

Review Articles

Role of gut inhabitants on vectorial capacity of mosquitoes

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ABSTRACT

Mosquito-borne diseases are spreading at an alarming rate. Globally millions of deaths occur due to the diseases transmitted by mosquitoes, next to AIDS and tuberculosis. Several methods have been used to control these vectors and the diseases caused by them. Earlier studies have shown the potential role of mosquito gut inhabitants on disease transmission. Their findings can be used as an innovative approach for devising strategies to modify the survival of mosquitoes by reducing their lifespan, reproduction and disease transmission abilities. In this study, microbiome of the three genera of mosquitoes, namely *Aedes*, *Anopheles*, and *Culex* along with their vectorial capacity have been reviewed for assessing their role in mosquito control and transmission. Relevant articles were accessed using different databases, including LILACS, Embase, Science Direct and PubMed from inception to June 2017. The search keywords included "*Aedes*", "*Anopheles*", "*Culex*", "gut inhabitants", "vectors", and "mosquito". The titles, abstract, and keywords of the retrieved articles were screened, and eligible research articles were sorted. The review indicates that paratransgenesis may be considered as a versatile and effective strategy to eradicate the spurt of mosquito transmitting diseases. *Enterobacter* species is the most common type of gram-negative bacteria associated with the gut of all the three genera of mosquitoes. It was found to have a beneficial effect on humans as it helps in destroying dreadful disease-transmitting vectors. These symbiotic qualities of the microbes need to be thoroughly investigated further to reveal their antipathogenic effect on the vector.

Key words *Aedes*; *Anopheles*; *Culex*; gut inhabitants; microbes; mosquito; vectorial capacity

INTRODUCTION

Mosquitoes, the hexapod invertebrates belonging to the Culicidae family of Insecta class, have profound influence on human beings. More than 3555 recognized mosquito species divided into two subfamilies (Anophelinae and Culicinae) and 112 genera have been recorded in different parts of the world¹. India, belonging to the oriental region is regarded as one of the richest biogeographic zones for different mosquitoes. A record indicates that Indian mosquito fauna includes 393 species divided among 49 genera and 41 subgenera². Most species of this holometabolous insect remains as nonpathogenic, while some are vectors of certain dreaded diseases like malaria, chikungunya, Zika, yellow fever *etc.* More than one million people die every year throughout the world due to mosquito-borne diseases³⁻⁶.

The vector competences of mosquitoes are highly dependent on the microenvironment of their gut which normally undergoes radical structural remodeling during each stage of the life cycle⁷. Hence, studies on gut content analysis of mosquito in terms of feeding (which includes

diverse form of microbial flora composed of commensal or symbiotic bacteria, algae, protozoans, organic debris *etc.*) are essential, as their feeding behaviour changes during metamorphosis from an aqueous larval stage to an aerial adult⁸. Studying the interaction between the gut microenvironment and vector competency might be helpful in controlling vector-borne diseases without disturbing the ecological balance. Accordingly, a systematic review was made, intended to reveal the characteristic features of microbial consortia residing in the mosquito gut. For this different published research articles and reviews were assessed using the online databases, *viz.* LILACS, Excerpta Medica data BASE (Embase[®]), Science Direct and PubMed[®] from inception to June 2017. Other sources consulted were the CDC, WHO, and NIH websites. The search keywords included "*Aedes*", "*Anopheles*", "*Culex*", "gut inhabitants", "vectors", and "mosquito". Articles retrieved for the study were absolutely in English. The titles, abstract, and keywords of the retrieved articles were screened, and eligible research articles were sorted. The selected articles were considered reliable, if they revealed one or more perspectives about the research

interest (microbial inhabitants in the gut of mosquito), irrespective of when or where the investigations or experiments were done. The study also included epidemiological and observational perspectives of gut inhabitants of *Aedes*, *Anopheles* and *Culex* mosquito genera, since these mosquitoes have profound effects on the public health.

Larval gut anatomy of mosquito

A prominent digestive tract appears from the larval stage of mosquitoes and it gets divided into foregut, midgut, and hindgut⁹. In all mosquitoes, the basic structure of digestive tract is similar, however, diverse modifications have been observed in this structure due to the differences in feeding mechanisms. Presence of macro- and micro-molecular nutrients like carbohydrate and proteins, along with micronutrients in the gut provide essential resources to the microbes for multiplication^{10–11}. For example, absorption of nitrogenous waste like uric acid in the hindgut provides nutritive environment for gut bacteria¹².

