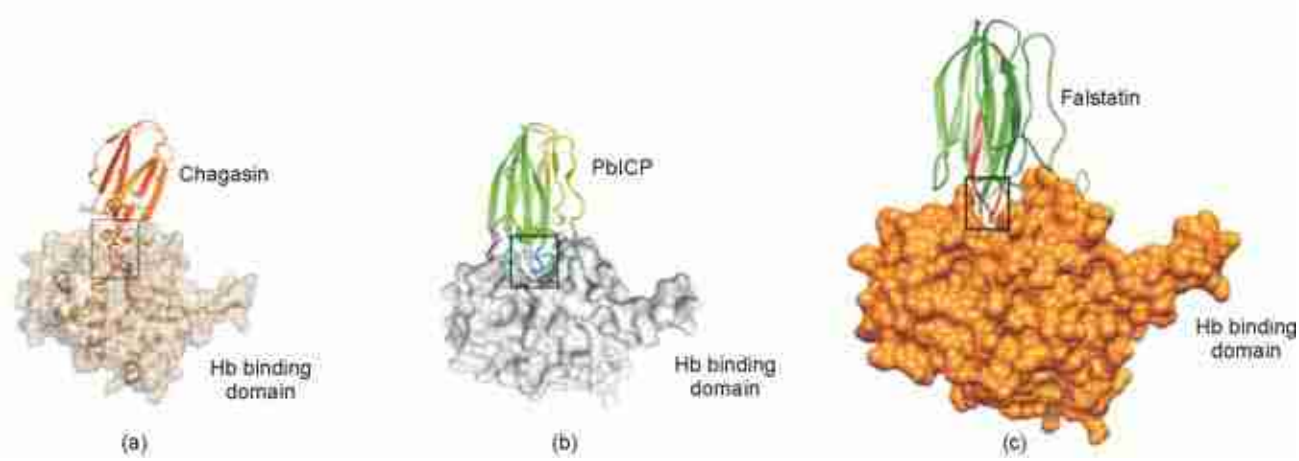


Fig. 16: Interactions of macromolecule inhibitors with cysteine proteases— Structure of Chagasin[43] (a); PbICP (b); and Falstatin (c) with FP2 were seen in the figure. The interacting loops of Chagasin, PbICP were shown in black boundaries. In case of Falstatin, only a BC loop (in red) was required for inhibition of FP2.

required for hemoglobin hydrolysis (Fig. 15f). In addition to its natural substrate, this study also focuses on the interactions between FP2 and its endogenous macromolecular inhibitor, Falstatin. Our previous study suggests that Falstatin inhibits falcipains by using only a BC loop (Fig. 16). In this study, there are evidences that multimeric units of Falstatin interact with 10 molecules of FP2 in a 1:1 stoichiometric ratio (Figs. 17 and 18). Targeting protein–protein interactions is a new field to explore in malaria. Therefore, new compounds that may block exosite mediated substrate interactions have gained interest as a novel class of inhibitors with enhanced selectivity and less likely susceptible to drug resistance.

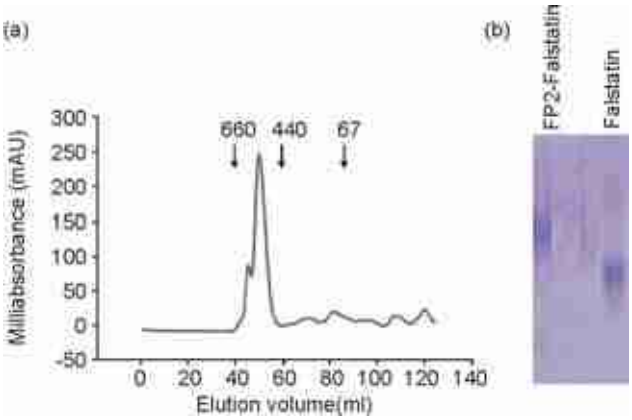


Fig. 17: Stoichiometric analysis of Falstatin-FP2—(a) An elution profile of Falstatin-FP2 complex was shown, and the positions of standard molecular weight markers were indicated (660, 440, 66 kDa). The apparent molecular weights for the Falstatin-FP2 complex and Falstatin alone were calculated as ~575 kDa and ~ 450 kDa, respectively; and (b) The complex of Falstatin-FP2 was also analyzed by native-PAGE analysis.

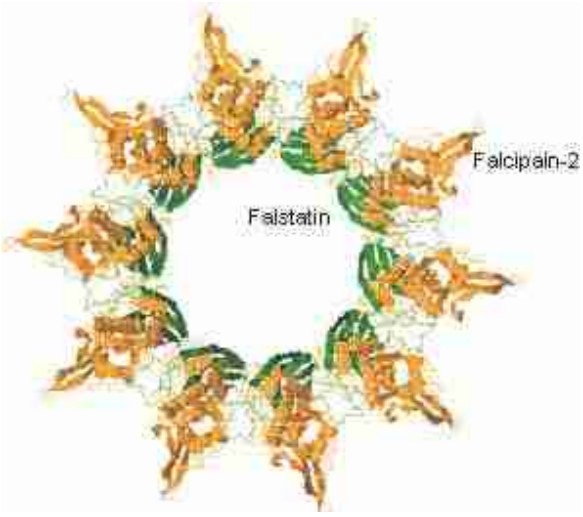


Fig. 18: Structural model of Falstatin-FP2— A model was prepared based on stoichiometric ratio. The multimeric units of Falstatin (10 mers) interacted with 10 molecules of enzyme with 1:1 stoichiometric ratio. The enzyme (orange) interacted with inhibitor (green) via BC loop.

2.6 Gametocyte production and expression of *Pfs25* gene in field isolates of *Plasmodium falciparum*

The malaria parasite life-cycle 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. This study was aimed to determine the subpatent population of *P. falciparum* gametocytes in their freshly cultured clinical isolates, and assessment of the ability of *P. falciparum* to produce gametocytes (*in vitro*) in natural infections and correlation of the expression of *Pfs25* gene in the isolates.

Fig 19: *Plasmodium falciparum* field isolates *in vitro* showing different gametocytes stages III, IV, and V.

The study involved collection of 20 field isolates, out of which only 12 isolates adapted in culture. After successful establishment of culture adapted samples, all the 12 isolates were put for gametocyte production *in vitro*. After 15 days of culturing in laboratory, 10 field isolates, *viz*. RNC 52, RNC 54, RNC 55, RNC 58, RNC 59, RAP 9, RAP 10, RAP 11, RAP 14 and RAP 16 produced gametocytes and various stages I-V were identified by microscopy. From Day 7 onwards (post sub-culture) different stages of gametocytes were seen in smears (Fig. 19). Among the 10 clinical isolates, four isolates (RNC 52, RNC 58, RAP 10 and RAP 16) harboured a higher frequency of gametocyte production in comparison to the other isolates. The expression of *Pfs25* gene in the adapted field isolates was also *in vitro* analyzed. It was observed that isolates which produce mature gametocytes *in vitro* also showed an increase in the *Pfs25* gene expression compared to the reference strain. The relative expression of the *Pfs25* gene in *P. falciparum* isolates ranged from 0.32 in RAP 11 to 4.56 fold in RAP 16 when compared to NF54 reference strain (Fig. 20). Also, the highest gametocytaemia was seen in RAP 16 and RNC 52 which showed a 4.56 and 3.34 fold increase in expression levels of *Pfs25,* respectively. Both these

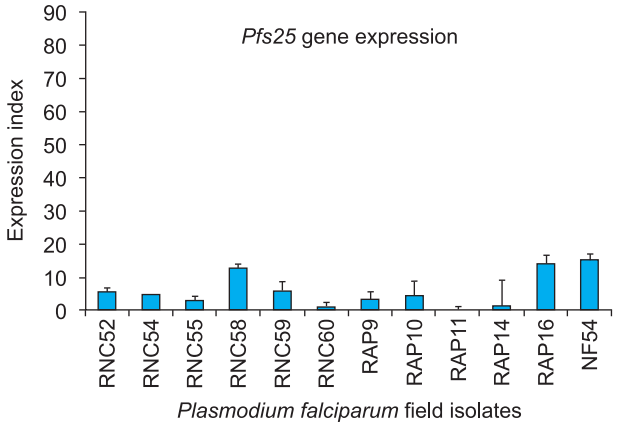


Fig 20: Gene expression pattern of *Pfs25* gene in 11 *P. falciparum* isolates when compared to the reference strain of *P. falciparum* NF54. Error bars indicate standard deviation and $p < 0.05$.

samples also showed high parasitaemia, *i.e.* 0.95 and 1.5%.

These results suggested that a correlation exists between *Pfs25* gene expression and the ability to produce gametocyte (mature) by the isolate. The results suggest that *Pfs25* gene has a role to play in the process of gametocytogenesis in the field isolates and disease transmission.

2.7 Studies on macrophage-T cells interaction in mouse model of malaria: Role of Th17 cells

It is well-known that the malaria parasite modulates both the wings of the immune system for its persistence. The outcome of a disease is believed to be dependent on both the innate as well as adaptive immunity. Immune response during lethal malaria parasite infection (*P. berghei*) and non-lethal parasite (*P. chabaudi*) was studied in the context with pro-inflammatory and anti-inflammatory cytokines. IL-12, a pro-inflammatory cytokine produced mainly by the professional antigen presenting cells (APCs), decreased in *P. berghei* infection, whereas non-lethal infection with *P. chabaudi* increased IL-12 production. The level of IL-1β was increased at the initial phase of infection, but gradually decreased in both *the P. berghei* and *P. chabaudi*-infected animals as the disease progressed as shown in Fig. 21. The production of IL-6 in *P. chabaudi* infection increased till Day 7 post- infection (PI) and decreased thereafter in both the *P. berghei* and *P. chabaudi*. However, much higher IL-6 was induced in *P. chabaudi* than that of *P. berghei*. The TGF-β level in *P. chabaudi*- infected animals was higher at initial phase of infection which rose further with the increase in parasitaemia and then decreased as the parasitaemia resolve. In contrast, level of TGF-β was lower at all time points of infection with *P. berghei*. The level of IL-10 was higher in mice-infected with *P. berghei* than observed in infection with *P. chabaudi*.

Further activation of T-cells requires ligation of T-cell receptor (TCR) with an antigenic peptide displayed with the major histocompatibility complex (MHC) molecules, and co-stimulatory molecules on antigen presenting cells (APCs). Binding of inducible co-stimulatory molecule (ICOS) to its ligand leads to the production of IL-10, which plays an important role in immune polarization. It was observed that on Day 10 PI,

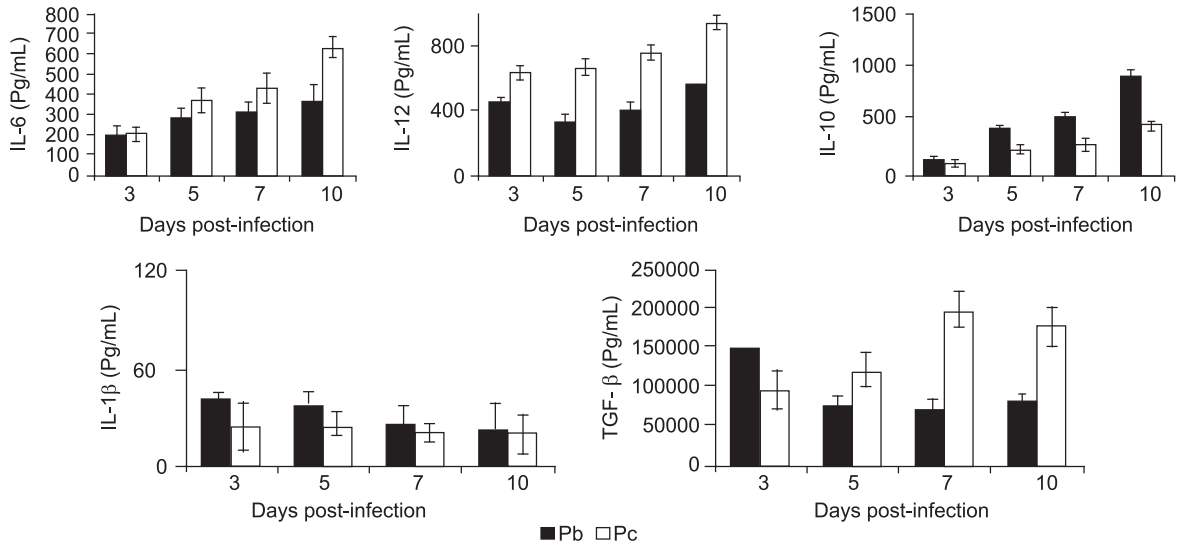


Fig. 21: Profile of pro-inflammatory and anti-inflammatory cytokine in serum secreted by various immune cells during infection with *Plasmodium* species (lethal and non-lethal) on Days 3, 5, 7 and10 post-infection using bead based array by Luminex.

large number of $CD4^+$ T-cells (~61%) expressed ICOS, while infection with non-lethal strain *P. chabaudi* induced expression of ICOS in ~31% $CD4^+$ T-cells. We also analyzed $CD11b^+$ macrophages cells for the CD40 expression, a pro-inflammatory co-stimulatory molecule as shown in Figs. 22a and b. It was observed that expression of CD40 molecule increased to 12% on macrophages on Day 10 PI with *P. berghei*. In contrast, *P. chabaudi* infection results in 2% expression on macrophages as shown in Figs. 22a and b suggesting that CD40 molecule might be helping in diseases exacerbation. It is known that $CD4^+ICOS^+$ cells expressing Foxp3 are highly

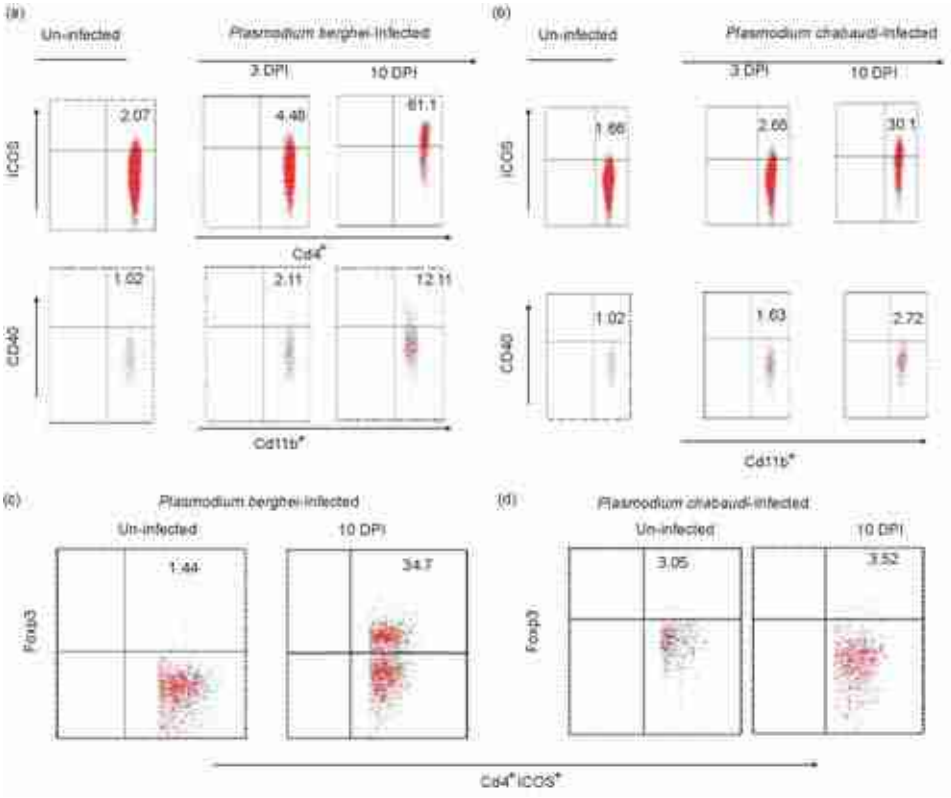


Fig. 22: Immune response in malaria-infected mice. Spleens cells stained with antibodies against CD4, CD11b, ICOS and CD40 on Days 3 and 10 post-infection of: (a) *P. berghei*; and (b) *P. chabaudi*. Treg cells were analyzed by flow cytometry staining with antibodies against CD4 and ICOS. Treg cells (gated on the $CD4^+$ $ICOS^+$ population) were also identified by staining for Foxp3 in (c) *P. berghei* infection; and (d) *P. chabaudi* infection.

immunosuppressive in nature and act as immunomodulatory T-cells. It was also observed that 50% of $CD4^+$ $ICOS^+$ cells express Foxp3 on their surface in *P. berghei* (Fig. 22c) and might be providing opportunity to lethal parasite to flourish in the host. However, expression of Foxp3 on $CD4^+ICOS^+$ T-cells was significantly low with self-resolving *P. chabaudi* (Fig. 22d).

