Mosquitoes are the medically most important group of insects, both in terms of the number of pathogens they transmitted and the magnitude of health problem caused by disease worldwide (Service, 1989). Mosquitoes act as a vector of various parasitic and viral pathogens, cause various diseases like dengue, chikungunya, zika fever, yellow fever, malaria, lymphatic filariasis, Japanese encephalitis, etc.

Mosquitoes belong to family Culicidae of order Diptera. This family comprises approximately 3523 species of mosquitoes in 111 genera worldwide, of which 372 species in 49 genera from India and 191 species in 28 genera from North East India. Three subfamilies: Toxorhynchitinae, Anophelinae, and Culicinae are recognized from family Culicidae (Harbach, 2013; Kettle, 1984; Abu Hassan et al., 1997). On the basis of blood sucking behavior, only subfamily Anophelinae and Culicinae are most important for disease transmission. The proboscis of family members of Toxorhynchitinae are curved shape due to which they are unable to suck the blood meal, hence are unable to transmit the parasites causing the disease (Service, 1996).

**Aedes (Diptera: Culicidae) mosquito**

The *Aedes aegypti* and *Ae. albopictus* are medically most important vectors of arboviruses, including dengue virus (DENV), zika virus (ZIKV), chikungunya virus (CHIKV), and yellow fever virus (YFV). Population growth, rapid urbanization, human travel and failures of preventative public-health measures are the major factors for increasing dengue fever cases (Adams et al., 2009; Chen et al., 2010; Gubler, 2002). It is also noticed that the dengue cases are increasing not only in urban areas but also in the rural areas (Ukey et al., 2010).

*Aedes* mosquitoes are the daytime biting mosquito and breeds in natural and man-made container with stagnant water which are generally within or in close proximity to households. Both species *Ae. aegypti* and *Ae. albopictus* are container-breeder however due to their distinct biology and behavior they occupied different niches (Eisen and Moore, 2013). It has been previously described that inter-species competition (O’Meara et al., 1995; Daugherty et al., 2000; Juliano et al., 2007) and/or non-reciprocal cross-species inseminations (Bargielowski et al., 2013) are responsible
for the local spreading of *Ae. albopictus* and decline *Ae. aegypti* populations. Other than using of containers to store water for egg laying, socio-economical factors including housing quality, use of air-conditioning and the rate of urbanization are the important factors which affect the distribution of *Aedes* mosquitoes (Ramos *et al.*, 2008; Astro’m *et al.*, 2012). Now in days, both *Ae. aegypti* and *Ae. albopictus* mosquitoes are present in most of the Asian cities and large parts of the Americas (Lambrechts *et al.*, 2011). Various other factors affect the distribution of *Ae. aegypti* and *Ae. albopictus* mosquitoes worldwide. Based on the broader range of climatic variables including precipitation, temperature, and humidity, researchers predicted the global distributions of *Ae. albopictus* and *Ae. aegypti* (Medley, 2010; Khormi and Kumar, 2014; Campbell *et al.*, 2015; Kobayashi *et al.*, 2002; Nawrocki and Hawley, 1987). Recently, it has been demonstrated that *Ae. albopictus* has higher survival rates than *Ae. aegypti* (Brady *et al.*, 2013) (Fig. 2.1).

![Fig. 2.1. Patterns of potential distributional overlap derived from ecological niche models of *Ae. aegypti* and *Ae. albopictus* worldwide (Courtesy: Campbell *et al.*, 2015).](image)

*Aedes (Stegomyia) albopictus* (Skuse)

Skuse (1894) first described the *Ae. albopictus* (Skuse) (Diptera: Culicidae) from India as “the banded mosquito of Bengal”. The Asian tiger mosquito, *Ae. albopictus*, is an aggressive, daytime biting insect. It is an exophilic mosquito and usually found in shady areas where it rests in shrubs near the ground (Carrington *et al.*, 2014; Koehler & Castner, 1997). Due to its sturdy nature and tolerance to a
broader temperature range, it is an ideal species for different climatic conditions (CDC, 2016) and acts as an emerging and potential threat in the outbreaks of arboviral diseases (Paupy et al., 2009, Rezza, 2012, Schaffner et al., 2013). It has been proved that the *Ae. albopictus* is the competent vector for about 22 arboviruses (Shroyer, 1987; Turell, 1988; Mitchell, 1995; Gratz, 2004).

*Ae. albopictus* is originally indigenous to Southeast Asia (Smith, 1956) and later on, during the last centuries, it was spread to Western Pacific and the Indian Ocean. It has been hypothesized that during the 17th and 18th centuries, immigrants came from Asia and plays a significant role in the introduction of this mosquito species (Delatte et al., 2009). During a decade of the 80s, the range of *Ae. albopictus* rapidly expanded to Europe, the United States and Brazil (Medlock et al., 2012; Carvalho et al., 2014). In the past 30–40 years, *Ae. albopictus* has colonized every continent except Antarctica (Caminade et al., 2012; Benedict et al., 2007) (Fig. 2.1). *Ae. albopictus* mosquitoes have been demonstrated to rapidly expand their territory worldwide (Kraemer et al., 2015).

*Aedes (Stegomyia) aegypti* (Linnaeus)

*Ae. aegypti* is highly anthropophilic and prefer to live close proximity to humans which makes it one of the most efficient vectors of arboviral diseases like dengue and chikungunya. It is known as an endophilic mosquito and mostly likes to live in and around the homes in tropical and subtropical regions (Carrington et al., 2014). *Ae. aegypti* is commonly distributed in urban areas with or without vegetation occupying densely populated locations.