The ectodermally derived foregut and hindgut are separated from the epidermal layer by a lining of exoskeleton (made of cuticular glycoproteins and chitin); which gets shed at each ecdysis. The foregut includes pharynx (used for filtering and swallowing) and oesophagus¹³. Hindgut is divided into an anterior portion termed as Malpighian tubules, followed by posterior fermentation chamber and rectum for holding faeces before defaecation. Endodermally derived midgut (Figs. 1 a and b) is the primary site for digestion and absorption. Histologically, it has an epithelial outer lining consisting of a basement membrane, followed by ciliated columnar epithelial and regenerative cells, and peritrophic membrane/matrix (Fig. 1c), which is a unique feature of the midgut. In mosquitoes, this matrix bifurcate midgut into ectoperitrophic

space and endoperitrophic space^{13–18}. Midgut consists of cardia, gastric caeca, anterior and posterior stomach. It remains as an important passage for the blood-borne parasite since it forms a barrier between the ingested parasite and haemocoel of the host. Mosquito parasites must penetrate host gut before completing their development in host tissue to remain as pathogenic. However, the midgut remains a hostile environment for the parasite. For example, in *Anopheles* mosquitoes, 50% of the mosquito midgut stages of the *Plasmodium berghei* die naturally by apoptosis before the gut invasion¹⁹. Therefore, an effective understanding of these factors can be used as a tool to control the parasitic vector microbes and check the disease transmission.

Earlier studies have reported two different types of peritrophic matrices of the midgut, one is Type I that lines the entire midgut when the food is ingested; and the other is Type II that is seen only in the anterior region of the midgut²⁰. This peritrophic matrix serves a variety of functions such as a barrier that protects the epithelium from mechanical damage by food particles, from exposure to large toxin molecules present in food, and also from microbial invasion to an extent. It also aids in concentrating food and digestive enzymes. As it takes 12–30 h for the complete formation of the peritrophic membrane, it does not remain as an intense barrier to filarial worms or parasites which can enter the midgut within few hours of invasion^{21–22}. Thus, basic architecture of gut can be modified depending on the specialized niche and feeding habits.

Microbial inhabitants of gut

Mosquito as a holobiont undergoes a metamorphic transition from larval to the adult stage. Microbial inhabitants of mosquito and its larvae refer to the microbial

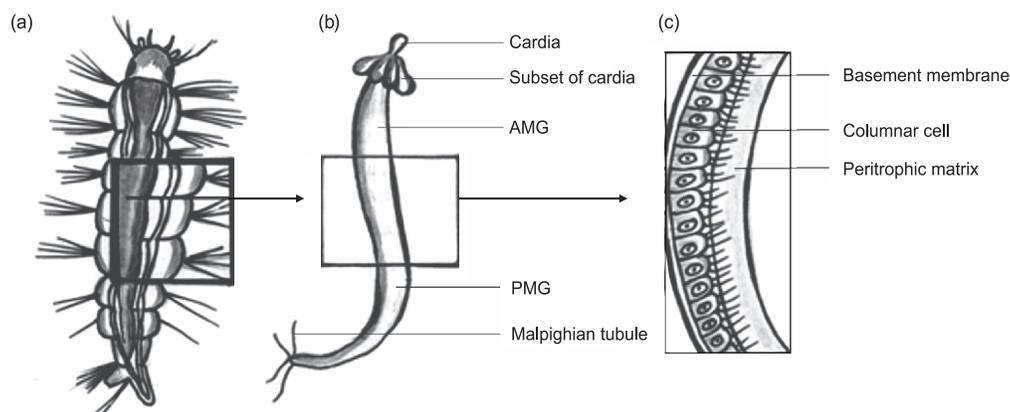


Fig. 1: (a) Larval stage of mosquito with external morphology; (b) Generalised midgut structure of mosquito larva; and (c) Epithelial lining of midgut; AMG—Anterior mid gut; PMG—Posterior mid gut.