Further, it was found that IFN-γ increased with the increase of parasitic burden in *P. berghei* infection, and a similar trend was observed during the course of infection with *P. chabaudi*.

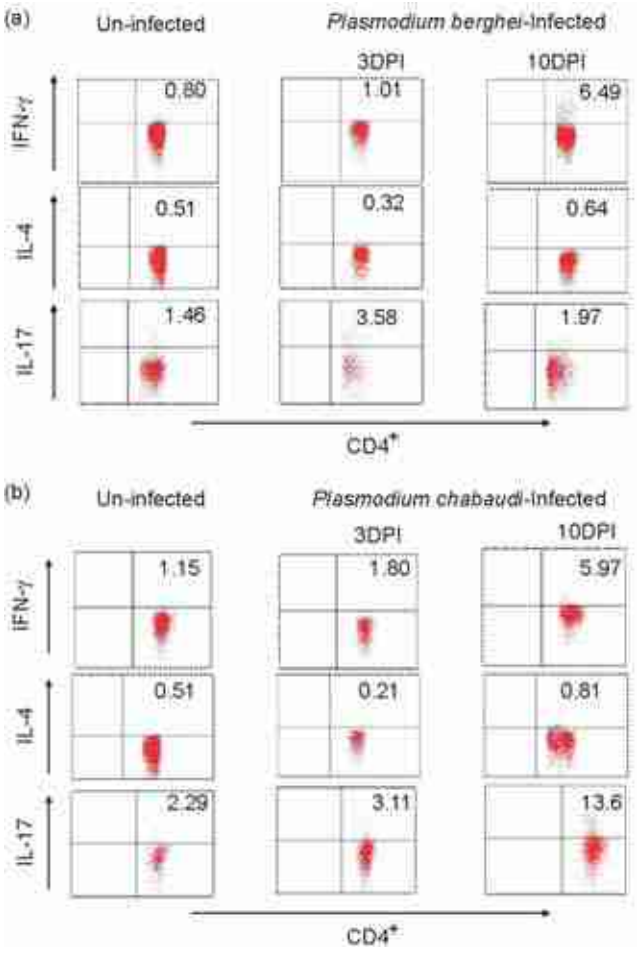


Fig. 23: Expression of cytokines IFN-γ, IL-4 and IL-17 checked by flow cytometer during the course of malaria infection on Day 3, and 10 post-infection— (a) *P. berghei;* and (b) *P. chabaudi.*

Plasmodium berghei infected mice show decreased IL-17 expression with increase in parasitic load, whereas in case of *P. chabaudi* infection expression of IL-17 increased even during decreased parasite load. No significant expression of IL-4 was observed in either case (Figs. 23a and b). Similar results were obtained with Luminex technique (data not shown).

2.8 Identifying the molecular marker(s) for relapse malaria in the *Plasmodium vivax*

Plasmodium vivax is a major public health problem in India. Relapse malaria has been reported with varying severity from India, indicating that there are different genotypes of *P. vivax* strains that cause the relapse. Therefore, there is need to understand the genotypes of *P. vivax* and their relation with relapse and new infection. All the 14 chromosomes of *P. vivax* were analysed and minisatellite markers were identified by Tandem Repeat Finder Version 4.07b. Initially four minisatellites were used for standardization. Of the four minisatellite markers, two were standardized and used for polymorphism analysis in *P. vivax* samples. Minisatellite marker from chromosome number one and chromosome number nine was used for molecular characterization. These minisatellite markers are highly polymorphic in nature. Polymorphic nature of these minisatellite markers suggests to imply for relapse and new-infection analysis of *P. vivax* (Fig. 24).

Fig. 24: PCR amplification of minisatellite marker showing base size polymorphism (Sample: Lane 1 to 24; M: 20bp DNA ladder).

❑

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 a multi-institutional Indo-Danish collaborative project being funded by the Department of Biotechnology, Government of India. NIMR's role in the project was to study epidemiological parameters from high and low transmission areas for which field visits were made in high (Jharkhand) and low (Haryana) malaria endemic areas for selection of study sites. Accordingly, Village Dumergarhi with a population of 913 spreaded over six hamlets under Angada CHC of Ranchi district, Jharkhand was selected from high transmission area, whereas Village Jujhuka with 600 population under Ujhina PHC of Mewat district, Haryana representing low endemic area was selected for immuno-epidemiological studies. Demographic details of the study area were compiled and patient codes were assigned to study individuals to maintain confidentiality and management of computer database.

A detailed work package for the age and exposure-related immuno-epidemiological profiling of IgG reactivity against malaria vaccine candidates was finalized. Sample size of the study population was worked out based on incidence rate. Accordingly, a cohort of 300 children (3–15 yr) and 100 adults (>15 yr) were enrolled in the study from high and stable malaria area of Jharkhand while 300 individuals of all the age groups were enrolled from low endemic area.

Detection of malaria cases through active and passive surveillance was carried out at regular intervals. Malaria prevalence surveys were carried out in low endemic study area of Mewat, Haryana during transmission season July-August 2014; and in high endemic study area of Jharkhand during October–November 2014. During the cross-sectional surveys, 142 individuals from low (Mewat, Haryana) and 388 persons from high transmission area (Ranchi, Jharkhand) were examined for malaria parasite irrespective of any clinical symptoms. The parasite rate in the low and high transmission area was found to be 2.8 and 46.6%, 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 the protocol. Further, studies on the malaria transmission dynamics are in progress.

3.2 Comprehensive case management pilot programme in Odisha, India

Comprehensive case management programme (CCMP) is an operational research study under the programmatic conditions. It is being implemented collaboratively by the Government of Odisha, National Institute of Malaria Research and the 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 focussing the districts: Dhenkanal (meso endemic), Bolangir (low endemic), Angul (high endemic) and Kandhamal (hyper endemic) (Fig. 1). Each district includes an intervention and control block (population of about 100,000).

The core components of the 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, namely introduction of microscopy at the primary health care centres (PHCs), use of patient cards for

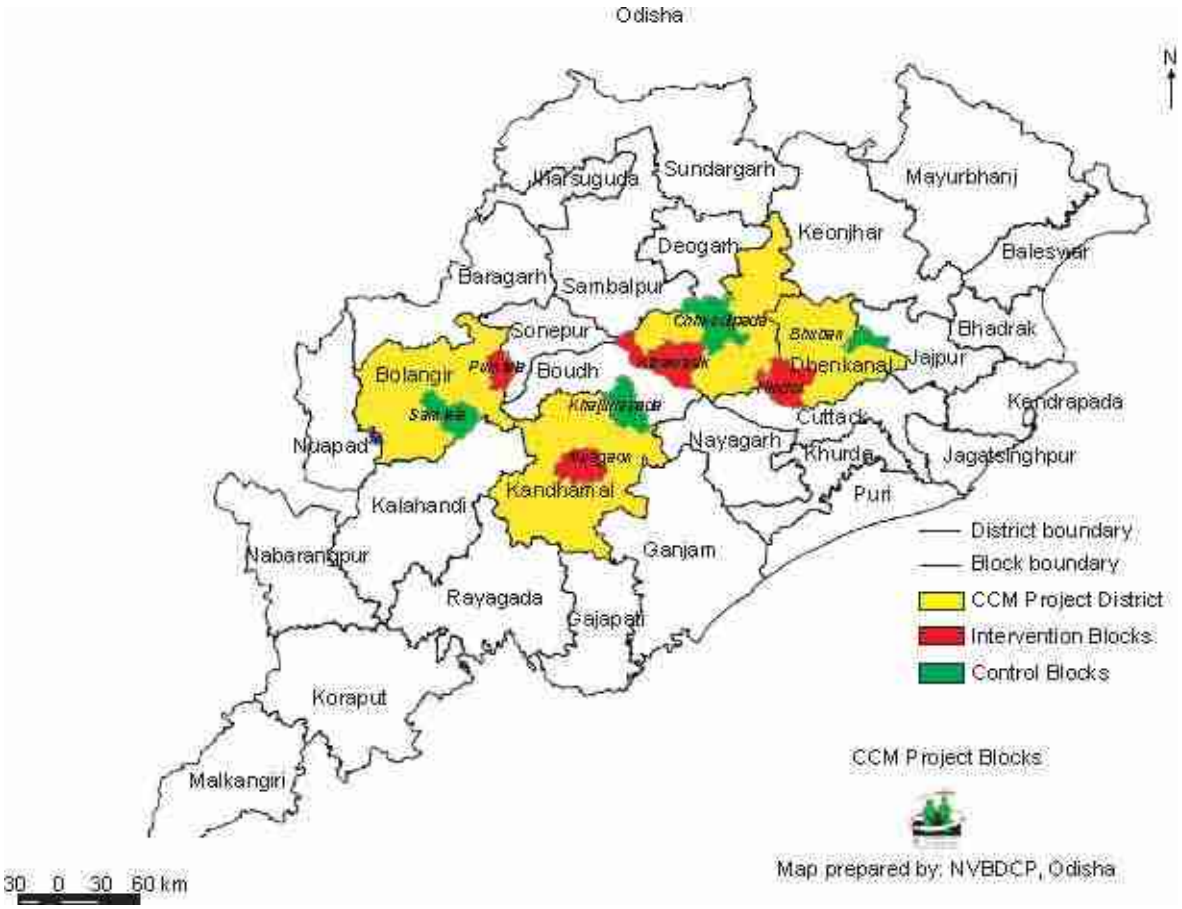


Fig. 1: Study areas for comprehensive case management programme in Odisha, India.

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.

CCMP approach is showing expected results in low endemic block. In other blocks, there has been a significant improvement in surveillance and early diagnosis and treatment which has permitted outbreak control and preventing complications (Fig. 2).

The capacity of ASHAs, who are an integral part of health delivery system at the community level, has been enhanced significantly with continuous capacity building on malaria diagnosis, treatment, case reporting and follow up. In areas, where ASHAs presence is weak, other volunteers, mainly *Anganwadi* workers (AWWs) have improved access to malaria services in hard to reach and low surveillance areas.

More than 90% of the malaria patients were followed up for complete treatment. Most cases are now diagnosed and treated at the ASHA level. The

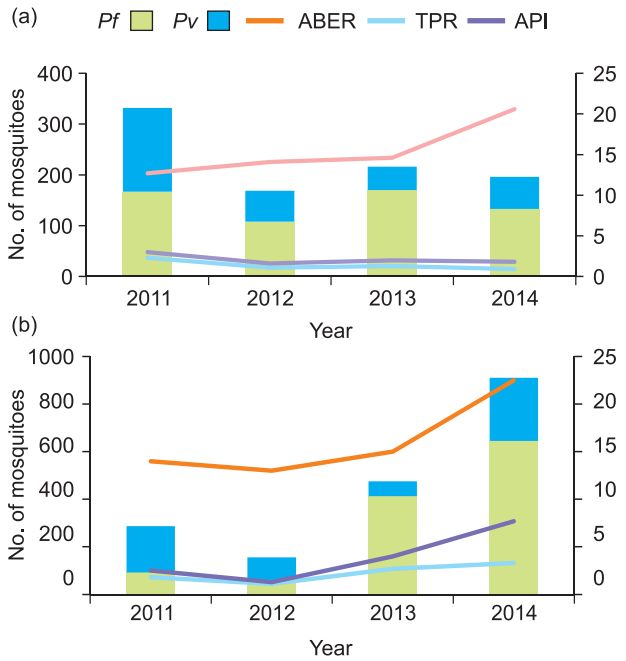


Fig. 2: Malaria status in (a) Puintala (intervention) and (b) Saintala (control) blocks of District Bolangir, Odisha. *Pv*– *Plasmodium vivax*; *Pf*– *P. falciparum;* ABER– Annual blood examination rate; TPR–Test positivity rate; API– Annual parasite incidence.

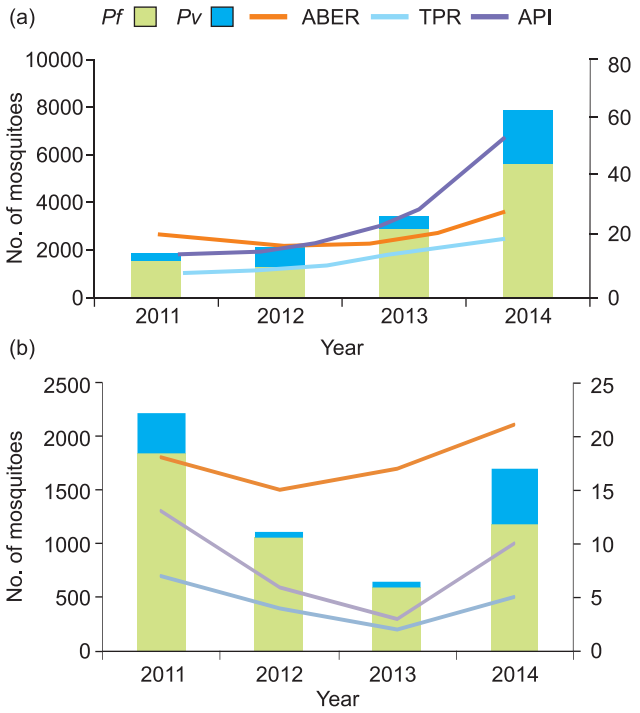


Fig. 3: Malaria status in (a) Madhapur (intervention) and (b) Kosala (control) blocks of District Angul, Odisha. *Pv– Plasmodium vivax; Pf– P. falciparum;* ABER– Annual blood examination rate; TPR–Test positivity rate; API– Annual parasite incidence.

annual blood examination rate (ABER) has increased in 2014. The time from onset of fever to treatment has decreased with the larger proportion receiving treatment within 24 h of onset of symptoms. In all the intervention areas the number of cases detected has increased except in the low endemic blocks of Bolangir which witnessed a 74% reduction in incidence from 2011 to 2014.

Improved surveillance including analysis by the sub-centre has helped in targeting efforts. An increase in positive cases in certain areas alerted the team, and prompted a mass survey which revealed asymptomatic reservoirs in these areas.

CCMP has led to a significant increase in access to diagnosis and treatment in all the intervention areas. It is too early to assess impact because surveillance is still improving in hard-to-reach areas. However, in the low transmission settings, improved case management is showing impact by way of reduced malaria.

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

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

During the year 2014–15 (14 April to 15 March), NIMR Study Centre, Jabalpur surveyed 160 villages of eight districts under nine dam projects in Narmada Basin area. In this cross-sectional survey, 438 blood slides were examined in the villages by the NIMR team. Rapid diagnostic kit was also used for diagnosis of symptomatic patients. A total of 25 cases (4 *P. falciparum* and 21 *P. vivax*) were found malaria positive which were treated immediately and report was discussed with the concerned health centre for follow up. Highest man hour density (46) of *An. culicifacies* was recorded in Matiyari dam project. Total five drinking water samples were tested using HiWater™ Test Kit (HiMedia), and all the samples were found positive for different microbes, viz. *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus*. In all, 109 IEC activities were held in different villages situated at different dam projects. Two health stalls were set up, and different slogans were painted on 10 walls in Narmada Basin area for spreading awareness about the vector borne diseases and their possible control. Along with this, 73 ASHAs/AWWs were given training to control vector borne diseases in the projected area.

Similarly, NIMR Study Centre, Bhopal surveyed 225 villages of seven districts under 10 dam projects in Narmada Basin area. In a cross-sectional survey, 752 blood slides were examined in the villages by the NIMR team, during April 2014 to March 2015. RDKs were also used for diagnosis of symptomatic patients. Total 20 cases (16 *P. falciparum* and 4 *P. vivax*) were found malaria positive which were given treatment immediately and reports were sent to the concerned health centre for follow up. Highest MHD (93) of *An. culicifacies* was recorded in proposed Shakkar project area. Total nine drinking water samples were tested using HiWater™ Test Kit (HiMedia), out of which only three were found positive for different microbes, viz. *Salmonella typhimurium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus*. Six water samples were safe for drinking purpose. Nine health camps were organized and slogans were painted on five walls

in Narmada Basin area for the awareness of vector borne diseases and their possible control. Along with this, 44 ASHAs/ANMs (Auxiliary nurse midwives) were given training to control vector borne diseases in the projected area.

NIMR Study Centre, Narmada Nagar, Khandwa surveyed 287 villages of six districts under 10 dam projects in Narmada Basin area. In this cross-sectional survey, 1302 blood slides were examined in the villages by NIMR team during April 2014 to March 2015. RDKs was also used for diagnosis of symptomatic patients. Total 45 cases (16 *P. falciparum* and 29 *P. vivax*) were found malaria positive which were treated immediately and report was discussed with the concerned health centre for follow up. Highest MHD (122) of *An. culicifacies* was recorded in the main project area. Total 30 drinking water samples were tested using HiWater^{TM} Test Kit (HiMedia), out of which only 29 were found positive with *Salmonella typhi-murium, S. enteritidis, Citrobacter freundii, Vibrio cholerae* and *V. parahaemolyticus*. One water sample was safe for drinking purpose. A health camp was organized and slogans were painted on 13 walls in Narmada Basin area for the awareness of vector borne diseases and their possible controls. A total of 131 ASHAs/ANMs were given training to control vector borne diseases in projected area.