It is believed that *Ae. aegypti* was originated from central Africa, where it was zoophilic tree hole mosquito and was known as *Ae. aegypti formosus* (Brown et al., 2014). Later on, it was distributed to various other parts of the world through sailing ship (Smith, 1956). In the late 21st century, dengue fever was first reported in Asia and it was believed that this was occurs due to the colonization of *Ae. aegypti* (Smith, 1956). It has believed that, the harsh condition along with the beginning of the slave trade, the *Ae. aegypti* mosquitoes were incorporated from Africa to the New World and subsequently extended worldwide to tropical and sub-tropical regions (Brown et al., 2014) (Fig. 2.1).
Life cycle of *Aedes albopictus*:

Life cycle is completed in 4 stages viz. egg, larvae, pupa and adult and takes about 7–9 days to develop from egg to adult in optimum conditions (Fig. 2.2).

**Egg:** Eggs of *Ae. albopictus* are black and oval with a length of 0.5mm, laid singly on the sides of water-holding containers such as flowerpots, birdbaths, tires, animal watering dishes, and natural holes in vegetation (Hawley, 1988).

**Larvae:** Larvae, also called wigglers, and feeds on the biotic material as well as fine particulate organic matter in the water. Finally, the forth instars larvae converted into the pupa.

**Pupa:** Pupa is comma shaped and comprises of a fused head and thorax. This is the non-feeding stage and follows 24-48 hours at 27°C for the emergence of the adult.

**Adult:** The adult male and female requires plant sap for their survival. Following the mating the female adult seeks a blood meal for ovary development and figures out a suitable place to lay eggs and cycle begins again.

![Life cycle of Aedes mosquitoes](image_url)

**Fig. 2.2** Life cycle of *Aedes* mosquitoes
Medical importance of *Aedes* mosquitoes

Dengue fever is caused by dengue virus (DENV) transmitted by *Ae. aegypti* and *Ae. albopictus*. Dengue occurs in two severe clinical manifestations—dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue fever is a severe, flu-like illness and affects infants, young children and adults. Dengue should be suspected when symptoms of high fever (40°C/ 104°F) with a severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash appear. In severe dengue cases, the plasma leak, fluid accumulation, respiratory distress, severe bleeding, or organ impairment causes deadly complication. Warning signs in severe cases start after 3–7 days of first symptoms combined with lowering in temperature (below 38°C/ 100°F) and including severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness, blood in vomit. This time and next 24–48 hours is the critical stage and it may be fatal. It is an emergency period and a proper medical care is needed to avoid complications and risk of death.

A dengue viral infection now causes more illness and death than any other arboviral disease, commonly in tropical and sub-tropical regions. It has become a leading cause of pediatric morbidity and mortality in some Southeast Asian countries (Bhatt *et al.*, 2013). In the recent decades, the disease transmission has increased drastically, mainly in urban and semi-urban areas (WHO, 2016). In 2010, about 96 million dengue infections were reported globally and out of which approximately 70% were from Asia alone. India alone contributed 34% (24–44 million infections) of the total globe (Chakravarti *et al.*, 2012; Kakkar, 2012) (Fig. 2.3). Bhatt and colleagues (2013) estimated that about 390 million dengue infections occurs annually worldwide (Bhatt *et al.*, 2013) and about 3900 million persons in 128 countries are at risk (Brady *et al.*, 2012; WHO, 2016).
Dengue fever was first documented from India in the year 1945 (Sabin, 1952). Subsequently, during 1963-64, it was reported from Eastern coast of India (Raheel et al., 2011). Later on, the cases were also reported from Delhi (in 1967) and Kanpur (in 1968) and gradually the whole country was involved with widespread epidemics of dengue fever (Balaya et al., 1969; Chaturvedi et al., 1970).

Fig. 2.4. Total number of dengue cases in the states and union territories of India from 2012 to 2016 (*Provisional till 31st December) (Courtesy: NVBDCP)
In the last few years, the incidence of dengue fever has been increased drastically. According to report of National Vector Borne Disease Control Programme (NVBDCP), dengue cases were recorded from 35 states including union territories of India (Fig. 2.4). Among last few years, maximum numbers of cases were recorded in 2016 (101388) in which West Bengal (11069) was the most affected and Sikkim (only 8) was least. Other states like Punjab, Orissa, Gujarat, Uttar Pradesh, Kerala, Maharashtra, Karnataka, Assam and Delhi were also highly affected with dengue fever in this year (Fig. 2.4) (NVBDCP, 2016). In 2015, Delhi (15652) was the highest endemic territory (Fig. 2.5).

In North East India, dengue activities were recorded from Assam (Darrang district) and Arunachal Pradesh (Lohit district) during 1963 (Rodrigues et al., 1977; Dutta et al., 2006). Later on, cases were also reported from other regions of Northeastern India, including Nagaland (Dutta et al., 2006; Barua et al., 1996; Sankari et al., 2012). Very recently, increasing number of dengue cases were reported from states of North East India, including Meghalaya, Nagaland, Assam, Manipur and Arunachal Pradesh, signifying the changing epidemiology of the disease in this part (Dutta et al., 2012). Khan et al., (2014) have also been reported the dengue outbreak in Pasighat, Arunachal Pradesh, North East India. Assam is highly endemic region among the entire North-east India. About 5654 cases were reported from North-east

![Fig. 2.5. Distribution of dengue cases in India in 2015 (WHO, 2015)
India in 2016., among them about 5075 cases were only from Assam and rest from 7 other states (Fig. 2.6) (NVBDCP, 2016).