communities which colonize in the target organism. The microflora associated with larvae is replaced in the adult mosquito with a new set of microbes. This variation in the microbiota is due to the significant changes in host mosquito according to the changes in the environment and feeding habits. This process of microbial cleaning and acquisition is termed as gut sterilization. During their larval stage mosquito mainly consume bacteria and planktons as nutritive resources. This paves the initial stage of bacterial colonization that adds to resident members. Among the microbes, the bacteria colonize more in the midgut than in salivary gland and reproductive organs^{23–25}. Later during adult stages, mosquitoes begin to feed on nectar and blood which triggers the proliferation of some types of microbes and the decline of the other bacteria. Thus, the host diet and its developmental stage plays a crucial role in shaping the gut microbiome²⁶. The gut microbiome is generally analysed by dissecting gut of IV instar larvae of field caught or laboratory reared mosquitoes. To enhance the microbial growth the macerated gut is grown in nutrient media. Later the microbial content are analysed by molecular techniques like pyrosequencing²⁷.

The resident communities inside the gut of mosquitoes can vary from microscopic dominant bacteria to even members of Protista (Table 1). This resident consortium can be changed by the influx of new microbes from their natural habitat. Mosquitoes such as *Anopheles*, *Aedes*, and *Culex* normally lay eggs in water that contains bacteria²⁶. The presence of aquatic plants influences the

microbial communities as they serve as a larval resource or provide cues for egg-laying adult mosquito, and many microbes of these plants also get transmitted to adult gut trans-steadily^{28–30}. These microbes have a significant impact on mosquito life traits like reproduction, fecundity, immunity and vector competence.

As per previous earlier studies, the general bacterial flora in mosquitoes includes gram-negative phylum Proteobacteria (Gammaproteobacteria, Alphaproteobacteria, and Betaproteobacteria) phylum Bacteroidetes, gram-positive phylum Firmicutes including *Clostridia*, *Actinomycetes*, *Spirochetes*, and other species. Naturally, a bacterial community in mosquito gut can reduce the development of *Plasmodium*, a human parasite (due to the presence of gram-negative bacteria). The outer membrane of the cell wall in these gram-negative bacterial cell wall contains lipopolysaccharides which acts as a physical barrier for harmful agents like hydrogen peroxide *etc*³¹, while gram-positive bacteria have no such barrier. Furthermore, different gram-negative bacteria have varying effects against *Plasmodium*. These variations may show certain differences in the production of certain metabolites. For example, red pigment prodigiosin produced by gram-negative bacteria is found to be effective against *Plasmodium*. One reason for this is the upregulation of immune genes that encode antimicrobial peptides (AMP) and a thioester-containing protein having an antiparasitic effect. These gut resident bacteria can be symbiotic or pathogenic (Table 1). The symbiotic microbes are ben-

Table 1. List of microbial interactions seen in mosquito

Mosquito species	Stage	Microbe	Type	Interaction	References
<i>Anopheles stephensi</i>	Adult	<i>Pantoea agglomerans</i>	Bacterium	Symbiotic	32
<i>An. gambiae</i>	Adult	<i>Pantoea agglomerans</i>	Bacterium	Symbiotic	33–34
		<i>Enterobacter</i>	Bacterium	Commensal	28
		<i>Pseudomonas</i>	Bacterium	Commensal	35
		<i>Serratia</i>	Bacterium	Commensal	27
		<i>Asaia</i>	Bacterium	Commensal	36
		<i>Elizabethkingia</i>	Bacterium	Commensal	33
<i>Culex tarsalis</i>	Larval	<i>Bacillus thuringiensis israelensis</i>	Bacterium	Predation	37–39
<i>Cx. quinquefasciatus</i>	Larval	<i>Bacillus thuringiensis israelensis</i>	Bacterium	Predation	40
<i>Cx. stigmatosoma</i> Dyar	Larval	<i>Bacillus thuringiensis israelensis</i>	Bacterium	Predation	39
<i>Cx. quinquefasciatus</i>	Adult	<i>Bacillus</i> spp	Bacterium	Symbiotic	41
		<i>Pseudomonas</i> spp	Bacterium	Symbiotic	41
		<i>Staphylococcus</i> spp	Bacterium	Symbiotic	42
		<i>Aeromonas culicicola</i>	Bacterium	Symbiotic	42
		<i>Klebsiella ozonae</i>	Bacterium	Symbiotic	42
<i>Cx. pipiens</i>	Adult	<i>Pseudomonas aeruginosa</i>	Bacterium	Symbiotic	42
		<i>Enterobacter agglomerans</i>	Bacterium	Symbiotic	42
		<i>Serratia marcescens</i>	Bacterium	Symbiotic	42
		<i>Aeromonas culicicola</i>	Bacterium	Symbiotic	32, 43
		<i>Asaia</i>	Bacterium	Symbiotic	34
<i>Aedes aegypti</i>	Adult	<i>Densovirus</i>	Virus	Symbiotic	44
		<i>Wickerharmomyces anomalus</i>	Yeast	Symbiotic	44
<i>An. gambiae</i>	Adult	<i>Metarhizium anisopliae</i>	Fungus	Symbiotic	45

eficial to host in many ways. It includes dietary supplementation, enhancement of in digestive mechanism, and tolerance towards environmental perturbation and protection from the parasite.