Some engineering problems were found in the form of 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 gutters surrounding the houses, *etc.* It results in high breeding potential of vectors and vector borne diseases. Control measures were suggested to Narmada Valley Development Authority (NVDA) and the state health department, *i.e.* de-weeding, introduction of larvivorous fishes, channelization of pools in main river and larvicidal spray to control the breeding. Health camps were organized involving health department in Narmada Basin area for the awareness of vector borne diseases and their possible controls.

The information of blood slides and water testing was given to the concerned health centre for immediate action. In each survey, detailed recommendations were submitted to NVDA and the state health department for necessary action for control of vector borne diseases.

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

Since, the first survey (November-December 2010) in 22 villages situated nearby NMC distribution network system, entomological and epidemiological surveys have been carried out so far in selected 64 villages within the command areas of NMC encompassing two control villages Meer-pura 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 were recommended. The releasing of larvivorous fish was started in February 2012. Meetings were organized with the concerned authorities to follow up the mitigating measures and to pursue recommendations.

After April 2014, interventions in 52 diggies were

Fig. 4: Larval positivity in diggies.

implemented and 12% positivity was found, with a larval density of 0.89 per dip by the end of quarter. In subsequent rounds carried out from August to November 2014, positivity found was 11.5%; from December 2014 to March 2015, positivity found was 19.2%, and larval density reduced to 0.71 per dip (Fig. 4).

3.5 Spatio-epidemiological analysis of dengue in Delhi

The aim of the present study was to investigate and evaluate the epidemiology of dengue infection and to estimate the rate of asymptomatic and symptomatic dengue infection in Delhi. A prospective community-based descriptive study of dengue asymptomatic cases was conducted in localities reporting index dengue cases as reported by MCD during the study period. Around 50 index cases, total 2125 individuals with or without dengue febrile illness, were finger-pricked and serologically confirmed as dengue positive and negative by using SD Duo Bioline Rapid Diagnostic Test (SD Inc, Korea) with NS1, IgM and IgG combo test, which detected dengue-specific antibodies Fig 5. Out of 2125 individuals, 768 (36.1%) individuals showed dengue test positive with past (25.5%), primary (1.88%) or secondary (8.8%) dengue infections. Higher percentage of IgG was found in age groups, 15–24 yr and 25–50 yr (36% each). Infants <1 yr presented higher incidence of new infections (22% of NS1 + IgM positives). Further, analysis revealed that out of the 226 newly infected cases (including NS1 and IgM positives), 142 were asymptomatic (63%) as per the WHO guidelines. The findings also suggest that 10.6% of the total population screened, conferred either primary or secondary dengue infection. All the three economic categories (high, medium and low) were found having 60, 55.6 and 70% of asymptomatic cases, respectively. Finger-pricked blood samples of all

Fig. 5: Team conducting a serological survey on households and neighbourhood.

Fig. 6: Entomological survey (*Aedes* breeding containers).

the individuals were also taken on filter paper for dengue viral confirmation by RT-PCR.

A door-to-door entomological survey (Fig. 6) was carried out to find out the *Aedes* breeding in all the types of water filled containers present in and around houses and their premises, and immature stages of *Aedes* mosquitoes were collected. In larval survey, different indices were used to record *Ae. aegypti* density level. In all the localities surveyed during transmission season, solid waste was observed to be most preferred breeding site, whereas overhead tanks (OHTs) and curing tanks were found to be the most preferred breeding containers during non-transmission season. Plastic containers (29%) in low income group (LIG); solid waste (27%) and plastic containers (26%) in medium income group (MIG); and solid waste (27%) and curing tanks (21%) in high income group (HIG) were found as the most preferred breeding containers for *Ae. aegypti*.

3.6 Isolation of dengue virus from *Aedes aegypti* in Delhi

On the request of Municipal Corporation of Delhi (MCD), New Delhi Municipal Corporation (NDMC) and Delhi Administration, *Aedes* breeding surveys were carried out in Delhi. As recommended by the Scientific Advisory Committee (SAC), a laboratory was established for detection of dengue virus in *Aedes* mosquitoes in January 2013 which became functional in July 2013. Training in IFA technique was also taken by two of the staff members from NIV, Pune. During 2014–15, a total of 2408 female and 1206 male *Ae. aegypti* were collected and tested by IFA technique. Samples were either provided by MCD or collected from different localities of Delhi, categorized in three economic groups, *i.e.* High, Medium and Low. Out of 2408 female *Ae. aegypti*, 14 were found positive with minimum infection rate (MIR) of 5.8 (Fig. 7).

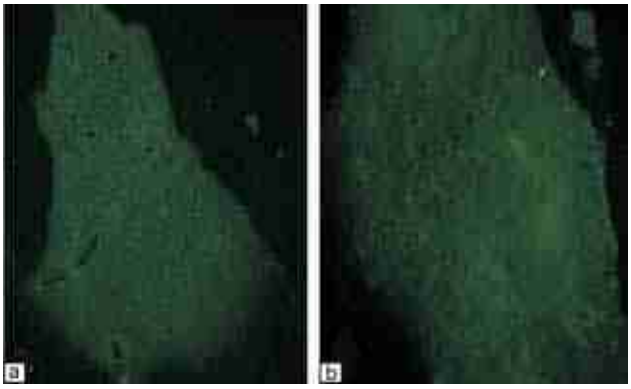


Fig. 7: IFA test performed on the head portion of the virus infected *Ae. aegypti* using monoclonal antibodies specific for dengue showing fluorescence collected from localities with highest MIR– (a) Buddh Vihar; and (b) Najafgarh (Jai Vihar) in Delhi.

Out of all 18 study areas, 11 localities were found positive for dengue virus infection and infection rate of *Ae. aegypti* mosquitoes was expressed as MIRs. LIG showed highest (9.8) mosquito infectivity followed by MIG (6.2) and HIG (1.3). No vertical transmission of dengue virus could be detected in 1206 male *Ae. aegypti* collected. Further, it is proposed to detect dengue virus in *Aedes* mosquitoes in non-transmission season which would help MCD in making dengue control strategies in Delhi.

3.7 Evidence-based assessment of biophysical determinants of malaria in the north-eastern states of India and development of framework for adaptation measures for malaria control under climate change scenario

It was a multidisciplinary, multicentric and multiinstitutional study for generation of evidence of change in climatic conditions as well as on anopheline vectors and malaria so as to suggest adaptation measures for addressing the adverse impact of climate change. The study was

undertaken at Nainital and Almora in Uttarakhand; Ramgarh and Ranchi in Jharkhand; Karbi-Anglong in Assam; and Kolasib and west Aizwal in Mizoram. Data on indoor/outdoor temperature, mosquito density, fever survey, detection of sporozoites from field collected mosquitoes, socioeconomics of communities and current adaptation measures being taken by the health system were generated.

Based on generated daily data of temperature at all the study sites, it was found that over a span of three years, changes in temperature were noticeable in study areas of Karbi-Anglong and Almora districts *etc.*, up to the tune of 2.69°C. Even decrease in temperature was also observed in Uttarakhand in winter months. Study provided insight for significance of using indoor temperature versus outdoor for determination of transmission windows and provided evidence for difference in temperature of breeding habitats (water body) and resting habitats of mosquitoes. Based on daily generated data of temperature, the days required for completion of sporogonic cycles could be determined which provided answer to high malaria endemicity in Ranchi (Jharkhand) against Bokajan (Assam). *Anopheles minimus* was encountered in Bokajan only on a few occasions; *An. culicifacies* were collected in high density (up to 82) from few villages which were linked to deforestation. Detection of *P. falciparum* in the month of February from Nishola and Bhorsa villages in Bhimtal PHC (hilly area) in Tadikhet (Almora), where from malaria is not reported by local health authorities, but provides evidence of knocking of malaria transmission in hilly areas. Sporozoites of *Plasmodium* were detected in *An. culicifacies* in May 2011 from Nainital district and *An. fluviatilis* collected in the month of October 2010 and November 2011 from Almora district, respectively indicating ongoing transmission when outdoor temperature was not conducive. The projection of probable months of malaria transmission by mathematical model based on generated data of temperature in the study villages and inputs from PRECIS model elicited that in 2030 and 2070 there is projected increase in TWs by 2–3 months. Based on Genetic programming mathematical model, the density of *An. culicifacies* was predicted for some villages under Nainital with R^2 values between 0.7 and 0.94 for observed and predicted density. Socioeconomic survey elicited through interview schedules (596) from two field sites revealed deficient knowledge, poor health seeking behaviour, non-acceptance of IRS and less use of bed nets, *etc.* at all the sites. Dissemination workshops were conducted at Bhimtal, Ranchi and Bokajan to disseminate the findings of the project to concerned state governments and lectures were delivered for building the capacity of stakeholders with latest developments in malaria epidemiology and control.

The overall findings revealed that climatic conditions are changing as compared to 1960 and 1990 and hilly areas are showing evidence of malaria transmission even in the month of November and January when outdoor temperature is not conducive. Current adaptation measures in practice at three field sites were assessed and scope of capacity building and strengthening of health system were identified for addressing the adverse impact of climate change.

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

The specific objective of the project was to generate risk maps of malaria from the viewpoint of malaria prevalence, climatic determinants, anopheline vectors' distribution and ecological risk.

Maps of hotspots of malaria, based on temperature and malaria endemicity provide insight that linearly progressing temperature is not likely to increase in high intensity of malaria. For determining the cut-off of rainfall for causing outbreak, it was found that the range of rainfall varied from 100-500 mm with varying lag period, *i.e.* month-to-month to three months lag.

The ecological risk at village level was determined based on IRS P6 LISS IV images, for one month before malaria peak in Geedam and Katekalyan blocks of Dantewada district, Chhattisgarh using supervised classification. Ground truth for satellite images was done by field visits in Dantewada and Koraput districts of Chhattisgarh and Odisha states, respectively. The criteria of labelling high and low malarious areas were water-bodies >1%, forest >35%, scrub land <30%, and fellow land <30% with further characterization of water-bodies, *i.e.* streams were related with high malarious areas. On the basis of the criteria, 16 sub-centres (out of 20) of Geedam block and all 12 sub-centres of Katekalyan block were identified as 'High' malaria potential risk sites, and only 4 sub-centres of Geedam were identified

as 'Low' malaria potential risk sites. There was mismatch between ecological risk mapping and reported API which was due to lack of surveillance in hard to reach areas which were ecologically suitable for high malaria. The urban centres, though ecologically not suitable reported high malaria.

A rapid assessment was undertaken to find out the reason of low malaria in Palakkad, Mallapuram and Kasargod districts of Kerala state. River bed pools were the major breeding habitats for anophelines while wells, small drains, cement tanks, seepage water bed pools, sintex tanks, overhead tanks, underground tanks, construction sites, tyres, discarded/broken containers, coconut shells and rubber latex were found suitable for *Aedes* mosquitoes. Malaria vectors *An. culicifacies* and *An. fluviatilis* were found only in Palakkad district. No case of malaria was detected. Work is in progress.

3.9 Malaria outbreak investigation at Tripura

The findings of investigation of malaria outbreak undertaken by the teams from RMRC, Dibrugarh and NIMR Field Unit, Guwahati were compiled and analyzed in consultation with the national programme. Additionally, the impact of satellite-derived temperature condition index (TCI) and vegetation condition index (VCI) in exacerbating the outbreak was also analyzed. The role of TCI in the month of April was found crucial in outbreak.

❑

Clinical Research

4

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

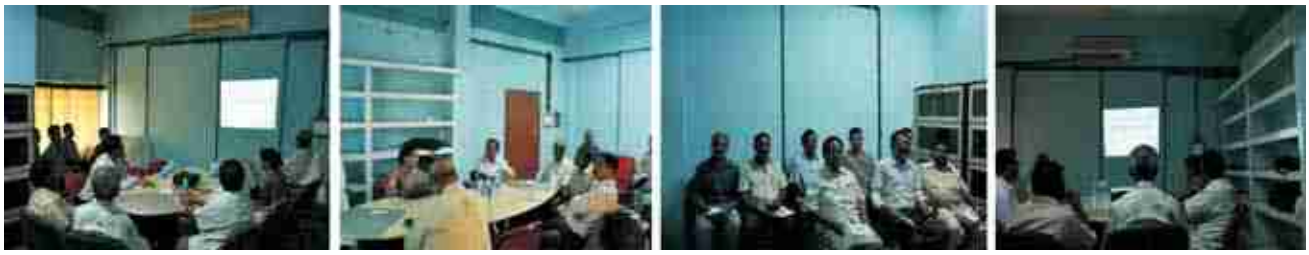
The National Institute of Malaria Research (NIMR) is involved in Quality Assurance programme for Malaria RDTs since 2009 and has been lot-testing the RDTs which are supplied to the NVBDCP. These RDTs are tested according to the WHO standard operating procedures (SOPs). The RDTs are subjected to pre-dispatch and post-dispatch testing. This project aimed at WHO recognition among Quality Assurance laboratories established and also to link with other lot-testing laboratories, so as to give credibility to the results of monitoring quality of malaria RDTs through external Quality Assurance scheme.

The procedures for the panel preparation were as per the current SOPs of the WHO. The WHO consultant has also visited the NIMR laboratory and the field collection site during panel collection and testing procedure, and the proficiency testing of NIMR was also carried out for malaria microscopy and PCR. Till date, 41 panels have been prepared including 25 *Plasmodium vivax* and 16 *P. falciparum* panels. These panels were characterized at an independent laboratory. The project has helped to build and incorporate an improved Quality Assurance programme of malaria RDTs. Before initiating the activities of panel preparation, proficiency testing for microscopy and PCR was conducted.

RDT lot-testing.

QA panels prepared from endemic areas.

Lot-testing in progress.



Orientation meeting at Senior Regional Director Office Guwahati

The samples were tested for HIV, hepatitis B virus and hepatitis C virus before processing further. Mono-infection with *P. falciparum, P. vivax* was confirmed by diagnostic PCR. Total 19 panels were characterized for HRP II and pLDH levels at Hospital Tropical Diseases, London.

Consultant from RITM, Manila visited NIMR and the sample collection site to train the staff and also to monitor the procedures.

Lot-testing of 10 RDT lots was conducted using the WHO SOPs. A total of 58 RDTs were tested from each lot using pre-qualified QC panel of *P. falciparum* and *P. vivax* with lower range of parasitaemia, i.e. 200 parasites/µl of blood. Among this 10 RDTs were tested with negative panels. Four QC panel of each *P. falciparum* were used for performing lot-testing for six RDTs with 200 parasites/µl QC aliquots (total 24 RDTs).The same procedure were used for *P. vivax*.

The RDTs were received through the national programme and regulatory authorities of India for lot-testing.

A consultant appointed by the WHO, visited the laboratory for assessing the performance as per the WHO SOPs.

4.2 Routine surveillance of the efficacy of two combinations in three sentinel sites across international borders of India

NIMR has played a pivotal role in guiding the national drug policy for treatment of malaria in India. Recent findings of high treatment failures of artesunate + sulphadoxine-pyrimethamine (AS + SP) in the northeastern (NE) states in studies conducted by NIMR led to change of treatment policy for *P. falciparum* to co-formulated tablets of artemether-lumefantrine [ACT-AL] in NE states.

