



## GUT BACTERIA DIVERSITY IN ANOPHELINE MOSQUITOES AND PROSPECTS IN VECTOR CONTROL- A REVIEW

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### ABSTRACT

**Anophelines have diverse kinds of gut bacterial communities which play significant roles during the life cycle stages of their respective hosts and perform potential roles in host nutrition, development and in reproductive biology. In addition, these bacterial communities play vital roles in determining the vectorial capacity of their respective mosquito hosts allowing their natural evolution. The removal of the symbiotic bacteria from the mosquito body or the use of genetically modified symbiotic bacteria would interfere with the spread of pathogens by their mosquito vectors or reduce the life span of host and their reproductive capacity. Control of symbiotic bacteria of mosquito vectors would be considered as a novel strategy for vector management.**

**Key words:** Mosquito vectors, anophelines, symbiotic bacteria, effects, pathogens, life cycle stages, reproductive capacity, vector management

The class Insecta consists of a large group of organisms with rich species diversity. The mosquitoes are small bodied nuisance arthropods that belongs to the order Diptera under the class Insecta. There are several mosquito-borne diseases like malaria, filaria, dengue, Japanese encephalitis which are of major public health concerns in various parts of India including West Bengal in particular. These diseases are transmitted by mosquitoes belonging to several genera like *Anopheles*, *Culex*, *Aedes*, *Mansonia* etc. (Porter et al., 1993; Poopathi and Tyagi 2006). Malaria is a major health hazard causing enormous morbidity and mortality continuously in many tropical and sub-tropical countries. In tropical parts of South and Southwest Asia *Anopheles culicifacies* Giles serves as the main malaria vectors (Zaimet al., 1993). *Anopheles subpictus* Grassi has been recognized as a potent vector of malaria in rural areas of West Bengal (Chatterjee and Chandra, 2000).

Several species of protozoan parasite *Plasmodium* are responsible for different types of malaria occurring throughout the world. Although susceptibility of anophelines to *Plasmodium* infection is genetically controlled but environmental factors also play a crucial role in determining the variation in number of oocytes among closely related mosquito species (Collins et al., 1986; Blandin et al., 2009). There are several evidences which suggested that mosquito bacterial communities could influence the competence of their respective

vectors (Favia et al., 2007; Briones et al., 2008; Rodrigues et al., 2010). Some studies have suggested that symbiotic bacteria might play a significant role to inhibit the infection of malarial parasites (Beier et al., 1994; Dong et al., 2009). Studies in both laboratory and field conditions have revealed that co-infection of malarial parasite *Plasmodium* with bacteria leads to reduction in the development of oocysts (Pumpuni et al., 1993; Meister et al., 2009; Cirimotich et al., 2011). Indiscriminate use of chemical insecticides viz. DDT, gamma-xene, malathion etc. to control the mosquito vectors ultimately led to the development of vector-resistance and also so many health and environment related serious emerging problems in India.

Present review focuses on gut bacterial diversity in larvae and adults of *Anopheles* spp., their positive and negative impact on host body and their potential use in the vector control programme. Greater understanding of these interactions between mosquito host and symbiotic bacteria may lead to novel vector control as well as disease control strategies.

### Diversity of gut bacteria

Amongst metazoans, insect species harbor different kinds of microbial community which interact with their intestinal epithelium in distinctive manners. The gut microbiota of mosquitoes are very dynamic, because during metamorphosis, mosquito swings from its

larval form in aquatic habitat to terrestrial adult forms. Bacterial diversity in the gut of adult mosquitoes is lower because of the phenomenon of gut renewal that occurs during the metamorphosis from pupae to adult.

Characterization of bacterial communities in the host body is a prerequisite to understand how a bacterial population functions in the gut micro-environment of mosquito body because microbiota play an important role in determining the outcome of infection of vector mosquitoes with their parasites (Cirimotich et al., 2011; Boissiere et al., 2012). Several studies have revealed gut bacterial diversity in different mosquito species either through culture dependent techniques or applying molecular approaches (Lindh et al., 2005; Terenius et al., 2008; Rani et al., 2009). The distribution and specificity of midgut microbiota depends on the species of mosquito concerned and also on the physico-chemical and organic condition of the breeding habitats. There are some bacterial genera which are found in majority of mosquitoes are referred to as 'mosquito midgut core microbiota'. These microbiota includes members of the genera *Asaia*, *Burkholderia*, *Serratia*, *Ralstonia*, *Acinetobacter*, *Staphylococcus*, *Pseudomonas*, *Sphingomonas*, *Shigella*, *Streptococcus* and *Escherichia*.

*Thorsellia* was a common gut bacterial isolate of *Anopheles arabiensis* (Lindh et al., 2005), *Anopheles stephensi* (Rani et al., 2009) *Anopheles gambiae* s.l. (Wang et al., 2011; Briones et al., 2008), and *A. culicifacies* (Chavshin et al., 2014). Osei-poku et al. (2012) reported that *Pseudomonas* population in Kenyan mosquitoes is very low whereas Chavshin et al. (2014) identified *Pseudomonas* as the most frequently isolated bacteria from *A. culicifacies* in Iran. Wang et al. (2011) depicted that the larval and pupal guts were dominated by the photosynthetic Cyanobacteria whereas the gut of adult mosquitoes were dominated by Proteobacteria and Bacteroidetes. Boissiere et al (2012) established the microbe-pathogen interactions showing strong correlation between richness of Enterobacteriaceae in the midgut of adult mosquitoes with the infection status by *Plasmodium* sp.