Nature of anopheline gut

Genus *Anopheles* which comprises approximately 550 species is cosmopolitan in distribution. Each mosquito genus has a specific ecological preference for selecting its habitat. For example, genus *Anopheles* are mostly observed in clear water exposed to direct sunlight⁴⁶. As non-selective filter feeder, *Anopheles* larvae indiscriminately feed inorganic matter like dust and organic matter such as filamentous algae, fungi, rotifers, cyanobacteria, zooflagellates and crustaceans present in water. They utilize mouth brushes or lateral palatal brushes as paddles to create currents by using energy for ingesting food particles⁴⁷.

The anopheline gut microbiome is strongly influenced by microbes suspended in its natural habitat. This has been proved by the thorough gut analysis of mosquito larvae by Howland⁴⁸ who dissected over 1000 larvae of eight species, identified the algae present, and ranked them by abundance in the food. She concluded that the abundance of algae in the larval food is correlated with algal abundance in the habitats. This has been also shown in another study on *Anopheles quadrimaculatus* larvae, a common vector of malaria in the Eastern United States⁴⁹ wherein the elimination of algae from a small pond with copper sulfate demonstrated its absence in their food. However, after recolonization the same pond, algal cells were again observed in the larval gut.

The anopheline gut is dominated by resident bacteria of genus *Pantoea* and *Asaia*. These bacteria have shown stable association with anopheline mosquitoes during different life stages. *Pantoea*, natural mosquito symbiont can cross-colonise several mosquito species and is readily transformed and cultured; this property of *Pantoea* has been proposed for paratransgenic applications⁵⁰⁻⁵¹. *Asaia* acts as an immunomodulator by producing antimicrobial peptides that interfere with the course of infection particularly its invasion to epithelial tissues and salivary gland²⁷.

Recent research on two *Anopheles* species *An. gambiae* and *An. coluzzii* from Ghana⁵² compared the midgut microbiota of mosquitoes during rainy and dry seasons from urban and rural breeding sites using 454 pyrosequencing. The data suggested that *An. gambiae* and *An. coluzzii* do not differ significantly in their gut microenvironment. Shewanellaceae family was observed in both the species. Bacterial families Enterobacteriaceae and Aeromonadaceae, were also associated with *Anopheles*

mosquitoes. The only difference observed was among *An. gambiae* collected from the different breeding site during summer. *Aeromonas*, *Shewanella*, and *Thorsellia* were other bacterial genera found to be significantly varying in abundance according to the breeding sites. This indicates that larval breeding site has a significant impact on the adult mosquito midgut composition. The presence of *Enterobacter* and *Serratia* strain in *Anopheles* mosquito gut have an antiparasitic effect on mosquito. *Enterobacteriaceae* that survived during the rainy season is found to be more in number than that of during the dry season. Two members of this family include *Enterobacter* species and *Thorsellia anopheles*. This gram-negative *Enterobacter* can directly act on *Plasmodium falciparum* and hinders the development of the parasite. *Thorsellia anophelis* was the dominant species in the midgut of *An. gambiae*. This symbiotic association with host mosquito vector attributes to its high tolerance for mosquito midgut alkalinity. *Serratia marcescens* HB3, isolated from laboratory-reared *An. stephensi* mosquitoes, inhibits *Plasmodium* development within the mosquito midgut by interrupting ookinete invasion through the midgut epithelial cells. Phenotypic variation at the cellular and structural levels was observed and directly correlated with the ability to induce resistance against *Plasmodium* invasion⁵³.

The prevailing environmental conditions have a great influence on the gut microbiome and host-vector competence. One such parameter is the influence of chemicals in regulating the bacterial fauna in mosquito gut. For example, *Pseudomonas aeruginosa* boost the larval development of *Culex quinquefasciatus* in phosphate-rich medium⁵⁴.