To assess the safety and efficacy of newly introduced artemisinin-based combination therapy-Artemether Lumefantrine (ACT-AL), studies were

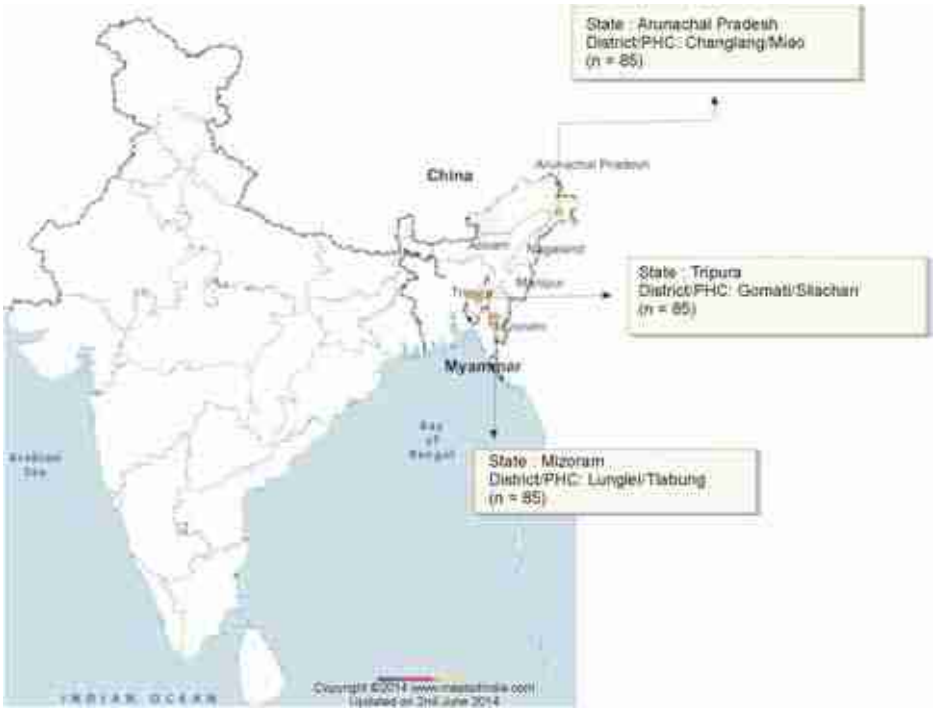


Fig. 1: Study sites evaluating therapeutic efficacy of ACT-AL.

initiated in April 2014 at three sites of NE states spread across International borders of the country. This was a one-arm prospective evaluation of clinical and parasitological response to directly observed treatment of ACT-AL in uncomplicated *P. falciparum* malaria. The study sites include Lunglei district (Mizoram), Changlang district (Arunachal Pradesh) and Gomati district (Tripura) in NE region (Fig. 1).

A total of 255 patients (85 from each site) satisfying the inclusion criteria were enrolled during the study period. All the eligible patients were between the age groups of 2 and 65 years. The parasitaemia ranged from 1040–99720/µl of blood and 80% patients had fever on Day 0. These studies have shown that the efficacy of ACT-AL ranged from 88.4–100% at three sites.

Mutation analysis was done in all the samples collected from three study sites. A total of 254 samples were subjected for mutation analysis in *Pfmdr1*gene (codons 86, 184 and 1246). The majority of the samples showed wild type (57.5%) followed by mutant (26.8%) and mix type (13%) pattern. For all the three codons, 2.8% samples could not be amplified.

The 76T mutation in *Pfcrt* gene was observed in higher frequency with highest in Changlang district in Arunachal Pradesh. The higher prevalence of 76T mutant in *pfcrt* at this site is probably due to the selection pressure by chloroquine (CQ), which is being used as first-line treatment for *P. vivax* cases, present in 97.6% cases at this site.

4.3 Effective and safe interventions for prevention of malaria in pregnancy in India: An assessment of burden of malaria 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 is 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 blocks) in Jharkhand state (Fig. 2)

In each block, all the sub-centres were randomly allocated to the control (PCD) or intervention arm (IST). Women attending antenatal care (ANC) 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

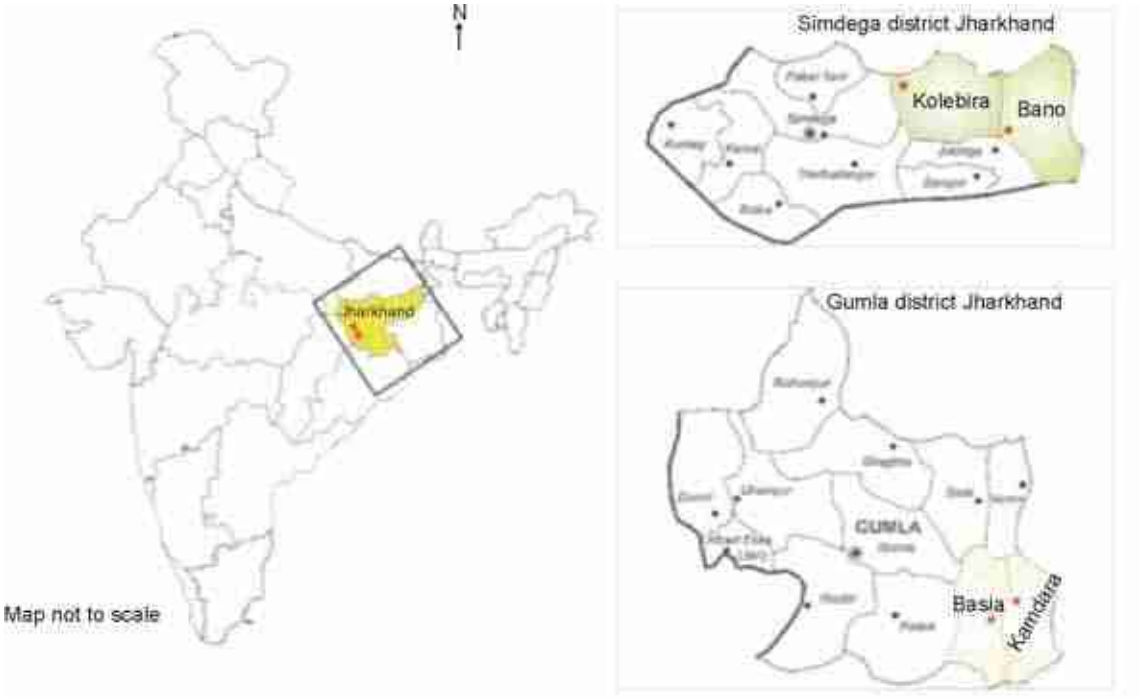


Fig. 2: Map of study area in the Jharkhand state.

using an RDT test at each ANC visit. Women were offered free delivery in the Study Hospital (St. Ursula Hospital, Konbir-Noatoli). All the women and child pairs were followed up after 2, 4 and 6 months post-delivery.

A total of 6859 pregnant women were enrolled, out of which, 5441 women had delivered and a total of 3136 (57.6%) placental biopsies were collected. The results of the interim analysis of data of women enrolled and delivered showed that IST is beneficial in comparison to PCD.

Data collection for component B was completed in November 2014 and data analysis will be completed by June 2015.The health care personnel as well as the community of Murhu block showed a very positive response to IST implementation. Across all the data collection methods, adherence to national guidelines for malaria case management and maternal health services was inadequate.

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

4.4 Biochemical and molecular analysis of G6PD deficiency in selected sites of India

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic disease among humans; affecting around 400 billion people worldwide. The main aim of this study was to explore biochemical and molecular aspects of G6PD deficiency and pattern of excessive haemolysis in malaria positive G6PD deficient patients treated with few antimalarial drugs like primaquine (PQ).

A total of 420 patients were screened for the G6PD deficiency out of which, 214 patients from Civil Hospital, Tlabung under Lunglei district of Mizoram and 206 patients from Rajapur PHC, District Ghaziabad, Uttar Pradesh were included in the study. Out of 420, 11 patients were found to be G6PD deficient (only from Mizoram site). Out of 11 G6PD deficient patients, blood filter papers were collected by finger-prick for the molecular studies except one patient (loss to follow up). A total of 16 samples (10 deficient and 6 non-deficient samples) were subjected for PCR-RFLP to detect the known mutations in G6PD gene in field isolates. All the samples showed wild type pattern for G6PD Mediterranean variant and G6PD Odisha variant.

For *in vitro* haemolysis, samples were collected from NIMR Clinic. In all, 13 samples were G6PD non-deficient and one sample was G6PD deficient. Haemolysis at normal dose with PQ was observed in very few samples but percent haemolysis increased with increasing dose; however, more observations are required to confirm the dose dependent increase. Haemolysis percent variation was also observed in G6PD non-deficient samples; thus factors influencing variation needs to be accounted for such observations. Haemolysis was also observed in G6PD deficient sample; however more number of deficient samples is required for conclusive result.

4.5 Studying artemisinin resistance in selected malaria endemic sites of India

Antimalarial drug resistance in *P. falciparum* malaria is the major cause of concern in fighting malaria problem throughout the globe. Currently, ACT is administered for the treatment of uncomplicated falciparum malaria based on WHO's recommendations. Resistance to artemisinin has already been reported from Pailin, western Cambodia which is marked by delay in parasite clearance time (PCT) after administration of artesunate.

In an effort to foresee decreasing artemisinin susceptibility in selected sites of India, this study was planned, where *P. falciparum* infected patients were treated with ACT (AS + SP) at sites from Odisha and Arunachal Pradesh states of the country and followed up to Day 42 to monitor the clinical outcome. Thin and thick blood smears and filter paper blood spots were collected on Days 0, 1, 2, 3, 7, and then weekly up to Day 42 of follow up. PCT was monitored over a period of three days on Days 0, 1, 2 and 3 by microscopy and real time PCR. Results obtained were compared and representative samples were analysed for important SNPs in *Pfatpase6* gene and copy number variations in *Pfmdr1* gene, the putative candidates related with artemisinin resistance.

Out of the total 72 patients enrolled at Bisra Community Health Centre (CHC), Sundergarh, Odisha, 64 patients could complete the study while others were withdrawn due to loss to follow up (LFU). All the patients were cured at the end of the

follow up attaining 100% adequate clinical and parasitological response (ACPR). In total, 43 patients having parasitaemia >10,000/µl of blood were analysed for real time measurement of PCT and results were compared with microscopic measure of PCT. At Miao Public Health Centre (PHC), Changlang, Arunachal Pradesh (n=42), nine samples were found out to be *Pf* reinfection, four were late parasitological failure (LPF) while two were late clinical failure (LCF). Therefore, high failure rate of 21.4% was observed in the isolates from the site of Arunachal Pradesh. Real time PCR was done on 27 of the total 42 samples which included all the failure samples (n=6) and those which had parasitaemia ≥10,000/µl of blood.

The results revealed that 12 samples at Odisha study site (n=43), while three samples at Arunachal Pradesh study site (n=27) have increased PCT according to the combined results of microscopy and real time PCR. The correlation of delayed PCT with high initial parasitaemia and Day 3 positivity confirmed by diagnostic PCR added weight to the findings. The results indicate that real time PCR can be deployed as an additional and useful tool in artemisinin resistant studies and that the drug should be preserved effectively for delaying resistance and combating malaria.

4.6 Effect of residual antimalarials in malaria patients enrolled for therapeutic efficacy studies and its effect on spread of drug resistant parasites in high malaria endemic districts in India

Emergence of antimalarial drug resistance is a major problem for the treatment of malaria. Post-treatment prophylaxis of long acting antimalarial drugs, self-intake of antimalarials, irrational treatment practices by the physicians and mass drug administration of antimalarials, *etc.* contribute to high drug pressure. By monitoring the residual levels of antimalarial drugs in the malaria patients, drug pressure in the community can be ascertained. Here we have studied the residual levels of antimalarials in malaria patients along with the mutation analysis in molecular markers for drug resistance.

Blood samples were collected for blood smear, residual levels of antimalarial drugs and PCR (molecular markers) on Day 0 from *P. falciparum* infected patients enrolled under therapeutic efficacy study at Bilaspur district, Chhattisgarh; Betul district, Madhya Pradesh; Simdega district, Jharkhand and Sundergarh district, Odisha. Clinical follow up was done as per WHO guidelines (2009).

A total of 295 samples were collected from Chhattisgarh, Madhya Pradesh, Jharkhand and Odisha. Out of these, 289 were processed for monitoring residual drug levels. Residual antimalarials levels on Day 0 were observed in 24.2% patients, with highest in patients from Madhya Pradesh followed by patients from Jharkhand, Chhattisgarh and Odisha. *Pfcrt* gene mutation at codon K76T has been associated with chloroquine resistance. Mutation in *Pfcrt* was observed in 76.1% samples. Mutation analysis in *dhfr* gene showed higher prevalence (72%) of double mutation (59R+108N) while *dhps* gene showed wild type genotype in majority of the samples.

In conclusion, it has been observed that patients with residual levels of sulphadoxine and chloroquine on Day 0, showed higher frequency of mutation in *Pfdhps* and *Pfcrt* genes as compared to patients without residual levels on Day 0, suggesting that the residual levels of antimalarials encourage the emergence and spread of drug resistant parasites.

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Highlights of Research Activities under IDVC Project

5

5.1 Bengaluru (Karnataka)

- Studies were carried out on Vector biology and bionomics of malaria vectors in the command areas of the Upper Krishna Project in Karnataka. The studies in riverine and non-riverine villages revealed differences in the abundance, distribution, behaviour and seasonality of malaria vectors *Anopheles culicifacies*, *An. stephensi* and *An. fluviatilis,* a finding which helped in understanding malaria transmission pattern in the project areas. The finding of high level of resistance in *An. culicifacies* led to change in insecticide and strengthening of biological control with larvivorous fishes.

- With the support of District Administration and involvement of 'Panchayats' larvivorous fish *Gambusia affinis* were introduced as a major intervention to control vectors of JE in Gorakhpur district, in eastern U.P. After two years of introduction, breeding and expansion of fish programme, breeding index of vectors *Culex tritaenorynchus* and *Cx. pseudovishuni* significantly declined in the study area.

- Small-scale evaluation of the efficacy and residual activity of alphacypermethrin WG (250 g a.i./kg) in comparison with alphacypermethrin WP (50 g a.i./kg) revealed that the dose of 30 mg/m^2 gave effective residual action for 16 weeks on most common indoor surfaces against Anopheles mosquitoes.

- A study involving patients suffering from cerebral malaria due to *Plasmodium falciparum* has been initiated to understand disease severity at transcriptome level.

- A WHOPES supported project on evaluation of the efficacy and duration of effectiveness of a biolarvicide, Bactivec® SC (*Bacillus thuringiensis* var. *israelensis* SH-14), has been initiated.

5.2 Chennai (Tamil Nadu)

- A study was carried out to assess the efficacy of chloroquine in the treatment of *P. vivax* showed adequate clinical and parasitological response (ACPR) in all the patients enrolled in Thangachimadam and Pamban PHCs of Ramanathapuram district, Tamil Nadu.

- A study was initiated to understand whether gametocytaemia can be used as a proxy and hence a monitoring tool for assessing early or delayed access to health care and treatment facilities.

- To study susceptibility status of *An. stephensi* in Coimbatore, field collections of vector has been initiated and insectary populations are being built up.

- Field studies were carried out on transmission potential of vector mosquitoes including adult indoor resting collections, vector incrimination, host blood meal preference, susceptibility status of operational larvicide (Temephos) against immature *An. stephensi*, household survey to find out the malariogenic conditions and incubator studies with varied temperatures to find out duration and emergence rate of *An. stephensi*.

- A field study was intitated to understand dynamics of malaria in Thiruvottriyur, an endemic area which has been inducted in the Chennai Corporation in 2012. The study will provide research evidence for malaria control activity by the Corporation.

- Monitoring of existing intervention tools/ methods was done for their optimal use in the programme for scaling down malaria in Rameswaram Island in Tamil Nadu.

- Malaria Clinic continued to function, catering to the needs of the public by providing early diagnosis and prompt treatment.

5.3 Guwahati (Assam)

- A cross-sectional study conducted to assess the current residual bio-efficacy and durability of both Olyset® and PermaNet® 2.0 LLINs-reinforced that LLIN-based intervention technology is appropriate in northeast India. A high coverage, replacement of nets every three years and strengthening of net fabric for better durability is advocated.

- Monitoring of therapeutic efficacy of newly introduced artemisinin-based combination therapy of artemether + lumefantrine (AL), extended follow up investigations up to 42 days were undertaken in Tlabung (Lunglei district) of Mizoram and Silachari (Gomti district) of Tripura sharing vast international border with Bangladesh. Based on the presented study results, high treatment failure rate (19-20%) was recorded across both locations but there was no evidence of reduced sensitivity to artemisinin. Most of the treatment failures had resurfaced follow up of 21-day onwards and occurred in 5-15 and >15 years of age groups.

- Malaria outbreak investigation conducted in Tripura revealed high smear positivity virtually in all the age groups ranging from 16-22%, most of which were *P. falciparum*. Cross-checking of smears for parasite positivity and species identification revealed high disparity calling for re-orientation of state technicians. Malaria outbreak was largely attributed due to: (i) inadequate disease surveillance; (ii) acute shortage of antimalarial medicines; (iii) sub-optimal vector control measures; and (iv) inaccessibility and difficult terrain calling for strategic reforms for strengthening health care services.

- Other activities included providing technical support to the control programme, viz. health education and capacity building measures, mass propagation and distribution of larvivorous fish (Guppy & Gambusia) in town areas, assessment of mass drug administration in filarial endemic districts, and in providing technical expertise on long-lasting insecticidal nets for procurement and supplies.

5.4 Hardwar (Uttarakhand)

- A study was carried out on prevalence of dengue vector in Hardwar during 2014. House, container and breteau indices were 47.8, 43.8 and 71.2, respectively. Percent composition of *Ae. aegypti, Ae. albopictus* and *Ae. vittatus* was 28.5, 46.5 and 25.0, respectively.