**Fig. 2.6. Dengue Cases in North East India since 2012 to 2016 (*Provisional till 31st December) (Courtesy: NVBDCP)**

Chikungunya is another most important viral disease caused by chikungunya virus (CHIKV), and transmitted by *Ae. aegypti* and *Ae. albopictus*. Time from the biting of infected mosquitoes with chikungunya virus to the onset of illness can be 2-12 days but is usually three-seven days. Silent infections of chikungunya virus or infections without symptoms of the disease do occur though it is still unknown how often. The symptoms of chikungunya fever resembles the dengue fever and it included a sudden onset of fever; a severe headache; chills; nausea; vomiting and joint pain. The chikungunya disease is characterized by severe joint pains. Sometimes it is constant for a long time but generally, it is not fatal. This prolonged joint pain and fatigue occur only in the case of chikungunya and these typical symptoms differentiated it from the dengue fever. A rash may sometimes occur, but a haemorrhaging is rare.

The first time the chikungunya viral disease was described during 1952 in southern Tanzania. Later on, Robinson and Lumsden in 1955 have reported that the disease was to be very similar to “Dengue-like fever” (Robinson, 1955; Lumsden, 1955). Since 1955, most cases of chikungunya infection have been described in Africa and India. However, the epidemic outbreaks of this infection have been reported in India, in several countries of Africa, the Indian Ocean region and Southeast Asia (Cavrini *et al.*, 2009) (Fig. 2.7).
In India, the chikungunya was first reported in 1963 in West Bengal (Shah et al., 1964) followed by several epidemics in Chennai, Pondicherry, Vellore, Visakhapatnam, Rajmundry, Kakinada, Nagpur and Barsi between 1964 to 1973, (Ray et al., 2012; Arankalle et al., 2007). Recently, the states of South India are highly affected with chikungunya infection. From coastal regions of Andhra Pradesh and Karnataka, the outbreak of chikungunya was started in 2005 (Schuffenecker et al., 2006) and about more than 1.3 million peoples have estimated to affected with this disease (Lahariya et al., 2006). Among the last few years, the highest number of chikungunya cases were recorded in 2016 (55639), and about 28 states including union territories of India were affected (Fig. 2.8). During 2016, Karnataka and Delhi were highly affected and only these two state/UT comprises about 45% of the total chikungunya cases in all over India (Fig. 2.8). Apart from these two states, other states like Maharashtra, Haryana, and Punjab were also highly affected. In 2015, a total of 27553 chikungunya cases were recorded from all over India among them 20763 cases were from Karnataka only (Fig. 2.8) (NVBDCP, 2016).
Fig. 2.8. Total number of chikungunya cases reported in India since 2012 to 2016 (*Provisional till 31st December)  (Courtesy: NVBDCP)

As compare to dengue fever, the incidence of chikungunya in North-east India is low. Among 8 states of North-east India, incidence of chikungunya was reported from four states only namely: Arunachal Pradesh, Assam, Meghalaya and Tripura. Since 2010, a total of 2036 chikungunya cases were reported from entire North-east India in which the maximum number of cases were from Assam followed by Meghalaya, Tripura and Arunachal Pradesh respectively (Fig. 2.9) (NVBDCP, 2016).

Fig. 2.9. Clinically suspected chikungunya cases reported in Northeast India since 2010 to June 2016 (*Provisional till 31st December) (Courtesy: NVBDCP)
Disease management strategies

Due to the noncurable nature of the disease, all efforts are directed to avoiding the proliferation of the mosquitoes population and ultimately the proliferation of parasites inside the mosquito’s body as well, resulting the prevention of disease transmission. Use of insecticides is a traditional approach and is routinely used for mosquito control. Chemical based insecticides such as organochlorine and organophosphate compounds are frequently used but it leads to resistance development among the mosquito vectors. Recently, the resistant development in *Ae. albopictus* and *Ae. aegypti* against temephose, malation, fenthion and several other insecticides have been reported (Husham *et al.*, 2010; Singh *et al.*, 2011).

Various other demerits of insecticides have been observed as follows:

- Insecticide-based control is temporary and frequent repetition is costly.
- Insecticides are also harmful to the environment and mankind.
- Insecticides may harm other organisms in the environment, including the natural enemies of mosquito.
- Insecticides are non-biodegradable in nature and remain in environment for prolong time.

Due to hazardous and toxic properties and persistence in the environment for long time, several pesticides are banned in many countries. Therefore, an urgent need to develop an effective, environment friendly and target specific techniques for overcome the detrimental effect of insecticides.

Alternative methods of vector Control

Several environment friendly methods involving use of insectivorous fishes, biopesticides, pheromones, sterilized males, refractory mosquitoes, etc. are being developed with various degree of success. But this technology is slow and highly specific in nature and moreover, different populations of the same species may respond differently to that particular semiochemicals in different geographical regions.

Genetic modification of vector mosquito is another effective technology which may be mainly used either to suppress or replace the wild populations of a vector so as to decrease vector populations or reduce vector ability to transmit. These
applications include release of reared mosquitoes in the environment to introduce modified genetic traits in wild populations. It is particularly significant to malaria where mosquitoes become resistant to insecticides and parasites to drug treatments and for dengue, where no any dedicated therapy are exists and disease prevention solely depends on the effective vector control (Hemingway et al., 2000; WHO, 2016).

GM encompasses multiple approaches which are broadly categorized into two types. The first category includes Gene drive systems for population replacement or manipulation and the second category is sterilized Insect technique (SIT) for population suppression.

**Gene Drive Technique**

In the Gene drive techniques, a desired genetic modification of an organism has been done which passed on to their offspring in the next generation and rapidly modify the entire population (Oye et al., 2014). Development of ‘CRISPR’ gene-editing technique helps to researchers for precisely genetic modification of the organism and makes it a realistic and highly potential for controlling the vectors.