Microbial diversity of gut microbiome in genus *Aedes*

Aedes, an arbovirus vector of dengue, chikungunya and Zika virus draws special attention due to their rapid geographical spread and increasing disease burden⁵⁵⁻⁵⁷. This is due to the association between certain gut microbes and potent human pathogens such as parasite, virus, and bacteria in host vector.

Zouache *et al*²³ explored the composition and diversity of mosquito-associated bacteria in relation to mosquito habitats from different geographical regions of Madagascar on wild *Ae. aegypti* and *Ae. albopictus*. This was done using the traditional culturing method and by denaturing gradient gel electrophoresis (DGGE) and sequencing of rrs amplicons of bacteria. This survey highlighted the variance in the relative abundance and composition of mosquito-associated bacteria during developmental stages. To know the influence of external microorganism with the internal gut microbe, the analyses were done on lab-

oratory-reared and wild targeted population. Wild *Aedes* gut generally had richer bacterial profile than laboratory reared ones consisting of *Serratia marcescens*, *Klebsiella ozaenae*, *P. aeruginosa*, *Enterobacter*, *Proteobacteria* and *Flavobacteriaceae* species depending upon the stages of development⁵⁸.

Extensive work of Coon *et al*⁵⁹ on *Ae. aegypti* and *Ae. albopictus* have shown that the I instar axenic larvae of these species were not able to develop properly in the absence of bacteria; though, they developed normally into adult in laboratory aquatic habitat recolonized with bacteria. Moreover, insect colonized microbial community also protects the host against pathogens. Interesting examples are available from different research reports such as *Wolbachia* an endosymbiotic resident bacterium in the gut can interfere the replication of chikungunya and dengue virus in *Aedes* mosquito. This is also supported by another result in which removal of the bacterial community in antibiotic fed *Anopheles* mosquitoes increases their susceptibility to malaria parasite infection. These observations suggest that the microbial gut flora of insect can be manipulated to control their vectorial capacity.

In India, attempts to survey the midgut microflora have remained mainly focused on two genus, *Culex*, and *Anopheles* mosquitoes, which act as vectors for Japanese encephalitis, filariasis, and malaria^{8, 45, 59–62}. In spite of being the major vector for dengue, midgut microbial diversity studies for different species of *Aedes* mosquitoes are rare from India. One such attempt has been performed on the midgut microbiota of *Ae. aegypti* and *Ae. albopictus* from the Arunachal Pradesh⁶³. This study, focused on the characterization of culture-dependent aerobic bacteria from the midgut of both species of *Aedes* mosquitoes, as the culturable bacteria can be used for further applications in the management of disease transmission such as paratransgenesis. The results showed maximum bacterial species of gram-negative Enterobacteriaceae family and gram-positive Bacillaceae family. This study also suggests that mosquito midgut bacteria are primarily inherited through vertical inheritance or through acquisition from the environment. The presence of these gut microbiota is essential for maintaining a fine-tuned balance for existence. This finding is important since in number of studies species belonging to *Enterobacter* genus has been reported to block the development of *P. falciparum* in *An. gambiae* and sporogonic development of *P. vivax* in *An. albimanus*^{35, 64} by inducing the immune responses.

Culex microbiome

Culex mosquitoes are filter feeders and consume bacteria and many other micro-organisms in the water col-

umn. A complex microbial community is a fundamental necessity for the normal survival and complete development of these mosquito larvae to adult⁶⁵. Earlier studies have shown that the microbial communities isolated from the mid-gut of laboratory-reared IV instar *Culex tarsalis* Coquillett (a vector of Western encephalitis and West Nile viruses) using conventional culturing techniques included several species, like *Lactobacillus*, *Micrococcus*, *Saccharomyces*, *Proteus rettgeri*, *Geotrichum*, *Pseudomonas*, and other unidentified gram-negative bacteria. Among these, *Micrococcus* species (Actinobacteria), *Lactobacillus* (Firmicutes : Bacilli) and *Pseudomonas* (Gammaproteobacteria) are commonly found in *Cx. tarsalis* guts. Most genera of bacteria found in the gut of larval *Cx. tarsalis* were also found in the adults, with the exception of *Aerobacter*, *Escherichia*, and *Flavobacterium*.