- A study on the assessment of the performance and effectiveness of ASHA in delivery of health services with reference to malaria in District Hardwar involving 372 ASHAs in two CHCs of District Hardwar was carried out with pre-structured proforma using interview technique. The study found both strengths and weaknesses in ASHAs specific to malaria control activities performed by them and necessary recommendations were made.

- Epidemiological investigations of malaria were carried out in flood-affected areas of Laksar CHC during the months of September/October 2014. The studies were carried out in six villages of 10,000 populations. Data revealed that malaria incidence was high in the flood-affected villages of the Laksar CHC.

- A study was carried out using remote sensing (RS) and geographical information system (GIS) based approach for mapping, monitoring, prediction of mosquitogenic

potential and probable determinants of malaria in District Hardwar of Uttarakhand state.

- A plant-based immersion oil was developed and tested for microscopy.
- A study was carried out on synthesis, pKa determination and *in vivo* toxicity of new promising antimalarial 6-methoxy-5,8-di-(4-amino-1-methylbutyl-amino)-quinoline.
- Botanical insecticide formulation of essential oils extracted from *Lantana camara* and *Valeriana jatamansii* and *Psoralea corylifolia* for the control of mosquitoes was developed.
- A controlled study of possible adverse effects of DDT, used for indoor residual spraying on human reproductive health with particular reference to lactation and pregnancy outcome was carried out.
- Entomological and parasitological investigation of malaria was undertaken in District Saharanpur, U.P.

5.5 Jabalpur (Madhya Pradesh)

- An assessment of durability of LifeNet long-lasting insecticide-treated nets (LLINs) in Madhya Pradesh was made in comparison with NetProtect and PermaNet 2.0 LNs in CHC Kundam of Jabalpur.
- A study was conducted on efficacy and safety of artemether-lumefantrine (AL) combination therapy for the treatment of uncomplicated *P. falciparum* malaria at four sites in India: Annuppur and Jhabua districts of Madhya Pradesh, Bastar district, Chhattisgarh and Simdega district, Jharkhand. No adverse effect was noticed in 262 enrolled patients.
- Extensive studies on bionomics of malaria vectors and their sibling species were carried out and their role in malaria transmission was studied in Chhattisgarh, India.
- A study was undertaken to assess rapid health risk following cyclone 'Hudhud' in Srikakulum district, Andhra Pradesh. The findings revealed that although a great deal of devastation occurred under the impact of cyclone, but no significant adverse impact was observed on health sector and no outbreak of diarrheal or vector borne diseases was reported. A list of suggestions was prepared.
- Three training workshops for 57 Medical Officers of different districts of Madhya Pradesh on vector borne diseases were organized during the year. The workshops were organized jointly by NIRTH, NIMR Field Unit Jabalpur and Directorate of Health Services Bhopal under the Enhanced Vector Borne Disease Control Programme (EVBDCP). Professors of Medical College Jabalpur, Scientists of NIMR, NIRTH and State Health Officers imparted training on different aspects of malaria and other vector borne diseases to the Medical Officers.

5.6 Nadiad (Gujarat)

- A detailed study was carried out in Kheda, Surendranagar and Patan districts of Gujarat in Phase-II command area of Sardar Sarovar project in the previous years. 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 districts included mosquito collection, peri-domestic and intra-domestic larval surveys, host preference and survivorship of malaria vector, cross-sectional mass blood surveys in sentinel villages.
- A large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet 3.0 long-lasting insecticidal nets (LLINs) compared with PermaNet 2.0 was initiated in Anand district. Adverse events following introduction of these two types of nets were studied.
- A research study on transmission dynamics and control of malaria in tribal area of Panchmahal district of Gujarat, was undertaken. Entomological surveys were

conducted to understand population build up, susceptibility status and breeding behaviour of principal vector *An. culicifacies* in this area.

- NIMR FU Nadiad is a site for Centre for the Study of Complex Malaria in India project funded by NIH, USA. A multi-site observational study to explore the clinical spectrum, outcomes and management of severe malaria in selected tertiary health facilities was undertaken. In parallel, cross-sectional and Reactive Case Detection studies were also carried out and clinical profile of malaria patients was recorded and compared between the three settings.

5.7 Panaji (Goa)

- Studies were carried out on proteogenomic analysis of 15 tissues of urban malaria vector *An. stephensi*. Besides, proteins expressed in diverse tissues and developmental stages, larvae and pupae were characterized using high-resolution LTQ-Orbitrap mass spectrometer. Life stage-specific proteome will enable selection of insecticide targets specific to these stages.

- Studies were carried out on characterization of dengue/DHF, chikungunya and yellow fever vector *Aedes aegypti* L. midgut proteome using high resolution fourier transform mass spectrometry. A large array of proteins of the midgut were detected and catalogued and their functional analysis and protein-protein interactions were studied.

- A research study was initiated to assess the role of gut microbiota in modulation of longevity, fecundity and fitness of *An. stephensi* as a malaria vector. The results showed that there exist good deal of variation in gut flora diversity between laboratory reared mosquito immature, adult male and female mosquitoes.

- A study is in progress on isolation, characterization and efficacy of naturally occurring mosquito pathogenic Bacilli in Goa. Preliminary bioassays of isolates showed >50% mortality in 24 hours of exposure against laboratory reared III instar larvae of *An. stephensi, Ae. aegypti, Cx. quinquefasciatus*.

- As mandated in International Health regulations, monitoring of vector mosquitoes with particular emphasis on dengue and chikungunya vectors was carried out in and around 400 meter perimeter of Dabolim Airport and Mormugao Sea port of Goa. House, breteau, container and pupal indices were calculated based on monitoring of immature populations covering a large variety of breeding habitats of mosquitoes.

- The Goa FU evaluated the efficacy of three biolarvicides, VectoMax FG, VectoLax FG and VectoBac GR in Phase-II and Phase-III field trials and activities of these three formulations in terms of >80% control, residual efficacy, and thus frequency of application was determined.

- NIMR FU Goa, one of the sites of project Malaria Evolution in South Asia in collaboration with University of Washington, Seattle has set up laboratories of International Center of Excellence for Malaria Research (ICEMR) funded by NIH, USA and carried out vector infection studies in wild caught mosquitoes in four malaria endemic geographical locations in Goa. During the studies, it has been observed that *An. subpictus* transmitted malaria in tandem with well-known urban malaria vector *An. stephensi*, a finding which will have implications on vector control strategy in Goa and possible other urban locations in India.

- On the request of the Commissionerate of Health, Gandhinagar, an investigation was undertaken in August 2014 to know the reason and suggest effective control measures in Morbi Taluka (Bharatnagar PHC) of Morbi district due to high prevalence of malaria cases reported there (599 malaria cases till June 2014). It was found that out of 171 *An. culicifacies* tested, 8 (4.67%) were having sporozoites. The report was sent to the concerned authority to take necessary control measures.

5.8 Raipur (Chhattisgarh)

- A study on large-scale (Phase-III) evaluation of efficacy, fabric integrity and community acceptability of Olyset Plus long-lasting insecticidal nets compared with Olyset Net in India was undertaken and it was found that Olyset Plus long-lasting insecticidal nets were more durable than Olyset Net and hence more acceptable although there was no significant difference in efficacy between them. However, attrition rate of insecticide was lesser in Olyset Plus as compared to Olyset Net, respectively.

- A study on 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 has been carried out. The project has been launched in 80 clusters (villages) of Keshkal block in Kondagaon district in southern Chhattisgarh. The study involved ASHAs and surveillance workers for malaria surveillance. The areas in two arms were match-paired on the basis of prevalence of malaria where IRS with Bendiocarb + LLINs and LLINs alone were used.

- A longitudinal study was conducted on survivorship and physical integrity of field distributed long-lasting insecticidal nets for malaria control in Chhattisgarh state. The information was collected using WHO recommended structured-questionnaire during house-to-house visits in selected villages. Analysis of data so far has been collected and further work is in progress.

- Studies on monitoring of insecticide resistance in malaria vector *An. culicifacies* in different districts of Chhattisgarh were carried out against DDT, malathion, alphacypermethrin, deltamethrin, permethrin and bendiocarb have been carried out using standard WHO test method. Monitoring has been completed in 20 districts so far. The results indicate widespread and high level of resistance in *An. culicifacies* to DDT 4%, malathion 5% and alphacypermethrin 0.1%, moderate level of resistance to deltamethrin 0.05% and permethrin 0.75% and low level of resistance (except Bilaspur and Dhamtari districts) to bendiocarb 0.1%.

- Cross-checking facility for malaria slides was provided by the Field Unit. During the reporting period, 3838 malaria slides received from 11 districts of Chhattisgarh through the State Programme Officer (VBD), Raipur were cross-checked at the Field Unit. Discrepancy of 1.2% was observed in positive slides and 0.5% in negative slides. Results were communicated to the concerned agency.

- A total of 31 persons with fever attended the Malaria Clinic at the Field Unit. One blood smear was found to be positive for *Pf* infection.

- The Field Unit has received a grant of Rs. 10 lakh from the State Health Department to organize 5-day refresher training courses in malaria microscopy for laboratory technicians working in the PHCs/CHCs. Refresher training was imparted in malaria microscopy to 102 laboratory technicians from 14 districts, posted in 34 CHCs, 60 PHCs, and 8 Hospitals, in eight batches from 8–12 &15–19 September; 24–28 November; 1–5 December 2014 and 12–16 & 19–23 January 2015. Training of more LTs has been planned.

5.9 Ranchi (Jharkhand)

- Mosquito fauna survey was undertaken with particular reference to anophelines in west Singhbhum district of Jharkhand state. From both the areas four malaria vectors, *An. culicifacies, An. fluviatilis , An. annularis* and *An. minimus* were collected. *Anopheles annularis* was found to be in low density (MHD 1-2) in Noamundi area. However, *An. minimus* continued to be the dominating mosquito species in Noamundi and Saranda forests.

- Outbreak of Malaria in Mahuadanr-CHC of the Latehar district, Jharkhand state, India was carried out on the request of the state vector borne diseases control programme. Situational analysis covering all the main epidemiological

factors was done and factors responsible for the outbreak were studied and remedial measures to stem the outbreak were suggested.

- Filariasis survey was carried out in West Singhbhum district (Jhinkpani PHC) of Jharkhand state during the month of May 2014. The district is dominated by Oraon, Munda, Santhal, Gonds, Bhumij, Kols and Karmalo tribes. Thorough investigation of *mf* patients revealed that in the study area in West Singhbhum district the *mf* rate was 6% while it was 10.86% in Kuchal PHC in Saraikela district after 9 rounds of MDA in the study villages.

- Baseline household surveys were carried out in 8 blocks of Jharkhand state during 2008 under the World Bank's project and surveys were completed, data analyzed and final report was submitted. Now, to track the results of various interventions undertaken during last few years, the endline surveys are in progress by NIMR/NIMS to analyze the impact of intervention carried out during 2009–13 by the NVBDCP with the World Bank support.

- To facilitate early diagnosis and prompt treatment, a Malaria Clinic is functioning at NIMR, Field Unit, Itki, Ranchi. All the cases from Itki PHC and TB Sanatorium Hospital were diagnosed. A total of 276 patients attended the Malaria Clinic during the year 2014-15, out of which 38 cases were found to be positive for malaria, of these 11 cases were positive for *P. vivax* and 27 cases for *P. falciparum*. Overall SPR was 13.76 and SFR 9.78%. Four *P. falciparum* positive patients showed gametocyte in their peripheral blood.

- A Filaria Clinic is functioning at IDVC Field Unit, Itki, Ranchi. A total of 40 patients of filariasis attended the clinic during the year. Most of the cases were of old filariasis cases. These cases were with acute manifestation of filariasis starting from hydrocele to elephantiasis. One case of epididymo-orchitis was observed, whereas eight patients had multiple manifestions (20%).

5.10 Rourkela (Odisha)

- A MMV funded Comprehensive Case Management Programme was launched in collaboration with the Government of Odisha, in four districts with the primary objective to assess the impact of comprehensive case management system of uncomplicated malaria on its transmission in different settings. In the intervention blocks of all the four districts *P. falciparum* and *P. vivax* cases were followed up on Day-5 and Day-14, respectively for drug compliance and adverse events.The overall compliance rates of follow up in Bolangir, Dhenkanal, Kandhamal and Angul districts were 96, 98.8, 99.5 and 85%, respectively which showed marked improvement in comparison to those of the previous year. However, the month-wise compliance rate of follow up in these districts ranged from 67.1 to 100%.

- A multicentric study on eco-epidemiology and transmission of complex malaria in India (under NIH funded CSCMi project) is being carried out with the objectives: to understand the basic eco-epidemiology of malaria; to determine the genetic diversity and population structure of *P. falciparum* and *P. vivax*; to quantify the role of environmental conditions in determining transmission intensity; and to evaluate the evolutionary response of key Indian vectors to the adoption of insecticide-based intervention.

- A Phase-II/III randomized clinical trial of the efficacy and safety of artesunate + sulphadoxin-pyrimethamine and artesunate + mefloquine to treat uncomplicated falciparum malaria in pregnancy was undertaken. The multicentric study was carried out simultaneously at Rourkela, Ranchi and TMH, Jamshedpur in order to find out the safety and efficacy of antimalarial drugs artesunate + sulphadoxin-pyrimethamine and artesunate + mefloquine in the treatment of uncomplicated *P. falciparum* malaria during pregnancy. In Rourkela, 83 patients were recruited for the study from a cohort of 3040. The range of parasite density on Day-0 was 80 to 85,280

per µl of blood. All the patients became negative on Day-3. All the 83 patients have completed 63 day follow up and there was no treatment failure. Only one patient had re-infection of malaria.

- A project was started in the year 2013 in Sundergarh district, Odisha when dengue cases were in a rising trend. Aedes mosquitoes and their larval surveys were carried out periodically. In the year April 2014 to March 2015, three surveys were carried out in those localities where the dengue cases were reported. The breteau index was 12.2, 31.1 and 21.3 during the surveys carried out in May and October 2014 and March 2015, respectively. The pupal index was 18.3, 30.1 and 21.3 during the corresponding period.

- A study on the susceptibility status of *An. culicifacies* to 4% DDT, 5% malathion and 0.05% deltamethrin was conducted in forest and plain areas of Sambalpur district. In the forest area, percent mortality of *An. culicifacies* on 4% DDT, 5% malathion and 0.05% deltamethrin was 16, 72 and 96%, whereas in plain areas it was 19, 76 and 95%, respectively. Alternative methods for the vector control are to be considered when the insecticide in question is no longer having the desired effect.

- During the year under reporting from April 2014 to March 2015, a total of 4860 patients with fever reported at the clinic run by the NIMR Field Unit, out of which 209 were found positive for malaria comprising of 127 *P. falciparum,* 79 *P. vivax* and 3 with mixed infection. The SPR, SfR and *Pf*% were 4.3, 2.7 and 62.2, respectively

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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.

Collection of biological materials

Till now, a total of 1341 isolates collected in the parasite bank, including 967 *P. falciparum*, 369 *P. vivax* and 5 *P. malariae* has been depicted (Tables 3–4). 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 Lambda-cyhalothrin (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. Total parasite samples collected year-wise

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	—	—
Total	967	369	5	1341

materials supplied from Parasite Bank and till now ₹ 4,39,000/- has been collected up to 2014-15.

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 Parasite Bank.

Table 4. 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

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 library-centre are completely computerized and indexed. It is probably one of the best in India in this field.