Another gene drive system is based on Homing Endonuclease Genes (HEG) which aimed to suppression of insect population. In the HEGs, a specific sequence of an essential gene is cutout due to which the function of that particular gene has been disrupted. This gene is the essential for transmission of the disease but not for the host (Burt, 2003; Deredec et al., 2008).

**Sterilized Insect technique (SIT)**

The sterilized Insect technique (SIT) includes the classical radiation induced male sterility, dominant lethal gene systems (RIDL) and Wolbachia mediated cytoplasmic incompatibility (CI).

The Sterile Insect Technique (SIT) is used only for the suppression of vectors population. This is a self-limiting system in which sterilized male (radiation sterilization) insects are released in the field and allow to mating with wild female counterparts, in result the reproductive potential of the target population decreases.

This technique has been successfully applied for the suppression of some flies population like moth and worms. In late 1980s, the New World screwworm (*Cochliomyia hominivorax*) from the Mexico and USA was successfully eradicated through SIT (Van der Vloedt). This techniques was also successfully and widely used
in the California and Florida to control the Mediterranean fruit fly population (*Ceratitis capitata*), codling moth (*Cydia pomonella*) and pink bollworm moth (*Pectinophora gossypiella*) (Lindquist *et al*., 1992).

There are some limitations also to use of SIT. Large number of sterilized male is required and the sterilization process directly affects the mosquito’s fitness. Sterilization process (radiation sources) and its security is highly expensive, make it a limited application. During the sterilization process, there are possibilities to release of fertile females in the field. However, during its trials, the diseases like dengue and malaria have been eradicated from the target area (Scolari *et al*., 2011).

**Release of insects carrying a dominant lethal (RIDL)**

RIDL technique is the genetic enhancement of the SIT which is developed by the British biotech company Oxitec. In the RIDL, a lethal gene has to be inserted in insects which produce the non-toxic, lethal protein (tTAV). This protein allows to development of larvae, but prevents the surviving of their offspring in the adulthood (Oxitec, 2016).

A successfully field trial of this techniques have been done in the Cayman Islands to control the *Aedes aegypti* and about 80% of the wild population was suppressed (Harris *et al*., 2011&2012). Another evident of this technology has also been obtained from field trials in Brazil with the reduction of 81% and 95% of local *Ae. aegypti* populations (Carvalho *et al*., 2015).

All the above-described technology is very effective to control the vector population but the replacement of wild populations is currently problematical from both scientific and ethical standpoints.

**Wolbachia mediated Cytoplasmic incompatibility**

During the last decade, one of the efficient strategies to reduce crop insect pests was the introduction of sterile males in a population that for instance, succeeded in limiting the expansion of the fruit fly *Ceratitis capitata* (Robinson, 2002). The incompatible insect technique (IIT) was developed based on *Wolbachia*-mediated cytoplasmic incompatibility (Werren *et al*., 2008). This process prevents infected males from producing viable progeny when mating with an uninfected female or a female infected with an incompatible *Wolbachia* strain (Fig. 2.10).
Fig. 2.10. Process of cytoplasmic incompatibility

As early as 1967, Wolbachia-induced cytoplasmic incompatibility (CI) was proposed as a tool for the control of Culex mosquito (Laven, 1967). In the 1970s this strategy was also trialed in India to eradicate the mosquitoes population, (Curtis and Adak, 1974). There has been some field testing, but although it has never been operationally implemented.

**Wolbachia mediated life-shortening and vector incompetency**

The life-shortening effect of Wolbachia strain wMelPop was first time discovered in *Drosophila melanogaster* (Min et al., 1997). Later on, it was reported in the *Ae. aegypti* mosquitoes where life-span become half in Wolbachia strain wMelPop-CLA recipient mosquitoes (Ricci et al., 2012). A female mosquito must survive an extrinsic incubation period to transmit the dengue virus or other pathogens. Wolbachia induced the shifting of population age structure toward younger females which incapable to pathogen transmission (Brownstin et al., 2003; Cook et al., 2008). This Wolbachia-based, biocontrol approach is a potential strategy to prevention of disease transmission and vector borne disease management.

Wolbachia strains wRi and wPip Istanbul significantly reduces the hatching rate in the *Ae. albopictus* mosquitoes (Fu et al., 2010; Atyame et al., 2011). Interestingly, no impact was observed on the fitness of wPip Istanbul transinfected *Cx. pipiens*, making this a more promising approach than SIT (Alphhey, 2002; Benedict et al., 2003; Atyame et al., 2011). Besides Wolbachia, other bacterial genus such as *Bacillus* and *Staphylococcus* importantly influences the fertility of the *Cx. pipiens*, although the mechanisms not yet determined (Fouda et al., 2001).

Wolbachia, direct provides the fitness benefit to their host through involvement in nutritional and development, fecundity or oogenesis, resistance to pathogens etc. (Brownlie et al., 2009; Hosokawa et al., 2010; Dedeine et al., 2001;
Bian et al., 2010; Glaser & Meola, 2010; Hedges et al., 2008; Kambris et al., 2010; Teixeira et al., 2008). Some Wolbachia strain can involve in blood-feeding alteration, interference to Plasmodium, DENV, CHIKV, bendy proboscis, increasing metabolism and increasing the inhibition activity of filarial nematodes etc. (Turley et al., 2009; Moreira et al., 2009a&b, Evans et al., 2009; Kambris et al., 2009; Iturbe-Ormaetxe et al., 2011).

Genetic modified mosquitoes (GMM) based techniques is highly effective to control of mosquitoes population however, there are several issues like, issues of transposon stability, drive mechanisms, sibling species complexes, requirement of mass-reared pests; expensive sterilization process and multiple subspecies of mosquitoes etc., prevents to bring it in the ground level. All these obstacles should be overcome by the development of new, cheap, environmental friendly and effective techniques. Paratransgenesis techniques (genetic modified symbiotic bacteria used for the expression of effector molecules in the insects gut), might be a most suitable, highly effective and environmental friendly solution for vector borne disease management.

