Chapter 2

Emergence and spread of insecticide-resistant *Anopheles (Cellia) culicifacies*: its bionomics and control in the context of malaria elimination initiative in India

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Abstract

Anopheles culicifacies s.l. is a predominant mosquito vector in rural India generating bulk of malaria cases (>60%) annually and maintains endemic malaria in areas of its influence. It is the most studied species complex for its sibling-species composition, distribution and their bionomical characteristics having implications in vector control. It is a highly adaptive and robust mosquito virtually resistant to all available insecticides including pyrethroids in large tracts of mainland India and fast invading new territories thwarting malaria elimination efforts. Control of *An. culicifacies* is a formidable challenge and containing its spread and transmission of drug-resistant malaria deserves utmost priority. Innovative technologies are warranted for sustained control of this vector species for achieving malaria elimination.Vector surveillance and monitoring insecticide resistance should be the continuing activity for formulating evidence-based and doable intervention strategies for effective vector management.

Keywords: *Anopheles culicifacies*, sibling-species complex, vector bionomics, insecticide resistance, malaria transmission, vector control, India

Introduction

India is malaria endemic and recently has been enlisted amongst 11 high burden countries by the World Health Organization for contributing 4% of the global estimated cases in 2017 [1]. The transmission is heterogenous across its landscape due to multiplicity of disease vectors, diverse ecology and contextual determinants [2]. Among six predominant mosquito vectors in India, *Anopheles culicifacies s.l.* is the most dominant one contributing >60% of the reported cases in the country [3, 4]. Historically, it was held responsible for devastating malaria epidemics in domain of its distribution in rural India and much of the malaria control efforts relate to containment of *An. culicifacies* alone even in the context of present-day malaria [5]. Malaria resurgence in 1970s may be attributed to the failure to control *An. culicifacies s.l.* inter-alia due to inadequate interventions and emergence of insecticide resistance resulting in rising densities and consequent predilection for human host [6]. Over the past three decades, a great deal of information has been generated in understanding its population genetics and bionomics for the benefit of the control programme helping devise appropriate control strategies [7-9]. It stands out to be the most studied mosquito species complex for its sibling-species composition, distribution, bionomical characteristics and role in disease transmission. India is currently experiencing rapid economic boom, population explosion/ migration, deforestation and infrastructure development resulting in expansion and ecological succession of other vector species in the altered ecology. *An. culicifacies* is one such species which is invading new territories and has grown multi-resistant inviting attention of programme planners and policy managers alike for its effective control [10-13]. Given the clarion call for malaria elimination in India by 2027 [14, 15], control of the malaria vector *An. culicifacies* deserves priority averting disease outbreaks and spread of drug-resistant malaria. We, hereby, present the overview of bionomics of this species complex in the context of malaria elimination for achieving sustainable control of this dominant mosquito vector in India.

Taxonomic considerations, sibling-species identification & distribution

An. culicifacies s.l is widely distributed in South and South-East Asia extending from Afghanistan to the far east to Thailand and Vietnam, with an eastern extension into the southern Arabic Peninsula and eastern Africa (Figure 1) [16]. It is a medium sized mosquito, adults of which can easily be distinguished from other species of the subgenus *Cellia* by diagnostic morphological characters (Figure 2) [17, 18]. Genetical investigations of this taxon revealed it to be a complex of five sibling species informally designated as species A, B, C, D and E based on species-specific diagnostic fixed paracentric inversions suggestive of pre-mating barriers in natural populations [7-9]. The five-sibling species are morphologically indistinguishable but can be substantiated by wide array of techniques including mitotic karyotype, Y- chromosome polymorphism, gene-enzyme variations, cuticular hydrocarbon profiles and diagnostic molecular assays. These sibling species are spread across India (Figure 3) and characterized to have distinct bionomical characteristics and distribution record with obvious implications for malaria transmission control [19]. Among these, species B is spread throughout rural India and occurs in sympatry with A, C and D. Whereas, species A and B are sympatric in north and south India; species A is

more abundant in the north than species B and vice versa in the south. In states of Uttar Pradesh, Bihar and north-east, species B is the only species that is prevalent or the most predominant. Species B and C are predominant in the west and east which overlap with species D in central and western Indian states. Species E has been exclusively recorded in south India and believed to be spreading to other endemic states.