Research conducted by Muturi *et al*⁶⁶ in 2016 on *Cx. pipiens* and *Cx. restuans*, the primary vectors of West Nile encephalitis in Champaign County in the USA by 16S RNA sequencing method provided a comprehensive analysis of microflora in these mosquitoes. It included Proteobactericea, viz. *Wolbachia* and *Spingomonas* species; and Firmicutes like *Alicyclobacillus* species. Even though the members of Proteobactericea dominated in both the species, *Cx. restuans* had more diverse population.

Mosquito mycobiome

Like bacteria and virus, eukaryotic fungus is an inmate of mosquito gut microbiome. Its role as a commensal, mutualistic or as pathogenic is inevitable in maintaining ecological balance in mosquitoes. During the metamorphic transition, mosquitoes are exposed to fungi in the form of mosquito larvae in water, or through the ingestion of fungi in sugar meal or by physical contact with conidia (adult mosquitoes)⁶⁷. Filamentous fungi and yeast are the common fungal isolates present in the mid-gut and other tissues of mosquitoes. A filamentous fungus comprises some species of *Aspergillus* and *Penicillium* as pathogenic forms and some genera of fungi like *Beauveria* and *Metarhizium* as entomopathogenic forms⁶⁸. Different genera of yeast like *Candida*, *Pichia* and *Wickerhamomyces* have been identified in *Aedes* and *Anopheles* mosquitoes through culture dependent and culture independent methods. Earlier explorations in mosquito mycodiversity were based on these types of the culture-dependent method²⁶. For example, a yeast strain *Wickerhamomyces anomalus* has been reported in the midgut and reproductive organ of *An. stephensi*, a primary vector of malaria⁶⁹. Recently, with the advent of high throughput sequencing (HTS) technique, the knowledge about mosquito mycobiome has widened⁷⁰. This HTS technique

was employed to analyze the mycobial composition in *Ae. triseriatus* and *Ae. japonicum*. The sequence revealed the presence of 21 distinct fungal operational taxonomic units (OTUs), out of which 15 were shared between these two species. Ascomycota phylum is the major fungal taxa in these two *Aedes* species. Even though the presence of mycobiome is evident in mosquito, a little is known about the tripartite interaction between vector, pathogen, and fungi.

Mosquito virome

Mosquito act as an exclusive host for a large group of virus which are insect-specific^{71–72}. A metagenomic approach was used to evaluate viral load by Shi *et al*⁷³ in two genera of mosquitoes *Aedes* and *Culex*. The comparison presented a striking difference in the virome of mosquitoes, where in genus *Aedes* showed a low viral diversity and less abundance than *Culex*. This metagenomic approach lead to the identification/discovery of different viral families in mosquitoes such as *Bunyaviridae*, *Rhabdoviridae*, *Orthomyxoviridae*, *Flaviviridae*, *Mesoviridae*, *Reoviridae*, and unclassified *Chuvirus*, and *Negevirus* groups. Most resident virome act as commensal microbe due to its inability to infect vertebrate cell lines, prolonged host infection and vertical transmission.

Influence of microbes on host vectorial capacity

Vectorial capacity is a quantitative measure of several factors like cellular, biochemical, behavioural, immunological, genetic and environmental parameters which can influence vector density, longevity and vector competence⁷⁴. All these factors are interrelated and can determine the pathogenicity and nonpathogenicity in mosquitoes.

Bacteria, a dominant member of gut microflora may interact directly or indirectly with invading pathogens. The indirect interaction is by activating innate immune response^{42, 75}. Normally pattern recognition receptors (PRRS) on the host cell recognizes the conserved surface determinants called pathogen associated molecular patterns (PAMPs) exclusively present/found in microbes. This binding triggers immune signaling pathways such as the toll pathway or the immune deficiency (IMD) pathway. In toll cell signaling pathway a cascade of events leads to the degradation of I κ B like transcription factor (Cactus), nuclear translocation of NF- κ B like transcription factors (Dif and Dorsal) that leads to the expression of antimicrobial peptides (AMP) genes. This AMP, produced in the fat body is secreted into haemolymph, where they directly kill the invading microorganism. Genetic analysis has revealed that the AMP gene expressions are

mainly controlled by the toll pathway and IMD pathway. The toll pathway is mainly activated by gram-positive bacteria, human *P. falciparum*, and DENV. The presence of gram-negative bacteria activates the IMD pathway which controls antibacterial peptide gene control⁷⁶.