Library collections

Books	8500
Bound Journals	4650
Journals (P+O)	29
Newspapers	10
Magazines	19
CD / DVD	30
Reprint documents	350
Theses	25
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
- Wi-Fi Internet Access Facility
- Health News Repository (English & Hindi)
- Current Awareness Service of Journals
- Abstract Services
- Photo Copying Services
- Theses Database
- Non-Print Material Database
- Print + Online Journals

E-consortia for Journals

- J-Gate@ICMR
- JCCC@ICMR
- NML-ERMED Consortia

Apprentice training

The NIMR Library & Information Centre trains and empowers students of library and information discipline by recruiting apprentices for one year. Apprentice library trainee service is approved by the ICMR, New Delhi. In the year 2014, 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 5341 member libraries and information centres across the globe. ❑

Inter-Institutional Collaboration

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Several collaborative projects were undertaken with the following ICMR/non-ICMR Institutes and Medical Colleges of the country and abroad:

1. A multisite observational study to explore the clinical spectrum, outcomes, and management of severe malaria in selected tertiary health facilities of India in collaboration with New Civil Hospital, Surat; Surat Municipal Institute of Medical Education and Research, Surat; DMO, Balaghat, Madhya Pradesh; DMO, Gadchiroli, Maharashtra; Ram Manohar Lohia Hospital, New Delhi; NIMR FU, Bengaluru; NIMR FU, Chennai; NIMR FU, Nadiad; SP Medical College, Bikaner, Rajasthan; BJ Medical College, Ahmedabad; and Civil Hospital, Ahmedabad, India.
2. Bionomics of malaria vectors and their sibling species, and establish their role in malaria transmission in Chhattisgarh, India in collaboration with NIRTH, Jabalpur, India.
3. Cross-sectional survey amongst children aged <5 years to define malaria endemicity in collaboration with Dr Chandramohan Daniel, London School of Hygiene and Tropical Medicine (LSHTM), London and Dr Irene Kuepfher, LSHTM & Liverpool School of Tropical Medicine, Liverpool, UK.
4. Observational study to explore clinical and laboratory presentation of dengue patients with different serotypes, in collaboration with Dr Ashutosh Biswas, AIIMS Hospital, New Delhi; Dr Sunita Bhatia, Dr Surendra Kumar and Dr Rakhi Dhawan, Kasturba Hospital, New Delhi.
5. Dynamics of malaria transmission in endemic areas of Chennai-Tiruvottiyur, Tamil Nadu in collaboration with Corporation of Chennai and Directorate of Health and Preventive Medicine, Govt. of Tamil Nadu, India.
6. Monitoring of existing intervention tools/methods in the programme for scaling down malaria in Rameswaram Island, Tamil Nadu in collaboration with Directorate of Health and Preventive Medicine, Govt. of Tamil Nadu, India.
7. Enhanced surveillance to reduce malaria burden towards elimination in targeted areas in Jharkhand state in collaboration with the State Programme Officer, Jharkhand and the Consultant, NVBDCP.
8. Establishment of a WHO-recognized laboratory for Quality Assurance of malaria RDTs in collaboration with Dr GS Sonal and Dr Awdhesh Kumar, NVBDCP.
9. Clinical and molecular surveillance for monitoring the emerging resistance to antimalarial drugs in *Plasmodium falciparum* in central India in collaboration with NIRTH and NIMR FU, Jabalpur, India.
10. A study on the role of gut microbiota in modulation of longevity fecundity and fitness of *Anopheles stephensi* as a malaria vector in collaboration with Dr Sandeep Garg, Deptt. of Microbiology, Goa University.
11. Computational assisted design and synthesis of novel antimalarial agents embodying structural diversity suitable for protease inhibitors in collaboration with collaborators Dr N Lalitha Associate Professor, Venkateswara College; Dr Brijesh Rathi, Asstt. Professor, Hans Raj College, Delhi University, New Delhi.

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Human Resource Development

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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; Kumaun University, Nainital; and Maharshi Dayanand University, Rohtak.

8.2 Students registered for Ph.D. Programme

In the year 2014, eight new students namely, Priya Gupta, Sanjay Tevatiya, Jagbir Singh, Praveen Kumar Shukla, Achyut Pandey, Purnima S Singh, Swati Nandeo and Sandeep Kumar were registered for Ph.D. degree under the supervision of NIMR scientists.

8.3 M.Sc. Projects/Dissertations

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

In total, 22 students namely, Srikant Singh, Preeti Pathak, Kavita Bhandari, Malvika Shah, Romila Moirangthem, Shalja Verma, Nikhil Dhariwal, Avantika Rawat, Pranesh Dwivedi, Riti Mann, Bhawna Aggarwal, Nisha Kumari, Himani Pandey, Kusum Nautiyal, Anchal Lodhi, Saba Parveen, Sagufta Parveen, Kritika Tiwari, Vaishali Rajpoot, Garima Dhawan, Sushant Kaushal and Neelu Dwivedi of M.Sc. successfully completed their projects/dissertations under the supervision of NIMR scientists.

8.4 Training Courses organized

NIMR has conducted regular training programmes as under:

1. Dr Kumar Ashwani organized the X Joint Annual conference of ISMOCD and IAE at NIMR Field Unit Goa in collaboration with Goa Medical College and Hospital at Goa from 10–12 February 2014.
2. Dr Mishra Neelima organized the Institutional Ethics Committee meeting at NIMR, New Delhi on 24 July 2014.
3. Dr Nagpal BN organized Reorientation training programme on Prevention and control of vector borne diseases and strengthening of vector surveillance of epidemiologists and entomologists of SDMC and EDMC (11 trainees) at NIMR, New Delhi from 23–25 January 2014.
4. Dr Nagpal BN organized Skill up-gradation/ hands on training programme on Diagnosis of malaria for laboratory technicians of SDMC (22 trainees) at NIMR, New Delhi from 27–31 January 2014.
5. Dr Nagpal BN organized training on Advanced entomology techniques in VBDs to Officers from Medical Research Institute, Colombo, Sri Lanka (5 trainees) at NIMR, New Delhi from 1–12 September 2014.
6. Dr Nagpal BN organized Induction training programme for VBD consultants of northeastern states of India (22 trainees) at NIMR, New Delhi from 20 November–29 December 2014.

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Research Papers Published
(January–December 2014)

9

1. Anushrita, Nagpal BN, Srivastava Aruna, Saxena Rekha, Kapoor Neera, Chand SK, Gupta Sanjeev Kumar, Dash AP, Dua VK, Valecha Neena. Health impact assessment – A retrospective study for prospective Approach in Madhya Pradesh, India. *Austin J Infect Dis* 2014; *1*(3): 7.
2. Anvikar AR, Arora U, Sonal GS, Mishra N, Shahi B, Savargaonkar D, Kumar N, Shah NK, Valecha N. Antimalarial drug policy in India: Past, present & future. *Indian J Med Res* 2014; *139*(2): 205–15.
3. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroth S, Pukrittaya-kamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcha-roen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ. The spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2014; *371:* 411–23.
4. Chauhan K, Pande V, Das A. DNA sequence polymorphisms of the pfmdr1 gene and association of mutations with the *pfcrt* gene in Indian *Plasmodium falciparum* isolates. *Infect Genet Evol* 2014; *26:* 213–22.
5. Dev V, Khound K, Tewari GG. Dengue vectors in urban and suburban Assam, India: Entomological observations. *WHO South East Asia J Public Health* 2014; *3*(1): 51–59.
6. Dhiman RC. Emerging vector-borne zoonoses: Eco-epidemiology and public health implications in India. *Front Public Health* 2014; *2*: 168.
7. Dixit J, Arunyawat U, Huong NT, Das A. Multilocus nuclear DNA markers reveal population structure and demography of *Anopheles minimus*. *Mol Ecol* 2014; *23*(22): 5599–618.
8. Dua VK, Srivastava A, Pandey AC, Gupta NC. Impact of Integrated vector control strategy on malaria incidence and disability adjusted life years (DALY) at Bharat Heavy Electricals Limited, Hardwar, India: Results of 25 years study. *Austin J Infect Dis* 2014; *1*(2): 1–7.
9. Elamathi N, Barik TK, Verma V, Velamuri PS, Bhatt RM, Sharma SK, Raghavendra K. Standardization of a bottle assay—an indigenous method for laboratory and field monitoring of insecticide resistance and comparison with WHO adult susceptibility test. *Parasitol Res* 2014; *113*(10): 3859–66.
10. Gupta P, Anvikar AR, Valecha N, Gupta YK. Pharmacovigilance practices for better healthcare delivery: Knowledge and attitude study in the national malaria control programme of India. *Malar Res Treat* 2014; 1–6.
11. Gupta P, Pande V, Das A, Singh V. Genetic Polymorphisms in VIR genes among Indian

Plasmodium vivax Populations. *Korean J Parasitol* 2014; *52*(5): 557–64.
12. Gupta P, Singh R, Khan H, Raza A, Yadavendu V, Bhatt RM, Singh V. Genetic profiling of the *Plasmodium falciparum* population using antigenic molecular markers. *Scientific World J* 2014; *2014:* 140867.
13. Jain S, Rana V, Shrinet J, Sharma A, Tridibes A, Sunil S, Bhatnagar RK. Blood feeding and Plasmodium infection alters the miRNome of *Anopheles stephensi*. *PLoS One* 2014; *9*(5): e98402.
14. 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* 2014; *29:* 6–14.
15. Kar NP, Kumar A, Singh OP, Carlton JM, Nutan N. Malaria transmission dynamics in forest ecosystems — A review. *Parasit Vectors* 2014; *7*(1): 265–76.
16. Keluskar P, Singh V, Gupta P, Ingle S. *Plasmodium falciparum* and *Plasmodium vivax* specific lactate dehydrogenase: Genetic polymorphism study from Indian isolates. *Infect Genet Evol* 2014; *26:* 313–22.
17. Masur U, Kumar H, Kumar A. Anti-larval effects of leaf and callus extract of *Dysoxylum binectariferum* against urban malaria vector, *Anopheles stephensi*. *J Nat Prod* 2014; *7:* 147–54.
18. Mbenda HG, Awasthi G, Singh PK, Gouado I, Das A. Does malaria epidemiology project Cameroon as 'Africa in miniature'. *J Biosci* 2014; *39*(4): 727–38.
19. Mishra N, Kaitholia K, Srivastava B, Shah NK, Narayan JP, Dev V, Phookan S, Anvikar AR, Rama R, Bharti RS, Sonal GS, Dhariwal AC, Valecha N. Declining efficacy of artesunate plus sulphadoxine-pyrimethamine in north-eastern India. *Malaria J* 2014; *13:* 284.
20. Ngassa Mbenda HG, Das A. Occurrence of multiple chloroquine-resistant *Pfcrt* haplotypes and emergence of the S(agt)VMNT type in Cameroonian *Plasmodium falciparum*. *J Antimicrob Chemother* 2014; *69*(2): 400–3.
21. Prajapati SK, Culleton R, Singh OP. Protein trafficking in *Plasmodium falciparum*-infected red cells and impact of the expansion of exported protein families. *Parasitology* 2014; *141*(12): 1533–43.
22. Prajapati SK, Singh OP. Identification of a vir-orthologous immune evasion gene family from primate malaria parasites. *Parasitology* 2014; *141*(5): 641–5.
23. Price RN, Von SL, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. *Lancet Infect Dis* 2014; *14*(10): 982–91.
24. Punita S, Swati S, Maurya RK, Das De T, Thomas T, Lata S, Singh N, Pandey KC, Valecha N, Dixit R. Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. *Parasit Vectors* 2014, ; *7* (1): 235
25. Raghavendra K, Barik TK, Sharma SK, Das MK, Dua VK, Pandey A, Ojha VP, Tiwari SN, Ghosh SK, Dash AP. A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India. *J Vector Borne Dis* 2014; *51*(3): 230–4.
26. Savargaonkar D, Shah N, Das MK, Srivastava B, Valecha N. *Plasmodium malariae* infection: A case of missed diagnosis. *J Vector Borne Dis* 2014; *51*(2): 149–151.
27. Saxena R, Das MK, Nagpal BN, Srivastava A, Gupta SK, Kumar A, Tomar AS, Sinha AT, Vidyotma R, Jeyaseelan AT, Baraik VK, Singh VP. Identification of malaria risk factors for control by focused interventions in Ranchi district, Jharkhand, India. *J Vector Borne Dis* 2014; *51*(4): 276–81.
28. Saxena R, Nagpal BN, Singh VP, Srivastava A, Dev V, Sharma MC, Gupta HP, Tomar AS, Sharma S, Gupta SK. Impact of deforestation on known malaria vectors in Sonitpur district of Assam, India. *J Vector Borne Dis* 2014; *51*(3): 211–5.
29. Shalini S, Chaudhuri S, Sutton PL, Mishra N, Srivastava N, David JK, Ravindran KJ, Carlton JM, Eapen A. Chloroquine efficacy studies confirm drug susceptibility of *Plasmodium vivax* in Chennai, India. *Malar J* 2014; *13:* 129.
30. Sharma P, Sharma S, Mishra A, Thomas T, Tanwee D De , Verma S, Vandana K, Lata S, Singh N, Pandey KC, Dixit R. Deep sequencing revealed plant like transcripts in

mosquito *An. culicifacies*: An evolutionary puzzle. *Cold Spring Harbor Labs J* 2014.
31. Sharma SK, Tyagi PK, Upadhyay AK, Haque MA, Agrawal OP. Efficacy, human safety and collateral benefits of alphacypermethrin-treated long-lasting insecticidal net (Interceptor®) in a hyperendemic tribal area of Orissa, India. *J Trop Dis* 2014; *2:* 135.
32. Singh N, Shukla MM, Chand G, Barde PV, Singh MP. Vector borne diseases in central India with special reference to malaria, filaria, dengue and chikungunya. *South-East Asia J Public Hlth* 2014; *3*(1): 28–35.
33. Singh R, Singh D, Gupta R, Savargaonkar D, Singh OP, Nanda N, Bhatt RM, Valecha N. Comparison of three PCR-based assays for the non-invasive diagnosis of malaria: Detection of *Plasmodium* parasites in blood and saliva. *Eur J Clin Microbiol Infect Dis* 2014; *33*(9): 1631–9.
34. Singh RK, Haq S, Kumar G, Mittal PK, Dhiman RC. Bionomics and vector potential of *Anopheles subpictus* as a malaria vector in India: An overview. *Int J Mosq Res* 2014*; 1*(1): 29–37.
35. Singh RK, Kumar G, Mittal PK. Insecticide Susceptibility status of malaria vectors in India: A review. *J Med Internet Res* 2014; *1*(1): 5–9.
36. Singh RK, Mittal PK, Dhiman RC. Evaluation of mosquito larvicidal efficacy of leaf extract of *Agave sisalana* (Family- Agavaceae*). J Entomol Zool Stud* 2014; *2* (1): 83–6.
37. Singh RK, Mittal PK, Kumar G, Dhiman RC. Insecticide susceptibility status of *Aedes aegypti* and *Anopheles stephensi* larvae against temephos in Delhi. *Int J Mosq Res* 2014; *1* (3): 69–73.
38. Singh RK, PK Mittal, Kumar G, Dhiman RC. Prevalence of *Aedes* mosquitoes in various localities of Delhi during dengue transmission season. Entmol Appl Sci Btt 2014; *1* (4):16–21.
39. Singh V, Gupta P, Pande V. Revisiting the multigene families: *Plasmodium* var and vir genes. *J Vector Borne Dis* 2014; *51*(2): 75-81.
40. Srivastava H, Huong NT, Arunyawat U, Das A. Molecular population genetics of the NADPH cytochrome P450 reductase (CPR) gene in *Anopheles minimus*. *Genetica* 2014; *142* (4): 295–315.
41. Sudheer Ch, Kumar D, Sohani SK, Malik A, Chahar BR, Dhiman RC. A support Vector Machine-Fire Fly Algorithm based forecasting model to determine malaria transmission. *Neurocomputing* 2014; *129:* 279–88.
42. Sukhthankar JH, Kumar H, Godhinno MHS, Kumar A. Larvicidal activity of methanolic leaf extracts of plant *Chromolaena odorata* L. (Asteraceae) against some vector mosquitoes in India. *J Med Internet Res* 2014*; 1*(3)*:* 33–38.
43. Sundaram B, Varadarajan NM, Pradeep AS, Ghosh SK, Nagaraj VA. Purification of a recombinant histidine-tagged lactate dehydrogenase from the malaria parasite, *Plasmodium vivax* and characterization of its properties. *Biotechnol Lett* 2014; *36*(12): 2473–80.
44. Sundararaj S, Saxena AK, Sharma R, Vashish Sharma S, Anvikar A, Dixit R, Rosenthal PJ, Pandey KC. Cross-talk between malarial cysteine proteases and falstatin: The BC loop as a hot-spot target. *PLoS One* 2014; *9*(4): e93008.
45. Tanwar OP, Rikta S, Marella A, Alam Md. M, Akhter M, Dua VK. Prediction and comparison of drug likeliness properties of primaquine and its structural analogues using *in silico* ADME and toxicity prediction tools. *J Adv Bioinfo Appl Res* 2014; *5*(3): 172–82.
46. Thomas T, Tanwee D De, Sharma P, Verma S, Rohilla S, Pandey KC, Dixit R. Structural and functional prediction analysis of mosquito Ninjurin protein: Implication in the innate immune responses in *Anopheles stephensi*. *Int J Mosq Res* 2014; *1*(4): 60–65.
47. Tyagi S, Pande V, Das A. Mitochondrial genome sequence diversity of Indian *Plasmodium falciparum* isolates. *Mem Inst Oswaldo Cruz* 2014; *109*(4): 494–8.
48. Tyagi S, Pande V, Das A. New insights into the evolutionary history of *Plasmodium falciparum* from mitochondrial genome sequence analyses of Indian isolates. *Mol Ecol* 2014; *23*(12): 2975–87.
49. Tyagi S, Pande V, Das A. Whole mitochondria genome sequence of an Indian *Plasmodium falciparum* isolate. *Korean J Parasitol* 2014; *52*(1)*:* 99–103.
50. Venkatesan R, Ravindran J, Eapen A, William J. Insecticidal and growth regulating activity

of crude extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Asian Pac J Trop Med* 2014; *4*(2): S578–S582.