**Paratransgenesis**

Paratransgenesis is defined as the genetic manipulation of microbes associated with hosts for the expression of effectors molecule that can block pathogens development inside host midgut (Ren et al., 2008; Coutinho-Abreu et al., 2010; Rasgon, 2011) (Fig. 2.11). This technology has been developed as a potential control strategy for malaria (Riehle et al., 2007).

The technique was first developed and successfully used to control the Chagas disease (Durvasula et al., 1997). A genetic manipulated symbiotic bacteria *Rhodococcus rhodnii* from midgut of kissing bug (reduviid) *Rhodnius prolixus*, expressed an effector molecule Cecropin A which reduces the intensity of *Trypanosoma cruzi* infection. Genetically transformed *Sodalis*, a symbiont of tsetse flies, the vector of sleeping sickness appears a promising strategy for reduction of African trypanosomes transmission (Cheng & Aksoy, 1999).
In 2007, *Escherichia coli* has been genetically engineered to display anti-*Plasmodium* effector molecules for inhibition of *P. berghei* development in *An. stephensi*. Though, its survivorship in mosquitoes is very poor (Riehle et al., 2007). Bisi & Lampe (2011) proved that symbiotic bacteria *Pantoea agglomerans*, express and secrete the anti-*Plasmodium* effectors proteins (SM1, anti-Pbs21, and PLA2).

The isolated bacteria *Asaia sp.* from the midgut of *Ae. aegypti* were successfully transformed and re-infect the adult mosquitoes through sugar or blood meals represents a candidate for expressing anti-pathogen molecules within the mosquito. It has been demonstrated that the bacteria *Asaia* can be vertically transmitted to the progeny by maternal, paternal and transstadial routes and horizontally transmitted among individuals with mating and co-feeding (Favia et al., 2007&2008; Damiani et al., 2010). This fact may make it possible to successfully introduce modified bacteria *Asaia sp.* into wild mosquito populations in the field (Ricci et al., 2012).

**Mosquito’s midgut and microbiota**

Midgut serves as the first contact point between parasites, midgut bacteria and the epithelial surfaces, where significant parasite numbers is reduced (Azambuja et al., 2005). The defense mechanism in mosquito against invading pathogens is believed to be provided by the midgut microbiota, by means of raising the immunity or by impeding the parasite development in the mosquito midgut. In the recent years, it is reported that some midgut inhabiting bacteria play important role in vectorial capacity of mosquitoes through interaction with host and parasites (Gonzalez-Ceron *et
al., 2003; Dong et al., 2009; Kambris et al., 2010; Cirimotich et al., 2011b; Fang et al., 2011; Rasgon, 2011).

The mosquito midgut has its own normal microbiota, and it can be infected with certain microbes that are known to augment the immune response of the mosquito (Meister et al., 2005&2009; Dong et al., 2006) and it believe that, immuno-competent mosquitoes are thought to be less likely to transmit the parasites such as malaria, dengue and chikungunya (Abdul-Ghani et al., 2012). Theoretically, it is possible to impair the vectorial competence by the genetic immunization of vectors to express pathogen-specific molecules (Beaty, 2000). Consequently, the use of certain microbes may help in producing vector incompetence, particularly with the advances in biotechnology and therefore, more interest should be given in microbial based control research on the feasibility of its deployment as a part of Integrated Vector Management (IVM) approach. One alternative vector management is not to kill vector, but to make it unable of transmitting the pathogen. Accordingly, there is current interest in the biocontrol approach using the midgut bacteria as a way to ensure sustainability. Biological control can exploit the weak link offered by the complexity of the parasite life cycle within the mosquito to make it incapable for pathogen transmission (Ghosh et al., 2009). However, the potential use of such approaches for vector management is still largely underexplored (Ferguson et al., 2010). Understanding the interactions in the tripartite link of endogenous or introduced microbes, mosquito and parasites may help us to improve certain vector control strategies (Abdul-Ghani et al., 2012).

Importance of midgut bacteria in relation to their various functional roles is still unexplored. It has been reported that some midgut bacteria involved in the disease prevention through various ways. Pumpuni et al., (1996) suggested that introduced or indigenous bacteria could modify vectorial competence of Anopheles through hindering the pathogen development and weaken the ability of Plasmodium to establish infection (Dong et al., 2009; Cirimotich et al., 2011b).

It has been reported that the Gram-negative bacteria involved in inhibition of sporogonic development in the midgut of mosquito (Pumpuni et al., 1993; Straif et al., 1998; Dong et al., 2006). Gonzalez-Ceron (2003) observed that midgut microbiota influences the parasite transmission through blocking the Plasmodium vivax sporogonic development in An. albimanus. The bacterial species Enterobacter species
generated the reactive oxygen species (ROS) in An. gambiae and blocks the development of P. falciparum (Cirimotich et al., 2011b).

The midgut bacteria may be used as the biolarvicides. Recently, the highly potent larvicidal activities have been reported from one anaerobic Gram-positive bacterium Clostridium bifermentans subsp. malaysia. The toxicity of bacteria is very high to Anopheles and comparatively low to Culex and Aedes larvae (Lee et al., 1990; Thiery et al., 1992). Lysinibacillus sphaericus contain two potent toxins, namely Mtx and Bin that have larvicidal properties (Berry, 2012). These toxins paralyzed the digestive system and disrupt the nervous system of the insects (Majambere et al., 2007; Nartey et al., 2013). Some other bacterial species Bacillus thuringiensis israelensis (Bti) and Bacillus sphaericus (Bs) are highly effective against mosquito’s larvae. Their protoxins in parasporal crystals and the spore coat are soluble in the alkaline pH medium in larval midgut and functions as stomach poison (Porter, 1996; Mittal, 2003; Raghavendra et al., 2011).