Application of microbes in mosquito vector control

Vector-borne diseases are spreading at an alarming rate. The traditional control strategies have achieved some progress in malaria control, but the incidence of arboviral diseases are on rise. The insecticide resistance among vectors and lack of vaccines are the two important reasons for this pandemic^{77–78}. So, the need of novel control strategies is essential to check emerging and re-emerging pathogens. In this regard, microbial based intervention is gaining due importance as a novel remedy to control these diseases.

Genus *Wolbachia* is an endosymbiotic, gram-negative, Alphaproteobacteria within the family *Rickettsiaceae*⁷⁹. *Wolbachia* strain has the capacity of cytoplasmic incompatibility (CI) which leads to production of sterile offspring, when an uninfected female mates with the *Wolbachia*-infected male mosquitoes. CI can also occur when mating occurs between mosquitoes infected with different, incompatible *Wolbachia* strains. This quality of *Wolbachia* has been exploited to control target vector mosquitoes by transinfection methods. The *Wolbachia* strain wMelPop present in *Drosophila melanogaster* has the quality to dramatically shorten the host longevity⁸⁰.

Enterobacter species are common gram-negative bacterial inhabitant present in genus *Aedes*, *Culex* and *Anopheles*. These bacterial strains produce reactive oxygen species that affect the development of oocyst from ookinetes. The reactive oxygen species interferes with the development of parasite leading to its death before its invasion to the intestinal environment⁸⁵.

Bacillus thuringiensis (Berliner) serovariety *israelensis* (*Bti*) de Barjac and strains of *Bacillus spherical* Neide are used as a nonchemical method to control mosquitoes⁹. It is a gram-positive spore forming bacteria observed in soil, aquatic areas, dead insects, grains *etc*^{37–40, 81–86}. They secrete delta endotoxin which has the capacity to kill the host organism. These principles are used to modify bacteria to kill disease-causing vectors⁸⁴.

Paratransgenesis, a new technique that attempts to eliminate a pathogen from vector populations through transgenesis of a symbiont of the vector has gained special attention. In this approach, microbes that reside within the gut of vectors are engineered to secrete anti-pathogen molecules. *Asaia* an *Alphaproteobacteria*, present in all

the developmental stages of host, in different regions (of host) make it an ideal choice for paratransgenesis^{32–34}. Attempts have been made to modify more strains of *Asaia* to secrete antiplasmodium effector molecules against malaria.

Entomopathogenic fungi can be used as a potential biological weapons agent against vector control. Normally spores or conidia which germinate on mosquito surface penetrate through the cuticle and reaches the haemolymph. *Beauveria bassiana* and *Metarhizium anisoplia* are naturally occurring filamentous fungi. *Beauveria bassiana* produces a toxin oosporein, which downregulate Duox expression in host midgut, and thereby slowly kills the mosquitoes⁸⁷. This slow killing process is an added advantage because it results in slow selective pressure for resistance, reduction in reproductive fitness, and prevent pathogenic transmission of vector.

CONCLUSION

The mosquito gut is inhabited by a large number of microbes. Understanding the symbiotic relationship between the gut microbiome and the host mosquito can facilitate novel intervention strategies for mosquito vector control. Paratransgenic strategy, wherein the symbiotic or commensal microbes of host mosquitoes/insects are genetically altered to express gene products that interfere with pathogen transmission, could act as valuable tool for control of vector-borne diseases. Bacteria, fungi and virus can be used as excellent candidates for paratransgenesis. This approach limits the adverse effects of many insecticides on nontarget species including humans, environment, soil and water; and also avert development of mosquito resistance. The tiny nature of densovirus remain as an attractive option to use it as a transgene with improved killing efficiency and capacity to reduce selective pressure for resistance to control mosquito population. Enhancing the ability of resident entomopathogenic fungi to shorten the mosquito life span by genetic manipulation also appears viable.

Techniques like introduction of *Wolbachia* in natural populations of mosquito, use of *Bti* strains, *etc.* are other promising vector control strategies. Knowledge and ability to manipulate the microbial diversity in mosquitoes can be potentially used to alter their competence and survival, as microbes have several desirable properties for applied control strategies, particularly the ability to disseminate through vector populations. The antipathogenic capacity of the microbes needs vast exploration to discover a breakthrough method of controlling disease transmission.

Conflict of interest

The authors declare that they have no conflict of interest.

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