51. Vijay S, Rawat M, Sharma A. Mass spectrometry based proteomic analysis of salivary glands of urban malaria vector *Anopheles stephensi*. *Biomed Res Int* 2014; *2014:* 686319
52. Wilson AL, Dhiman RC, Kitron U, Scott TW, Van den Berg H, Lindsay SW. Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: A systematic review and meta-analysis. *PLoS Negl Trop Dis* 2014; *8*(10): e3228.

Books

1. Sharma SK. Long-lasting insecticidal nets in malaria control: A review. In: *Major tropical diseases*: *Public health perspective*. Editors: Kumar A, Rodrigues S, Dias A. Broadway Publishing House 2014; pp. 98–114.

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Other Activities

10

10.1 Information, Education and Communication

To create awareness in general public about vector borne diseases and identification of various breeding sites of mosquitoes in the households and nearby places is important. In this context, attempts were made for direct interaction with the households of unauthorized colonies such as new modern Shahdara, Budh Bazaar and Ram Nagar of east Delhi area and Bagdola village, Sector 8, Dwarka of west Delhi, where mosquito breeding sites are in abundant and cases of dengue and malaria have been reported.

Public lectures and group discussions were organized in these places including house-to-house interaction with the households. Information about various interventional methods of personal protection was imparted. Efforts were also made to improve habits and bring behavioural changes for source reduction of mosquito breeding sites in these places.

Public lecture and group discussion with households of new modern Shahdara, east Delhi.

Group discussion with the households of Budh Bazaar, east Delhi.

Identification of various breeding sites of mosquitoes at Budh Bazaar area of east Delhi.

Group discussion with households of Ram Nagar area of east Delhi.

10.1.1 Documentation Cell

In Documentation Cell, the following works were carried out:

Public lecture/group discussion with households of Bagdola village, Sector 8, Dwarka of west Delhi.

1. Updating of various information enlisted for intramural and extramural projects undertaken by NIMR along with their status, *i.e.* ongoing or completed, extension period (if any) granted, and budget details based on the minutes of the Scientific Advisory Committee (SAC) meeting for the year 2014-15 as well as inputs provided by individual principal investigators/co-principal investigators.
2. Updating of NIMR research publications list and compilation of the list for the year 2014-15.
3. Following services were provided to various Divisions of NIMR as well as ICMR for day-to-day research activities:
   (i) Updated list of ongoing Intramural/ Extramural projects was provided to Dr Aruna Srivastava for repository.
   (ii) Project IDs were provided to all the SAC approved Intramural/ Extramural projects.
   (iii) Information on updated list of SAC approved IDVC projects of Dr MK Das was provided to Mr KK Gupta, AO, IDVC.
   (iv) Updated list of SAC approved Intramural/ Extramural projects was provided to Mr Rashid Pervez, ALIO for preparation of Annual Report 2014-15.
   (v) Project reports of completed Intramural/ Extramural projects along with budget details were provided to the Accounts Division for audit purpose.
   (vi) Information on research publications of NIMR scientists for the last 10 years 2005-2014 and details on Projects (Intramural/ Extramural), and their collaboration were provided to Dr Anup Anvikar for preparing status report on NIMR for university status of ICMR.
   (vii) List of completed and ongoing projects was provided to Dr OP Singh for preparation of Results—Framework Document (January-March) 2014-15.

10.1.2 Photography & Videography

The photography section carried out different photography works on various occasions/meetings/ trainings/workshops/field surveys and functions held at NIMR and ICMR.

Some major activities which included photography works are: Malaria eradication or control: Getting the balance right (by Prof. Green Wood) in June 2014; Photography of training programme on "Prevention and control of vector borne diseases and strengthening of vector surveillance of epidemiologists and entomologists of SDMC & EDMC" (June 2014); Photography of expert group meeting for brainstorming session on polycystic ovary syndrome (September 2014); *Hindi Pakhwada* and *Hindi Samaroh Kavi Sammelan* (September 2014); ICMR workshop on Medical diagnostics and devices innovation partnership at India International Centre, Lodhi Road (September 2014); *Swachh Bharat Abhiyan* at ICMR (2nd October 2014) and NIMR (monthly); Independence Day, Republic Day and Annual Day celebrations of NIMR and ICMR; IDVC, RAC and SAC meetings; Farewell functions at NIMR and ICMR; ICMR- FORTE workshop on Ageing and health (November 2014); Release of a popular publication of NIN, Hyderabad (February 2015) *etc.*

In addition to photography, some audio-visual works were also carried out on the occasion of various functions and field work activities. Video films on Life-cycle of mosquito and malaria parasite were made based on field collected shots for using in the production of videos on *Malaria Bukhar* in English as well as Hindi. Editing and special effects were imposed in the videos made. Video recording was carried out for following activities: Inaugural function of *Swachh Bharat Abhiyan* on 2nd October 2015 at NIMR; Public speech on cleanliness and hygiene at New Modern Shahdara, Budh Bazaar and Dwarka, New Delhi.

Besides above Video DVDs on malaria, laboratory diagnosis of malaria, dengue and related subjects produced at NIMR were distributed to the

participants of different training programmes organized by NIMR, NVBDCP, MCD. The DVDs were also sent to other states on demand and given to interested visitors.

10.1.3 Swachh Bharat Abhiyan

Hon'ble Prime Minister of India, Shri Narendra Modi launched *Swachh Bharat Abhiyan* on 2 October 2014. On this occasion an oath taking programme on cleanliness and hygienic practices was organized by the Director, NIMR. A committee was also constituted for *Swachh Bharat Abhiyan* under the supervision of Dr Nutan Nanda, Scientist F, for carrying out various activities such as identification of spots which are vulnerable to unhygienic conditions, handling of biohazard materials in the laboratories, arrangement for log chart in toilets/washrooms and carrying out of maintenance work, weeding out of files, digitization of files, auctioning of the old equipments, furniture, proper arrangements and placements of files and almirahs. Organization of voluntary *Kar Sewa*/ *Shram Dan*, seminar on hygiene and cleanliness-related topics, dissemination of IEC materials and initiation of model project on hygiene and cleanliness-related topics are also being carried out.

Oath taking ceremony

Weeding out of files at the Institute

Shram Dan activities on weekly and monthly basis

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 including Science Citation Index Expanded, MEDLINE, PubMed, Scimago Journal Ranking, DOAJ, *etc.*

During the year 2014, all the issues of the journal were published on timely basis. More number of articles was published in comparison to previous years. The full articles of the journal can be accessed online through the NIMR'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. 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. The issues were primarily focused on climate change and human health, problems of insecticide resistance in treating malaria, activities under *Swachh Bharat Abhiyan* and *Hindi Pakhwada* celebrations.

Plasmodium Newsletter

Plasmodium Newsletter of the Institute was also brought out by the Division. It highlighted some of the current research advancements in the field of malaria, particularly about the new malaria diagnostic tools and techniques, and important

activities of the Institute and its field units during the period under report.

Monographs/Books

The Division published the revised 3rd edition of *Guidelines for Diagnosis and Treatment of Malaria in India* in the year 2014. The guidelines were prepared collaboratively by the ICMR, NVBDCP, NIMR, New Delhi and experts from different parts of the country with the aim to guide the medical professionals on the current methods of diagnosis and treatment of malaria, based on the national drug policy. This manual deals with the treatment of uncomplicated malaria and specific antimalarials for severe disease. The warning signs of severe malaria have been listed so as to recognize the condition and give the initial treatment correctly before referring to a higher facility. It is hoped that these guidelines will be useful for health care personnel involved in the diagnosis and treatment of malaria at different levels.

Annual Reports

Besides above, the Division also undertook the publication of multicoloured Annual Reports of the Institute (NIMR) as well as IDVC project for the financial year 2014–15.

10.3 Seminars/Conferences/Workshops/ Training Courses/Meetings attended

Anvikar Anup

1. Delivered series of Training Course Curriculum for Laboratory Technicians at South Delhi Municipal Corporation from 27–31 January 2014.
2. Attended meeting to revise Guidelines for diagnosis and treatment of malaria on 12 February 2014.
3. Attended workshop of EIS officers at National Centre for Disease Control, New Delhi from 26-28 February 2014.
4. Attended meeting to prepare Guidelines for Apex Referral Laboratories and Sentinel Hospitals for dengue and chikungunya at National Vector Borne Disease Control Programme, New Delhi on 4 March 2014.
5. Delivered series of lectures during a workshop on Antimalarial resistance monitoring at NIMR, New Delhi from 19-23 March 2014.
6. Attended meeting of mentors of EIS officers at US Embassy, New Delhi on 29 March 2014.
7. Delivered a talk on Antimalarial drug resistance in a symposium at NCDC at New Delhi on 7 April 2014.
8. Delivered talk on Need for Pharmacoepidemiology in India and malaria control in a workshop on Pharmacoepidemiology at Mumbai on 18 April 2014.
9. Regional consultation on Development of Global Technical Strategy at New Delhi from 28–30 April 2014.
10. NIMR-MMV-PATH Workshop: Implementing G6PD testing to endure safe radical cure of *P. vivax* malaria at New Delhi on 1 May 2014.
11. Training course at South & East Delhi Municipal Corporations from 23–25 June 2014.
12. Participated in Malaria stakeholders meeting on GMAP at New Delhi on 14 July 2014.
13. Delivered talk on Malaria treatment to clinicians at Wenlock Hospital, Mangalore on 22 August 2014.
14. Delivered series of lectures during Advanced Entomological Techniques in VBDs from 1–5 September 2014.
15. Attented X Joint Annual Conference of ISMOCD & IAE being held at Goa from 10–12 October 2014.
16. Attented Inter-country meeting to address the Threat of artemisinin resistance in South Asia, WHO-SEARO, New Delhi from 9–11 December 2014.
17. Delivered series of lectures during induction training of District VBD Consultants from 22 November to 29 December 2014.

Das Aparup

1. Delivered a talk on the X Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases (ISMOCD) & Indian Association of Epidemiologists (IAE) at Goa from 10-12 October 2014.

Das Ram

1. Attended the conference "X Joint Annual Conference of ISMOCD & IAE and presented the poster entitled "Developing minisatellite marker for genotyping of *Plasmodium vivax*" at Panji, Goa from 10–12 October 2014.
2. Participated and demonstrated the Techniques for malaria diagnosis in India International

Trade Fair (IITF) at Ministry of Health & Family Welfare Pavilion in Pragati Maidan, New Delhi from 14–27 November 2014.

Dev Vas

1. Presented a paper on The dominant mosquito vectors of human malaria in India and also chaired a session on Medical Entomology in an International Conference on Entomology held at Punjabi University, Patiala from 21–23 February 2014.
2. Participated in a workshop on Delaying artemisinin resistance in India at New Delhi jointly organized by NIMR and Public Health Foundation of India from 24-25 March 2014.

Dhiman RC

1. Delivered a lecture at the International Conference on Entomology at Punjab University, Patiala from 21-23 February 2014.
2. Delivered a talk on the X Joint Annual Conference of Indian Society for Malaria and other Communicable Diseases (ISMOCD) & Indian Association of Epidemiologists (IAE) at Goa from 10-12 October 2014

Dixit Rajnikant

1. Attended International Conference on Entomology at Punjabi University, Patiala from 21-23 February 2014.

Eapen Alex

1. Attended and presented a research paper on Challenges for sustainable control of malaria in India in an International Conference on Vector borne diseases combat and control at Chennai, India from 22-23 January 2014.
2. Attended and presented a research paper on Epidemiology and transmission of perennial malaria in Chennai: A metropolitan city in India in the keystone symposium on the Science of malaria eradication at Merida, Yucatan, Mexico from 2-7 February 2014.
3. Attended and presented a paper on 'Urban malaria and its control in Chennai' at the Fourth Annual workshop of International Centers of Excellence in Malaria Research (ICEMR) of NIH at Lima, Peru from 26-28 August 2014.
4. Attended and presented an invited scientific paper on 'Deployment of reactive case detection in Chennai, India in 63rd Annual meeting of American Society of Tropical Medicine and Hygiene (ASTMH) at New Orleans, USA from 2-6 November 2014.

Ghosh SK

1. Attended a meeting with the Chief Executive Officer, Zilla Parishad, Dakshina Kannada district at Mangalore on 20 March 2014.
2. Attended the World Health Day on the theme of Vector-borne diseases: Small bite, big threat at Bengaluru on 7 April 2014.
3. Attended & presented a paper on Planning containment of Japanese encephalitis (JE) vectors with larvivorous fish in Gorakhpur district, eastern Uttar Pradesh, India in the brainstorming session on JE/AES at Madurai organized by CRME from 26-27 June 2014.
4. Attended and presented a paper on Malaria in the command areas of Upper Krishna Project, Karnataka in the X Annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Gupta SK

1. Attended and presented a paper on *Aedes* breeding: Transmission season vs non - transmission season on the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Gupta P

1. Attended and presented a poster on Virulence *vir* gene and drug resistance gene in severe *Plasmodium vivax* in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Kumar Ashwani

1. Presented (Invited: Plenary Session) a paper entitled "Impact of insect growth regulating (IGR) compound, Novaluron 10% EC (mosquiron), spraying in Goa, India: Phase-III evaluation of the effectiveness on mosquito vector control in urban settings" in an International Conference on Entomology organized by the Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India from 21–23 February 2014.
2. Organized the X Joint Annual Conference of ISMOCD & IAE at Goa from 10-12 October 2014.

Kumar G

1. Attended and presented a poster on Climatic variables and malaria transmission in South 24 Parganas district, West Bengal in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Kumar V

1. Attended and presented a poster on Bionomics of malaria vector and its role in malaria transmission in Jharkhand, India in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Mishra Neelima

1. Attended meeting of the Greater Mekong subregion (GMS) Therapeutic efficacy studies (TES) Network, at Hanoi from 20-21 May 2014.
2. Attended meeting of the Center for the Study of Complex Malaria in India Scientific Advisory Group meeting at Seattle, USA on 10 July 2014.
3. Organized an Institutional Ethics Committee meeting at NIMR, New Delhi on 24 July 2014.
4. Delivered invited talk on Tracking artemisinin resistance in northeastern region of India in X Joint Annual Conference of ISMOCD and IAE on 11 October 2014.

Nagpal BN

1. Attended meeting and delivered a lecture on Surveillance and control of *Aedes* breeding in non-transmission season in Delhi: A case study organized by SDMC, Public Health Department (HQs), New Delhi on 7 April 2014.
2. Attended launch of Antimalaria/Dengue month campaign-cum-sensitization programme for Municipal Councillors organized by SDMC at New Delhi on 31 May 2014.
3. Attended the meeting to review the preparedness of Apex Referral Centres and SS Hospitals for diagnosis and management of dengue patients in Delhi organized by Govt. of NCT, Delhi at Delhi Secretariat, New Delhi on 24 July 2014.

Nanda Nutan

1. Participated as faculty member in two training courses on Skill up-gradation/Hands on training programme on Diagnosis of malaria for laboratory technicians of SDMC at NIMR, New Delhi from 20-24 January and 27-31 January 2014.
2. Participated as faculty member in the workshop on Antimalarial drug resistance monitoring for clinicians/research officers/ technical officers organized by NIMR in collaboration with NVBDCP from 19-23 March 2014 and delivered a lecture on life-cycle and morphology of human malaria parasites.

Prasad KM

1. Attended and presented a poster on Detection of parathyroid resistance by bioassays and biochemical assays in *Anopheles culicifacies* in Chhattisgarh India in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Rani A

1. Attended and presented a poster on Effect of urbanisation of the intensity of malaria transmission in Ghaziabad in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Raghavendra K

1. Attended meeting on Implications of insecticide resistance (IR): National Project Coordinators at Nairobi, Kenya from 27-30 January 2014.

Savargaonkar Deepali

1. Participated as a faculty member in the workshop on Antimalarial drug resistance monitoring for clinicians/research officers/ technical officers organized by NIMR in collaboration with NVBDCP from 19-23 March 2014.
2. Attented sixth meeting of the Subject Review Committee of National Formulary of India (NFI), on 12 August 2014 in the Department of Pharmacology, AIIMS, New Delhi.
3. Attented Inter-country meeting to address the Threat of artemisinin resistance in South Asia, organised by WHO-SEARO, New Delhi from 9–11 December 2014.

Sharma SK

1. Participated in the Country Consultation meeting on the Second Global Malaria Action Plan from 18–19 June 2014 at Hotel Royal Plaza New Delhi. The meeting was organized by Roll Back Malaria partnership and Caritas India.

Singh A

1. Attended and presented a poster on Health impact assessment of Narmada basin dams RR colonies in Madhya Pradesh in the X joint annual conference of ISMOCD and IAE at Goa from 10-12 October 2014.

Singh KR

1. Attended 63rd annual meeting of American Society of Tropical Medicine and Hygiene, New Louisiana, USA from 2-10 November 2014.