It has also been reported that the midgut bacteria also influences the susceptibility to some viral disease. In the presence of midgut bacteria Serratia odorifera, immune response of Ae. aegypti has been suppressed, due to which susceptibility of Ae. aegypti to dengue and chikungunya virus increases (Apte-Deshpande et al., 2012&2014). Susceptibility of Ae. aegypti to DENV-2 also increases when fed with the Aeromonas sp. and Escherichia coli (Rani et al., 2009).

Midgut bacteria can produce some compounds that can directly assimilated by the host. Bacteria can improve the digestion by producing some degrading enzymes which facilitate the assimilation of complex molecules. In phytophagous insects, microbiotas generally provide vitamins, amino acids and sterol that complement limited plant diets. The best known example is the involvement of the bacterium Buchnera in providing essential amino acids to aphids (Douglas, 1998). Another interesting example is the bacteria that provide vitamin B which is not present in vertebrate blood, the sole nutrient source of Glossina tsetse flies (Aksoy, 2000).

It has been demonstrated that Serratia and Enterobacter containing the hemolytic enzymes and play an important role in blood digestion in mosquito (Gusmao, 2010; Campbell, 2004; De Gaio, 2011). Minard et al., (2013) described that bacteria, Acinetobacter baumannii, and A. johnsonii could be involved in both blood
digestion and nectar assimilation in *Ae. albopictus*. *Acinetobacter* strains of mosquito’s midgut were able to metabolize the amino acids α-ketovaleric acid and glycine, which are blood components, as well as 4-hydroxybenzoic acid and xylose, which are common constituents of plant sap. The bacterial species *Asaia bogorensis* isolated from *An. stephensi* was shown to be phototrophic with respect to vitamins, suggesting it may provide the vitamins to mosquito (Crotti *et al*., 2010).

It has been reported that bacteria released some compounds which are essential for mosquito’s larval development. For instance, it has been demonstrated that a high level of *Pseudomonas aeruginosa* improved larval growth of *Cx. quinquefasciatus* in a phosphorus-rich medium while that of *Cx. tarsalis* was slowed down (Peck & Walton, 2006). The level of phosphorus in breeding sites could be a factor explaining how mosquitoes can adapt to a specific condition according to their bacterial load, possibly with a trade-off between the nutritional and toxic roles of bacteria. Differential tolerance of larvae to putative toxins present in *P. aeruginosa* could explain why the two mosquito species are not found in the same aquatic habitat (Minard *et al*., 2013).

Some studies have demonstrated a link between the presence of bacteria in insect hosts and their ability to degrade some insecticide molecules ingested inside of mosquito’s midgut. The acquisition of these bacteria by each generation could be an easy way for an insect to detoxify itself the insecticide without any genetic cost (Kikuchi *et al*., 2011).

The midgut bacteria in mosquitoes acquired both from the aquatic larval stage and by nectar feeding in the adult stage. In aquatic larval stage, it might be inserted from breeding sites and passed on to the adults (Díaz-Nieto *et al*., 2016; Osei-Poku *et al*., 2012; Smith *et al*., 1998). Midgut microbes in the adult mosquito are thought to be acquired through vertical inheritance as well as from the surrounding environments (Buck *et al*., 2016; Minard *et al*., 2013; Moro *et al*., 2013).

It has been proved that microorganisms are the important food source for larval development and in absence of microorganisms, the larval growth be hampered. *Ae. aegypti* larvae cannot be grown on sterile media (Rozeboom, 1935). It also affects the normal larval growth and in absence of bacteria the size was smaller than the larvae with water containing bacteria (Wotton *et al*., 1997). In contrast, one report
showing the successful rearing of *Cx. quinquefasciatus* larvae in water with tetracycline (Mourya *et al.*, 2002). These studies indicate that bacteria are very important, if not essential for larval development of mosquito.

**Diversity of midgut microbiota of mosquitoes**

Diversity of midgut bacteria of mosquitoes was started in late sixties and the first time midgut microbiota was isolated from laboratory reared *Culex* mosquitoes (Chao & Wistreich, 1959&1960, Ferguson & Micks, 1961). Later on, Jadin *et al.*, (1966) described the midgut bacteria *Pseudomonas* from *Anopheles* which plays important role in sporogenesis of hematozoon of malaria (Jadin *et al.*, 1966). After a large gap of almost 20 years, in 1987, Seitz *et al.* described the detrimental effects of midgut bacteria *Serratia marcescens* on the *Plasmodium berghei* in *Anopheles stephensi* (Seitz *et al.*, 1987).

In 1987, study of midgut microbiota in *Aedes* mosquitoes was started by Hung *et al.*, (1987) and reported a novel bacterial species *Spiroplasma culicicola* from Ae. *sollicitans*. Later on, another novel bacterial species *Spiroplasma taiwanense* (in 1988) and *Spiroplasma diminutum* (in 1996) was also reported from *Culex* (Abalain-Colloc *et al.*, 1988; Williamson *et al.*, 1996). It has been proved that, *Spiroplasma taiwanense* significantly reduces the survival rate and flight capacity of adult *Ae. aegypti* and *An. stephensi* (Humphery-Smith *et al.*, 1991).

There is a possibility to modulation of vectorial capacity of *Anopheles gambiae* by external acquired and midgut microbiota through inhibition of *Plasmodium* and other pathogens development. However, the mechanism is still not understood (Dong *et al.*, 2009). The diverse microbiota affects the development of *Plasmodium* and intensity and prevalence of *Plasmodium* infection were significantly reduced by the natural bacterial isolates (Tchioffo *et al.*, 2013; Ngo *et al.*, 2015).