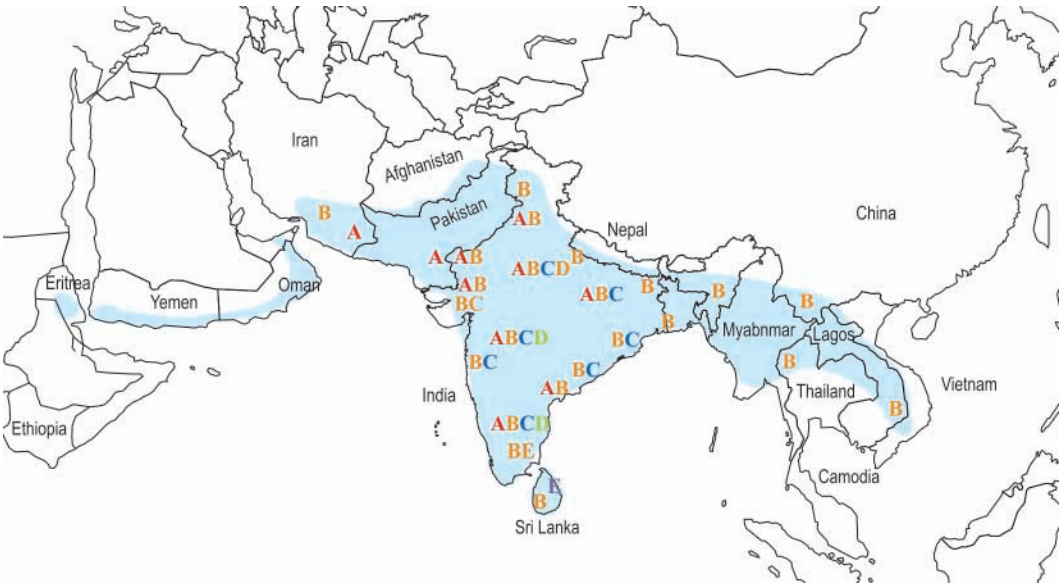

Figure 1: Distribution of sibling-species of *Anopheles culicifacies s.l.* in the world [sketch map not necessarily in conformity with political boundaries].

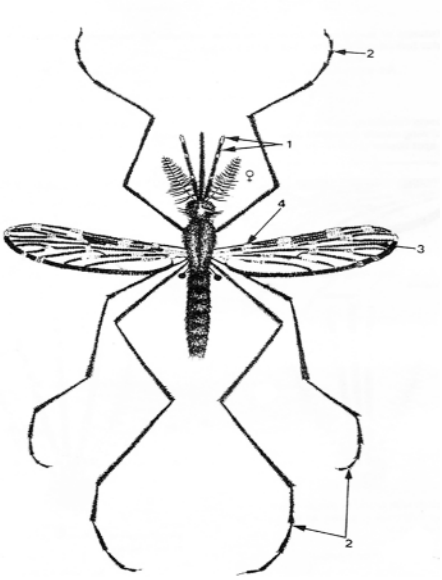

Figure 2: *Anopheles culicifacies:* morphological distinguishing characters of mosquito adult: (1) apical band nearly equal to pre-apical dark band on palpi, (2) tarsomeres without bands, (3) vein - 3 mainly dark, (4) inner costa interrupted. Source Reference [18].

Figure 3: Geographical spread of sibling-species of *Anopheles culicifacies* complex (species A,B,C,D,E) in India. Source Reference [19].

Seasonal abundance, breeding and resting habitats

An. culicifacies s.l is widely abundant during monsoon and post-monsoon months in rural and peri-urban India. It occurs in low densities at high altitudes (1000 - 2000 msl), but most commonly found in plains receiving heavy to moderate rainfall. It is responsible for unstable malaria in large tracts of forested tribal belts [20], and given the ideal climatological conditions, it attains high densities resulting in focal/regional epidemics. It is a prolific breeder and habitats are diverse and numerous. These include irrigation channels, seepage-water streams, unused wells, river-bed pools, rice fields, miningand borrow pits, rocky pools and other fresh water collections (Figure 4). All member species rest indoors in human dwellings but known to rest outdoors as well in cattle sheds.