Singh Neeru

1. Attended annual review meeting at ICMR, (HQs), New Delhi to review the Progress of the VDLs at RMRIMS, Patna; RIMS, Ranchi; KIPM, Chennai; SMS, Jaipur and RMRC, Jabalpur on 28 January 2014 and regarding establishment of new laboratory at Raipur and Field Unit visit at District Hospital Korea and Janakpur from 29–30 January 2014.
2. Attended meeting on Malaria situation in Madhya Pradesh with the Principal Secretary, Ministry of Tribal Welfare, Govt. of Madhya Pradesh, Bhopal on 12 February 2014.
3. Attended Joint monitoring mission meeting at WHO, SEARO on Vector borne diseases in India from 1–10 March 2014.

Singh OP

1. Attended and presented a paper on Pyrethroid resistance and presence of two knockdown resistance mutations (*kdr* mutations), F134C and a novel mutation T1520I in *Aedes agypti* in the X joint annual conference of ISMOCD and IAE at Goa from 10–12 October 2014.

Singh SK

1. Attended and presented a poster on Prevalence and distribution of vector mosquitoes in Indira Sagar and Omkareshwar dam projects in the X joint annual conference of ISMOCD and IAE at Goa from10–12 October 2014.

Singh Vineeta

1. Nominated as member of the committee of Vigyan Prasar (VP) & OSDD-CSIR to create awareness about Malaria on 8 January 2014.
2. Delivered a lecture on the Dynamics of drug resistance malaria: An overview in a national seminar on Pharmaceutical approaches for malarial targeting and resistance at JSS College of Pharmacy, Ooty, India from 14-15 February 2014.

Srivastava HC

1. Attended meeting with DMOs for control of Vector borne diseases at Civil Hospital, Ahmedabad on 9 April 2014.

Tiwari SN

1. Participated in one-day CME programme on Vector borne diseases at MS Ramaiah Institute of Medical Sciences, Bengaluru on 12 April 2014.
2. Attended a review meeting on Vector borne diseases chaired by the Hon'ble Health Minister, Govt. of Karnataka at Bengaluru on 19 July 2014.

Valecha Neena

1. Attended Technical Specification Committee meeting of Miltefosine capsule under the chairmanship of DGHS (PH) at Nirman Bhawan, New Delhi on 16 January 2014.
2. Attended 25th Scientific Advisory Committee meeting of CRME at CRME, Madurai from 4-5 February 2014.
3. Delivered a guest lecture on Dengue epidemiology and future challenges for its control at Punjab University, Patiala from 21–23 February 2014.
4. Attended meeting on Access to quality medicines and others technologies Taskforce at Sydney, Australia from 11-14 March 2014.
5. Attended meeting of Working group on Pharmacoepidemiology network on creation of representative database with a view to provide a framework & road map and advice & guidance for capturing information for Pharmacoepidemiology at ICMR (HQs), New Delhi on 21 March 2014.

6. Attended meeting of three ICMR Institutes (VCRC, NIMR, RMRC) to develop a comprehensive action plan proposal of vector related to malaria transmission, identifying appropriate insecticide for supporting local government to assist malaria control programme at RMRC, Bhubanseswar, Odisha from 28-29 March 2014.
7. Participated as invited speaker on Is it feasible to contain emergence and spread of drug resistance in malaria? an Informal Expert consultation on Vector borne diseases WHO-SEARO at WHO, New Delhi from 7-8 April 2014.
8. Organised Regional consultation meeting on Development of malaria global technical stragtegy at Hotel Le Meridien, New Delhi from 28-29 April 2014.
9. Organised NIMR-MMV workshop on Implementing G6PD testing to ensure safe radical cure of *P. vivax* malaria at Hotel Le Meridien, New Delhi on 1 May 2014.
10. Attended meeting with the national manager, Australia along with CDSCO officials and state drug controllers at FDA Bhawan, New Delhi on 13 May 2014.
11. Attended Technical Resource Group (TRG) meeting on Household survey for malaria in endemic districts of seven northeast states under Global fund supported integrated malaria control project-II (IMCP-II) at NIHFW, New Delhi on 15 May 2014.
12. Participated in the meeting to Rectify the comments on final draft of SOP and common protocol for introduction of public health pesticides at ICMR (HQs), New Delhi on 26 May 2014.
13. Participated in 2nd meeting of the Access to quality medicines and others technologies task force at Manila, Philippines from 9-10 June 2014.
14. Attended meeting of Developing the 2nd Global malaria action plan (GMAP2) at WHO, New Delhi from 16-20 June 2014.
15. Attended TEG meeting on Malaria chemotherapy: II Technical consultation at Geneva, Switzerland from 25-27 June 2014.
16. Attended the meeting with Hon'ble Minister of Health and Family Welfare to discuss and share strategies and to work together to prevent disease outbreak in the states of Uttar Pradesh, Rajasthan, Haryana and NCR Delhi at Nirman Bhawan, New Delhi on 2 July 2014.
17. Attended the meeting Establishment of model rural health research unit in the state of Uttarakhand with principal secretary and other health officials at Dehradun, Uttarakhand on 28 July 2014.
18. Attended 18th meeting of group under the chairmanship of union secretary (H&FW) to Mandate the use of DDT under NVBDCP for the year 2014-15 at Nirman Bhawan, New Delhi on 1 August 2014.
19. Attended the Expert Committee meeting to Evaluate proposals for ICMR Advanced Centers of Excellence for Clinical Pharmacology at ICMR (HQs), New Delhi on 13 August 2014.
20. Attended the workshop on Malaria burden estimation at NIMR, Delhi on 2 September 2014.
21. Attended the meeting on Malaria Policy Advisory Committee (MPAC) Salle D, World Health Organization at Geneva, Switzerland from 10-12 September 2014.
22. Attented Medicine for Malaria Venture (MMV) ESAC from 14–15 October and Product Management Advisory Committee APMAC at Geneva, Switzerland from 16–17 October 2014.
23. Attended Expert Committee meeting to Review the reports of investigations of malaria outbreaks in Tripura at Nirman Bhawan, New Delhi on 24 November 2014.
24. Attended meeting with Prof. Balram and Australian Group on Medical and Health Robotics at AIIMS, New Delhi on 6 December 2014.
25. Attended Inter-country meeting to address the Threat of artemisinin resistance in south Asia from 9-11 December 2014.
26. Attended Immuno-Biological Diagnostic Kits Sectional Committee MHD19 on 23 December, 2014 at Bureau of Indian Standards, Bahadur Shah Zafar Marg, New Delhi.

10. 4 Awards and Prizes

Dr Alex Eapen

Honoured with 'Travel Award' by the American

Society of Tropical Medicine and Hygiene to attend the 63rd Annual meeting of ASTMH at New Orleans, Louisiana, USA from 2–6 November 2014.

Dr Neena Valecha

Awarded with Indian Medical Association's Diamond Jubilee IDPL Oration Award for "Malaria control in India: Challenges and opportunities" in 89th All India Medical Conference, Ahmedabad on 27 December 2014.

Hon'ble Health Minister Shri JP Nadda presenting Dr Neena Valecha with the IDPL Oration Award.

Dr K Padhan (NIMR FU Rourkela)

Awarded as the best organization for promoting health awareness among people by Youth Hostel Association of India. Shri K Padhan, OIC, NIMR, Rourkela Field Unit received the award and citation from the CEO, Rourkela Steel Plant and Director, NIT, Rourkela on 25 May 2014.

Shri K Padhan, receiving the award from the CEO, RSP, Rourkela

10.5 Books edited

Dr Ashwani Kumar along with Dr Savio Rodrigues and Dr Amit Dias edited book entitled, "*Major tropical diseases: Public health perspective*"

Book release during the Xth Joint Annual Conference of ISMOCD and IAE.

which was released by Dr VM Katoch, Secretary DHR and Dr Jagdish Prasad, DGHS, Govt. of India during X joint annual conference of ISMOCD and IAE held at Goa on 11 October 2014.

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संस्थान में राजभाषा विकास संबंधी गतिविधियाँ

11

संस्थान में वर्ष 2014–15 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 बैठकें, *मलेरिया पत्रिका* (हिन्दी) का प्रकाशन करने के साथ राजभाषा विभाग द्वारा लागू प्रोत्साहन योजनाएं कार्यान्वित की गईं इसमें निदेशक महोदया द्वारा लागू की गईं अधिक शब्द सीमा की प्रोत्साहन योजना जारी रही एवं संस्थान के प्रवेश-स्थल पर एक नवीन हिन्दी-अंग्रेजी शब्द एवं सुविचार लिखने का नवीन प्रयास इस वर्ष भी जारी रहा जो कि राजभाषा के प्रति रूचि जागृत करने का प्रयास था।

इसके साथ ही प्रतिवर्ष की भांति इस वर्ष भी हिन्दी पखवाड़ा दिनांक 15 सितम्बर से 24 सितम्बर 2014 तक पूर्ण उत्साह के साथ मनाया गया। हिंदी पखवाड़े के अवसर पर हिन्दी कार्यशाला, टिप्पण-प्रारूपण प्रतियोगिता, निबंध प्रतियोगिता एवं कर्मचारियों और अधिकारियों के लिए पृथक-पृथक वाद-विवाद प्रतियोगिताओं का आयोजन किया गया। संबंधित प्रतियोगिताओं का आयोजन संस्थान की निदेशक महोदया डॉ. नीना वलेचा के निर्देशन में संस्थान की सहायक निदेशक (रा.भा.) एवं राजभाषा कार्यान्वयन समिति के विभिन्न सदस्यों द्वारा किया गया।

इसी क्रम में दिनांक 18 सितम्बर 2014 को प्रशासनिक वर्ग के अधिकारियों एवं कर्मचारियों के लिए पूर्वाह्न 10 बजे से 1 बजे तक अर्द्धदिवसीय हिन्दी कार्यशाला का आयोजन किया गया। इस कार्यशाला में प्रथम व्याख्याता के रूप में डॉ. महेश चन्द्र गुप्त को आमंत्रित किया गया था। सर्वप्रथम डॉ. गुप्त का पुष्प भेंट कर विधिवत् स्वागत किया गया। तत्पश्चात् हिंदी कार्यशाला के संचालक श्री सी.एस. नम्बूदिरि, प्रशासन अधिकारी ने डॉ. गुप्त का परिचय देते हुए उन्हें व्याख्यान हेतु आमंत्रित किया। डॉ. गुप्त ने अपने व्याख्यान में "विज्ञान एवं प्रौद्योगिकी के विकास में हिन्दी की भूमिका" विषय की शुरूआत राजभाषा हिन्दी एवं अंग्रेजी के शब्दों के बीच तुलनात्मक अवलोकन करते हुए राजभाषा की गरिमा व महत्ता पर अत्यन्त रोचक ढंग से विचार रखे जिनकी निदेशक महोदया एवं डॉ. आर.सी. धीमान ने विशेष रूप से भूरि-भूरि प्रशंसा की। उन्होंने हिन्दी भाषा के विज्ञान एवं प्रौद्योगिकी में सहज रूप से बढ़ते कदमों की ओर संकेत कर विस्तार से जानकारी प्रदान की। कार्यशाला के द्वितीय चरण का आरंभ अपराह्न 12 बजे हुआ जिसमें राजधानी कालेज, दिल्ली विश्वविद्यालय के हिन्दी प्रभाग के एसोशिएट प्रोफेसर डॉ. राकेश त्रिपाठी को आमंत्रित किया गया था। उन्होंने "सरकारी कामकाज में सहज, सरल हिन्दी का प्रयोग" विषय के माध्यम से सरकारी कामकाज में प्रयुक्त होने वाली राजभाषा हिंदी के बारे में अनेक उदाहरणों के माध्यम से अत्यंत रोचक व ज्ञानवर्द्धक जानकारी दी। इस पखवाड़े के दौरान उल्लेखित गतिविधियों के अलावा दिनांक 25 सितम्बर 2014 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 परिषद मुख्यालय से श्री टी.एस. जवाहर,

हिन्दी कार्यशाला का शुभारंभ करती निदेशक महोदया

वरिष्ठ उपमहानिदेशक (प्रशासन) को मुख्य अतिथि तथा श्री सुरेश जी यादव, उपायुक्त, नजफगढ़ क्षेत्र, नई दिल्ली को सम्मानित अतिथि के रूप में आमंत्रित किया गया था जो कि उपायुक्त के उच्च पद पर आसीन 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 श्री अली हसन, राष्ट्रीय स्तर के कवि को भी आमंत्रित किया गया था। इस समारोह का शुभारंभ मुख्य अतिथि, सम्मानित अतिथि, कवि और संस्थान की निदेशक महोदया को पुष्प भेंट कर किया गया। स्वागत समारोह के पश्चात् संस्थान के डॉ. बी.एन. नागपाल, श्री सी.एस. नम्बूदिरि एवं डॉ. अरूण शर्मा द्वारा क्रमशः माननीय अतिथि, सम्मानित अतिथि एवं कवि महोदय को शॉल भेंट कर सम्मानित किया गया। इसके बाद संस्थान की निदेशक महोदया ने अपने संबोधन में कहा कि गृह मंत्रालय द्वारा प्रेरणा एवं प्रोत्साहन की नीति द्वारा भी तभी सफलता मिलेगी जब आपके भीतर हिन्दी में काम करने की प्रबल इच्छा-शक्ति होगी। जिस प्रकार हम कार्यालय के अन्य नियमों का पालन करते हैं उसी प्रकार राजभाषा नियमों का पालन करना हमारा कर्तव्य है। उन्होंने यह भी कहा कि हमने राजभाषा नियम, अधिनियम के भली-भांति पालन हेतु राजभाषा संबंधी निर्देश का पोस्टर तैयार किया है जिसे विभिन्न अनुभागों में इस आशय से लगाया जाएगा ताकि हर रोज ये निर्देश हमें राजभाषा के प्रति अपने दायित्व की याद दिलाएंगे। निदेशक महोदया ने अपने संबोधन के पश्चात् पोस्टर का विमोचन किया जिसे संस्थान के विभिन्न अनुभागों में लगाया गया।

तत्पश्चात् श्री सुरेश जी यादव (सम्मानित अतिथि) ने सभा को सम्बोधित करते हुए कहा कि वर्तमान समय में हिन्दी तेजी से अपना एक विशेष स्थान बनाते हुए आगे बढ़ रही है। इस संबंध में उन्होंने अपनी कुछ कविताओं को भी प्रस्तुत करते हुए कार्यक्रम को एक नया मोड़ दिया। उन्होंने हिन्दी भाषा के सरकारी कामकाज में बढ़ते हुए वर्चस्व स्थापित होने की बात भी कही।

पुरस्कार वितरण के पश्चात् मुख्य अतिथि श्री टी.एस. जवाहर ने सभा को संबोधित करते हुए संस्थान में आयोजित पुरस्कार वितरण समारोह में सभी अधिकारियों एवं कर्मचारियों

संबोधित करती हुईं निदेशक डॉ. नीना वलेचा

राजभाषा संबंधी पोस्टर का विमोचन करती निदेशक महोदया

अतिथि डॉ. सुरेश जी यादव संबोधित करते हुए

मुख्य अतिथि श्री टी.एस. जवाहर संबोधित करते हुए

के उत्साह को देखकर हर्ष जाहिर किया। उन्होंने राजभाषा हिंदी के प्रयोग संबंधी संवैधानिक प्रावधानों पर प्रकाश डालते हुए सरकारी कामकाज में हिंदी का अधिक से अधिक प्रयोग करने हेतु प्रेरित किया।

अंततः कार्यक्रम का विधिवत् समापन करने हेतु संस्थान की वैज्ञानिक 'एफ' डॉ. नीलिमा मिश्र, ने हिन्दी पखवाड़े के दौरान आयोजित गतिविधियों का सफलतापूर्वक संचालन करने हेतु सभी संचालकों को धन्यवाद ज्ञापित करने के साथ ही समग्र कार्यक्रम के आयोजन में संस्थान के हिंदी अनुभाग के योगदान की सराहना करते हुए उन्हें हार्दिक धन्यवाद ज्ञापित किया। इसके साथ ही उन्होंने उपस्थित श्रोताओं एवं विजेताओं को भी धन्यवाद दिया जिनके सहयोग द्वारा ही इस कार्यक्रम का सफलतापूर्वक आयोजन किया जा सका।

संस्थान ही नही वरन् संस्थान की क्षेत्रीय इकाइयों में भी राजभाषा कार्यान्वयन के प्रति उत्साह जागृत करने के उद्देश्य से हिन्दी दिवस के उपलक्ष में विभिन्न प्रतियोगिताओं का आयोजन किया गया, जिसमें नडियाड, 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 कार्यों 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 ❑

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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 Arun Sharma
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Dr PK Atul
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Dr Jyoti Das
Dr AK Mishra
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Dr AK Mohanty
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