Pumpuni *et al.*, (1993) reported that gram-negative bacterial strains partially or completely inhibit the oocyst formation, while gram-positive bacteria do not show any inhibitory effect. The *An. funestus* females, which harbored gram-positive bacteria, were likely to be more infected with sporozoites compared with those with no cultivable bacteria or gram-negative bacteria in their midguts (Straif *et al.*, 1998). In 2003, Gonzalez and colleagues, isolated midgut bacteria from field-collected *An. albimanus* Weidemann and observed that infection of *Plasmodium vivax* decreases in
Anopheles mosquitoes, when it was co-infected with midgut bacteria, *En. cloacae, En. Amnigenus* 2 and *S. marcescens* (Gonzalez et al., 2003). Cirimotich et al., (2011) have also proved that Enterobacter interfere the *P. falciparum* development through generation of reactive oxygen species (ROS) which shows anti-*Plasmodium* activity.

Fouda et al., (2001) demonstrated that symbiotic bacteria influence the potential of reproduction (fertility and fecundity), pre-oviposition and blood meal digestion in the Cx. *pipiens* mosquitoes. They have reported that *Bacillus* and *Staphylococcus* are essential for normal and high fecundity of Cx. *pipiens*. It has been also proved that gut bacteria are essential for the normal development of the embryo (Fouda et al., 2001).

In 2012, Boissiere et al. demonstrated that gut microbial communities are one of the major components of mosquito innate immune responses and significantly influences the *Plasmodium* infection in An. *gambiae* (Boissiere et al., 2012). Some gut bacterial isolates are able to activate the mosquito’s immune system in *Anopheles* mosquito. The bacterial isolates also affect the life-span of the mosquitoes. It has also been observed that *Serratia marcescens* was efficiently colonized in the mosquito’s gut and comprises the survival and inhibition of sexual and asexual stages of *Plasmodium* through secreting some effector molecules (Bahia et al., 2014; Tchioffo et al., 2015).

The gut microbial diversity of mosquitoes varies with various developmental stages (Demaio et al., 1996; Wang et al., 2011; Ngwa et al., 2013; Minard et al., 2013). Ngwa et al., in 2013, have also demonstrated that diversity of midgut microbiota decreases during the development of mosquitoes from egg to adult. They have also identified a dominant gram-negative bacterium *Elizabethkingia meningoseptica* in midgut of male and female adult An. *stephensi* which can be transmitted from one generation to another. The variation of midgut bacterial communities also depends on the mosquito’s sex (male and female) and ecological factors (Zouache et al., 2011; Minard et al., 2013).

Midgut microbiotas of adult have been determined by the native breeding sources of the larvae where they were grown. Mosquito-associated microbiota depends upon different environmental condition from where the bacteria have been acquired (Zouache et al., 2011; Boissiere et al., 2012; Minard et al., 2013; Tchioffo et al., 2015; Buck et al., 2016). Mosquitoes of different environments have a specific bacterial profile. This is the reason for variations in the midgut microbiota among the adult
mosquitoes which breeds at distinct sites. It has also been described that bacterial profiles associated to mosquitoes provided precise and predicting information about the spatial dynamics of the mosquito population (Buck et al., 2016).

It has been also described that bacterial communities vary according to different feeding regimes. Wang et al., (2011) have also proposed that mosquito’s food like sugar meal and blood meal significantly affects the microbial structure in the midgut of adult *Anopheles gambiae* and reported that blood meals favour the enteric bacterial communities and reduces the community diversity. In 1996, Pumpuni et al. also described that the number of bacteria in *An. gambiae* increased 11 fold by 24 h after a blood meal, while in *An. stephensi* it increased 40 fold. 2000 fold increase in bacterial count was also observed in *Culex* mosquito. In the presence of a blood meal the bacterial population increases, but after 3-5 days it decreases to pre-blood meal level (Pumpuni et al., 1996). After post blood meal an interspecies competition between the bacterial isolates occurs in the female mosquito’s midgut and the numbers of coexisting bacterial species become low. Terenius et al., 2012 have also suggested that midgut bacterial dynamics among the mosquito’s midgut is due to the possible existence of co-adaptation between midgut bacteria and their host (Terenius et al., 2012).

In 2005, Lindh et al. isolated the midgut bacteria from field collected *An. gambiae* and *An. funestus* and described that several identified bacteria were known to be symbionts in other insects. Favia et al., 2007 worked on *An. stephensi*, *An. maculipennis* and *An. gambiae* and found that *Asaia* was the dominant mosquito-associated bacterial genera. Apart from midgut of adult male and female, genus *Asaia* was also colonized in the reproductive system of male and larval gut. Chouaia et al., (2010) also reported that, *Asaia* was the dominant bacterial genera in four different mosquito species, *An. gambiae*, *An. stephensi*, *Ae. aegypti*, and *Ae. albopictus* and play a beneficial role in the normal development of *An. stephensi* larvae (Chouaia et al., 2012).

Gusmao et al., (2010) identified the bacterial genera *Serratia*, *Klebsiella*, *Asaia*, *Bacillus*, *Enterococcus*, *Enterobacter*, *Kluyvera* and *Pantoea* from *Ae. aegypti* and among them, genus *Serratia* was dominant. All the above described genera were isolated from the midgut except *Enterobacter* which was identified from eggs only. Apart from midgut the bacterial genera *Asaia* and *Pantoea* were also identified in
eggs and ovary respectively. Apart from the bacterial isolates they have also isolated two yeasts namely: *Pichia* from midgut and *Candida* from midgut and ovary.