Host preferences, infectivity and disease transmission

All sibling species are predominantly zoophilic, except species E which is observed to be highly anthropophilic in Rameswaram Islands of Tamil Nadu and incriminated as malaria vector having high sporozoite infectivity [21]. Species A is reported to have relatively high anthropophilic index compared to B and D, and C having intermediate level of human blood index [7-9]. All member species of the Culicifacies complex are night biting, peak activity hours, however, varied anywhere between 18:00 – 23:00 h. *An. culicifacies s.l* has been repeatedly incriminated as vector by detection of gut and salivary gland infections across range of its distribution [18]. Sporozoite infection rates, however, varied amongst sibling species in range of their distribution. Among these, species A, C and D were proven vectors by immunoradiometric assays by cumulative infection rates of 0.51%, 0.3% and

0.4% respectively [22, 23]. However, species B is a non-vector or poor vector evidenced by low prevalence of malaria in areas of its occurrence. These observations were further substantiated by fitness studies for reproductive potential, sporogony and insecticide susceptibility [24]. Disease transmission is largely seasonal during monsoons and post-monsoon months corresponding to build up of high vector density.

Figure 4: Larval habitats of *Anopheles culicifacies*: (A) Irrigation channels with residual water pools, (B) Rainwater collections in ditches/pools (courtesy: Neeru Singh), (C) seepage-water streams, (D) river-bed pools (courtesy: Rinzin Namgay).

Insecticide resistance and vector control

Insecticide resistance in malaria vectors is a global phenomenon [25, 26]. The Indian National Vector Borne Disease Control Programme relies heavily on application of residual insecticides for vector control [27]. DDT proved to be an "angel" during 1953 – 1960 resulting in dramatic decline of cases from 75 million in pre-DDT era to less than one hundred thousand cases in 1960s in India [28]. However, in 1970s, malaria re-emerged with vengeance to six million cases on record, which was largely attributed to development of resistance to DDT in *An. culicifacies* maintaining endemic malaria [6]. The phenomenon of emerging resistance was unstoppable across arsenal of insecticides including cross-resistance with organo-chloride compounds (Dieldrin), and other classes of insecticides, i.e., organophosphate (malathion) and more recently pyrethroids (deltamethrin) making

control of rural malaria a difficult enterprise (Figure 5) [29-31]. These data are corroborated by low frequency of the *kdr* allele (mostly in heterozygous condition) in field populations that confer resistance to DDT and pyrethroids [32]. *An. culicifacies* is presently resistant to virtually all available insecticides and worse that multi-resistant populations are now proliferating and spreading to newer territories. It is reported in high densities in degraded forests of north-east India replacing susceptible populations of *An. minimus* resulting in ecological succession sharing similar resting and larval habitats [10-13, 33].

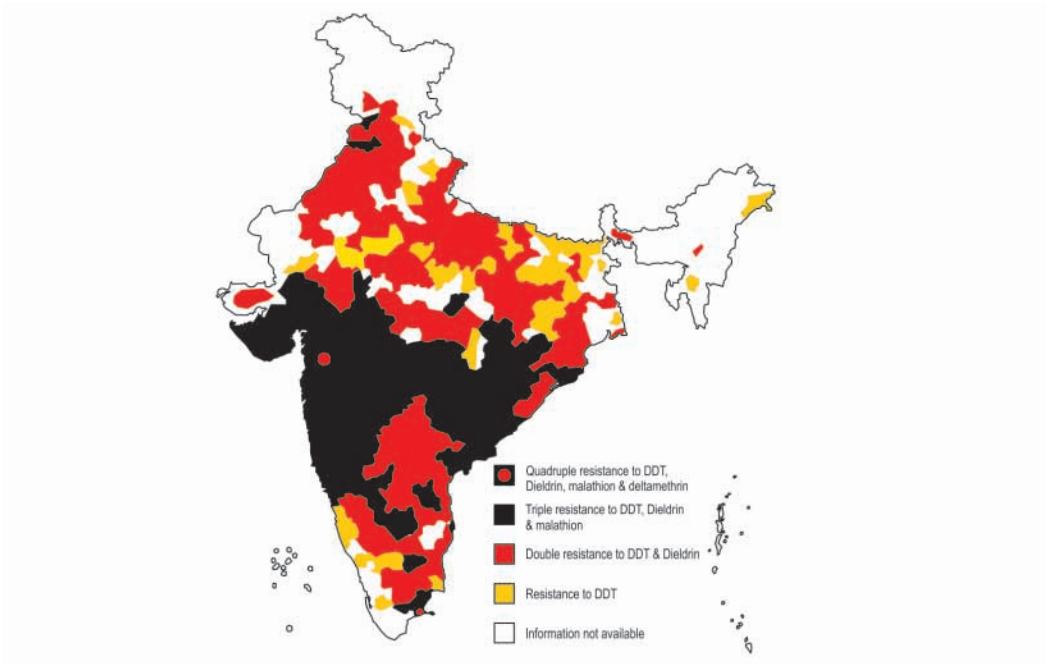

Figure 5: Status of insecticide resistance in *Anopheles culicifacies* in India for data based on 2006. Since then, *Anopheles culicifacies* has invaded north-east India and is resistant to DDT and Dieldrin in almost all parts of the country, and to DDT, Dieldrin and Malathion in large parts of central and western states, and to all insecticides including pyrethroids in certain parts of Gujarat and Tamil Nadu.Source Reference [29].

Among high-burden countries, India holds the distinction for reporting steady decline in cases over the past few years registering 24% decline in 2017 compared to 2016 [1, 34]. It can be largely attributed to the combination of interventions including induction of insecticide-treated netting materials/long-lasting insecticidal nets for vector control, artemisinin-based combination therapies (ACT) circumventing the development and spread of drug-resistant malaria, dipsticks for on-the-spot rapid diagnostic tests (RDT) ensuring early case detection, human-resource development in strengthening healthcare services in the periphery, and above all external financial assistance by international agencies for enhanced coverage that made the difference in rolling back malaria to less than a half million cases in 2018, and counting towards malaria elimination in the foreseeable future [15]. However, threat looms large for expansion of insecticide-resistant populations of *An. culicifacies*, which would spell doom to the programme evidenced by rising proportions of *Plasmodium falciparum* each passing year to presently >60% of total reported cases in the country [35]. There is an imperative need for monitoring insecticide resistance and developing risk-maps for focussed coverage for keeping its populations at bay.

Sibling species paradox and implications in malaria vector control

It is ironic that given the wealth of added information in understanding the sibling-species composition and bionomics of *An. culicifacies*, nowhere this information has been utilized in the national control programme. The techniques applied for confirmed identification for sibling-species A, B, C and D are not diagnostic except that of fixed inversions readable in the polytene chromosome karyotype (Table 1). Besides, there being a great deal of overlap in the geographical range of distribution of sibling species (Figure 3); identification is far more a skilled exercise well beyond the capacity of field-health workers making it an operationally difficult choice for control interventions in resource-poor settings.

Table 1. Techniques applied for characterization of sibling-species of *Anopheles culicifacies* complex

Sibling species	Polytene chromosome inversion genotype	Mitotic karyotype Y- chromosome	LDH enzyme alleles*	Species specific DNA probes	PCR-RFLP**	ASPCR***
A	$X+^{a}+^{b}$; $2+g^{1}+h^{1}$; $+i^{1}/i^{1}$	Submetacentric	Fast	Yes	Yes	Yes
B	Xab; 2g1+h1	Acrocentric, Submetacentric	Slow	Yes	Yes	Yes
C	Xab; 2+g1h1	Acrocentric, Submetacentric	Slow	Same as B	Same as B	Same as B
D	X+a+b; 2i1+h1	Submetacentric	Fast	Not tested	Same as A	Same as A
E	Xab; 2g1+h1	Submetacentric	Slow	Not tested	Same as B	Same as B

LDH: lactate dehydrogenase; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; ASPCR: allele specific polymerase chain reaction. Adapted from source Reference [9].