Mosquitoes exposed to a variety of microbes during their lifecycle and some of which are essential for their successful development. There is a reciprocal interaction between the midgut microflora of mosquitoes and dengue virus and certain bacteria in midgut of mosquitoes are responsible for the reduction of susceptibility to dengue virus infection (Ramirez *et al.*, 2012). Joyce *et al.*, (2011) also proved that midgut bacteria from *Ae. albopictus* reduced the infectivity of La Crosse virus (LACV) Vero cells and about 50% isolated bacteria played this significant role.

Apte-Deshpande *et al.*, (2012) proposed that polypeptide of midgut bacteria *S. odorifera* blocked the midgut surface molecule prohibitin which presents on the surface of females and this is responsible for the enhancement of the susceptibility of DENV-2 to females *Ae. aegypti*. In 2014, Deshpande *et al.*, observed that susceptibility to CHIKV in adult *Ae. aegypti* increased when *Ae. aegypti* was co-infected with *S. odorifera*.

Rani *et al.*, (2009) studied the midgut microbiota of laboratory reared and field collected *An. stephensi*. They have determined that uncultured *Paenibacillaceae* was the dominant bacteria in field-caught adult male *An. stephensi* while *Serratia marcescens* was in larvae and female adults. On the other hand, *Cryseobacterium meningosepticum* and *Serratia marcescens* were found to be abundant in lab-reared mosquitoes. In 2011, Djadid *et al.* also identified the midgut microbiota associated with *An. stephensi* and *An. aculipennis* and a majority of the identified bacteria belonged to the Gamma-proteobacteria (Djadid *et al.*, 2011). Using the midgut microbiota of *Anopheles* mosquitoes, they proposed for development of paratransgenesis-based techniques to control the transmission of malaria.

Moro *et al.*, (2013) determined the midgut microbial diversity of field-caught *Ae. albopictus* populations using culture depending techniques. They found that phylum Proteobacteria was dominant in female adult *Ae. albopictus* while, Actinobacteria was the most abundant phylum in male mosquitoes. They have also observed that the variation among the midgut bacterial composition and their abundance occurs and it depends on the collection sites of the mosquitoes.
Chandel *et al.*, in 2010 have identified a novel gram-negative bacteria strain *Chryseobacterium culicis* from the midgut of *Culex quinquefasciatus* (Kampfer-Chandel *et al.*, 2010). In 2013, they worked on midgut microbial community of female *Cx. quinquefasciatus* mosquitoes collected from a large geographical area of India and found that bacterial species were dominated by phyla Proteobacteria followed by Firmicutes and Actinobacteria (Chandel *et al.*, 2010).

**Bacterial identification using 16S rRNA**

The using of 16S rRNA gene sequences has been by far the most common housekeeping genetic marker to study the bacterial phylogeny and taxonomy. The prokaryotic organisms contain three types of rRNA genes *viz.* 5S, 16S, and 23S rRNA genes, in which the 5S rRNA is relatively small (120 bp), while 23S rRNA is a very large molecule (3000 bp) long. The size of 16S rRNA gene is 1500 bp which not too short and not too long. Therefore the 16S rRNA gene is a molecule of choice as it possessed the optimum attributes.

16S rRNA gene is most commonly used housekeeping genetic marker for phylogeny and taxonomy study of bacteria. The common reason for using the 16S rRNA gene are (i) it is found in almost all bacteria, often existing as a multigene family, or operons; (ii) over time the function of the 16S rRNA gene has not changed. It suggested that random sequence changes are a more accurate measure of time (evolution), and (iii) for informatics purposes the 16S rRNA gene (1,500 bp) is large enough (Janda *et al*., 2007; Patel, 2001).

The microorganisms are regularly identified, classified and quantified from complex biological mixtures by using 16S rRNA gene sequences. Nine “hypervariable regions” are present between 16S rRNA gene sequence of bacterial species that exhibit considerable sequence diversity among distinctive bacterial species which can be utilized for identification of bacterial species (Van de Peer *et al*., 1996).

The 16S rRNA molecules consists a mosaic of conserved and variable regions (Rossello’-Mora & Amann, 2001; Woese, 1987). Amplification of target sequences of bacterial species using PCR techniques is enabled by the presence of conserved regions, flanked the hypervariable regions in most bacterial species (Baker *et al*., 2003; Lu *et al*., 2000; McCabe *et al*., 1999; Munson *et al*., 2004). Generally, the
conserved regions of 16S rRNA are used for designing of universal primers making it possible to amplify the gene in a wide range of different microorganisms from a single sample.

The conserved regions carry information about phylogenies at the higher taxonomic levels since they have evolved slowly and are highly similar among the different taxa, whereas the variable regions have undergone more mutations during evolution, and are more useful for classification at the intraspecies/strain level (Woese, 1987). The rRNA genes are essential because important constituents of protein synthesizing machinery are functional and evolutionary conserved and therefore present in all organisms. Their conserved structure makes it easy to identify homologous molecules by their size. Furthermore, the rRNA provides sufficient sequence information to permit statistically significant comparison. There are no artifacts of lateral transfer between contemporaneous organisms in rRNA genes, so these relationships between rRNA reflect an evolutionary relationship of organisms. Finally, after many years of intensive sequencing, the current sequence databases comprise a huge amount of ribosomal DNA sequences, encompassing cultured but also many uncultured species.

It has been clear that the midgut microbiota significantly involves in several important functions of pathogens and vectors but no any data reported on the midgut bacteria of Ae. albopictus from the North-east India regions. Hence a comprehensive study of midgut microorganism is required in relation to the mosquito’s biology and disease transmission prevention as an important component of the system.