Seemingly, there are lapses in reaching correct identification except the existence of A and B (initially proposed by Chris Green) validated by post-zygotic fertility data marked by hybrid male sterility, reduced fertility and atrophied gonads [36]. However, similar dataon reciprocal crosses between remaining sibling species are far from adequate, *inter-alia*, which would have been a clinching evidence to prove their existence beyond doubt [37]. Moreover, application of PCR based r-DNA diagnostic assays clearly clubbed sibling-species into two distinct groups, i.e., group I (species A/D) and group II (species B/C/E) [38]. Besides, based on the cumulative data on mitotic chromosomes karyotype, gene-enzyme system, species-specific DNA probes and distribution records, it is imperative that true prevalence of species C, D and E requires introspection (Table 1). It seems as if species A and D are similar; species C is akin to B (except polytene chromosome karyotype); instead species B and E are the same corroborated by homosequential polytene chromosome karyotype and sequencing of ITS2 and D3 regions of 28S rDNA [39]

In essence, species A and B are only the two true breeding species requiring species-specific control interventions for effective vector management. The existence of possible morphological differences between sibling-species have not been explored and need to

be prioritized enabling binomial nomenclature. Apparently, there are glaring gaps of information, which have been clearly overlooked [16]. In summary, *An. culicifacies* species complex is far from being resolved and for control of its populations, irrespective of prevalence of any of its sibling-species in given area, intensification of interventions has become of paramount importance in order to eliminate residual populations preventing re-establishment of active transmission in malaria-free territories.

Priority areas of research

The expanding range of distribution of *An. culicifacies* in north-east India is an emerging threat to the control programme for continued transmission and spread of multi-drug resistant malaria. The proportions of drug-resistant *P. falciparum* malaria are steadily risingfor which north-eastern region is considered high-risk zone for proliferation and spread to peninsular India [35, 40]. Low-grade artemisinin resistance has already surfaced in north-east along international borders evidenced by detection of *kelch-13* mutations [41-43]. *An. culicifacies* is robust, invasive and highly adaptive species in varied ecological contexts for which interventions should be strengthened for universal coverage ensuring maximal compliance. The emergence and spread of this species must be contained by innovative technologies aiming reducing its density below threshold and defeating insecticide resistance. There exists scope for alternate mechanisms for insecticide resistant management like insecticide rotation [44, 45], and newer technologies, viz., eave-tubes, attractive toxic sugar baits (ATSB), mosaic long-lasting insecticidal nets, which must be put to field-evaluation in different transmission settings [46]. These interventions combined with other bio-environmental approaches, e.g., larvivorous fish (guppy and *Gambusia*) and above all behavioural change communication educating communities would help achieve sustainable control of vector populations [40]. Early biting behaviour of mosquito vectors associated with out door transmission are seen as crucial challenges, which must be addressed by appropriate technologies, viz., insecticide-treated plastic sheeting/ hammocks, use of repellents, to cite a few, in context of malaria elimination efforts in the South-East Asian region of the World Health Organization [47, 48]. North-east India shares wide international border with Myanmar to the east, Bangladesh to south and Bhutan to the west having similar ecology and disease vectors. These borders are porous and ill equipped to meet the complex emergencies permitting mix of parasite strains and resultant focal disease outbreaks and propagation of drug-resistant strains. Cross-border initiative with these member countries deserves priority for coordinated action for vector control operations to arrest the spread of drug-resistant varieties of malaria emanating across borders [49].

Conclusions

An. culicifacies has grown multi-resistant to the insecticides. The emergence of spread of this invasive speciesis a formidable challenge control of which has regained importance in the wake of malaria elimination efforts in the Asia Pacific Malaria Elimination Network

of countries (APMEN). To contain its spread, country-led response is mandated for vector surveillance, monitoring insecticide resistance, data sharing and cross-border initiative for coordinated action; all these should be the core-activities for mitigating impending disease outbreaks and spread of drug-resistant malaria [50]. In keeping with 'high burden to high impact' flagship initiative of WHO, much more can be achieved in transmission reduction by strategic application of technologies that are evidence-based, community-oriented and doable, and above all political commitment for strengthening healthcare services in the periphery/high-risk foci ensuring universal access to realize the envious goal of malaria elimination in India in due time [51-53].

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