Till today, it is still not properly understood that how these commensal bacteria are acquired by *Anopheles* mosquitoes. During the primitive stage of development of mosquito larvae, the main source of these bacteria is their aquatic environments (Straif et al., 1998). Microbial community in the adult stage is significantly affected by the type of diet such as sugar

meal or blood meal. On the other hand studies by egg smearing of *A. gambiae* and *A. stephensi*, revealed that there is a vertical transmission of  $\alpha$ -proteobacterium of the genus *Asaia* spp. that present in the larvae and adults of *A. stephensi*, an important vector mosquito of main malaria agent *Plasmodium vivax* in Asia (Favia et al., 2007), whereas horizontal and transstadial transmission occurs in case of *Elizabethkingia* spp. (Lindh et al., 2008; Briones et al., 2008), which indicate an attractive feature to build up the potential design for the novel mosquito/malaria biocontrol strategies (Akhouayri et al., 2013).

### Role of symbiotic bacteria on host

Interactions between the mosquito host and their symbiotic microorganisms have a great impact to understand the extended phenotypes of mosquitoes. The bacterial communities associated with mosquitoes are categorized into two broad categories: primary symbionts, which coevolved with their hosts and secondary symbionts, which are not obligative and associated with their host during evolution. These endosymbiotic bacteria play many significant roles in nutrition, reproduction and subsequent development of their hosts and even protection of their hosts from enemies (Toft et al., 2010).

**Nutrition:** There are various ways by which insect associated bacteria contribute to the nutrition of their host. There are several evidences which suggest that bacteria are involved in host's nutrition. In some cases, mid gut bacteria provide their host with essential amino acids, when their host lack these on their food such as in plant sap (Douglas et al., 1998). In addition, mid gut bacteria produce several enzymes such as cellulase, amylase, protease etc. that help to degrade several complex substances that are consumed by the host and thus improve the digestion of host. In hematophagous insect gut bacteria involved in blood digestion (Pais et al., 2008). Reduction of gut bacteria by antibiotic treatment led to incomplete red blood cell lysis and deprived the mosquitoes from essential nutrients (Gaio et al, 2011). Some gut bacterial species of mosquitoes could be involved both in the assimilation of nectar and digestion of blood by the mosquito host (Minard et al., 2013). From *A. stephensi*, the isolated bacterial species *Asaia bogorensis* was found to be prototrophic with respect to vitamins, which indicated that this bacterial strain could afford vitamins to their host mosquito (Crotti et al., 2010).

**Reproduction:** Previous studies have shown that

Table 1. Gut bacteria in anopheline mosquitoes

S No.	Name of Mosquito species	Name of Gut-bacteria	Country	Reference
1	<i>Anopheles funestus</i>	<i>Nocardia corynebacterioides</i> , <i>Anaplasma</i> sp.,	Suba district, Western Kenya	Lindh et al., 2005
2	<i>Anopheles stephensi</i>	<i>Asaia</i> sp.	Asia	Favia et al., 2007
3	<i>Anopheles darlingi</i>	<i>Enterobacter hormaechei</i> , <i>Pseudomonas putida</i> , <i>Aeromonas</i> sp.	Amazonas, Brazil	Terenius et al, 2008
4	<i>Anopheles stephensi</i>	<i>Paenibacillaceae</i> , <i>Serratia marcescens</i> , <i>Cryseobacterium meningosepticum</i>	Asia	Rani et al, 2009
5	<i>Anopheles maculipennis</i>	<i>Pseudomonas mendocina</i> , <i>Aeromonas punctate</i> , <i>Aeromonas bivalvium</i> , <i>Lysinibacillus sphericus</i>	Sistan and Baluchestan in South East Iran and Guilan in northern Iran	Djadid et al, 2011
6	<i>Anopheles stephensi</i>	<i>Bacillus pumilus</i> , <i>Brevundimonas aurantiaca</i> , <i>Lysinibacillus sphericus</i> , <i>Sphingomonas paucimobilis</i> , <i>Rahnella aquatilis</i> , <i>Pantoea agglomerans</i> , <i>Pantoea stewartii</i>	Sistan and Baluchestan in South East Iran and Guilan in northern Iran	Djadid et al, 2011
7	<i>Anopheles gambiae</i>	Photosynthetic Cyanobacteria, Proteobacteria and Bacteroidetes	Kenya	Wang et al., 2011
8	<i>Anopheles stephensi</i>	<i>Myroides</i> , <i>Chryseobacterium</i> , <i>Aeromonas</i> , <i>Pseudomonas</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Shewanella</i> , <i>Alcaligenes</i> , <i>Bordetella</i> , <i>Exiguobacterium</i>	Southern Iran	Chavshin et al., 2012
9	<i>Anopheles gambiae</i>	<i>Asaia</i> , <i>Sphingomonas</i> , <i>Burkholderia</i> , <i>Ralstonia</i> , <i>Serratia</i> , <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Acinetobacter</i> , <i>Streptococcus</i> , <i>Sphingomonas</i> , <i>Staphylococcus</i> , <i>Shigella</i> and <i>Escherichia</i>	Cameroon	Boissiere et al., 2012
10	<i>Anophelegambiae</i>	<i>Elizabethkingia meningoseptica</i>	London	Akhouayri et al., 2013
11	<i>Anopheles gambiae</i>	<i>Delftia</i> , <i>Aeromonas</i> , <i>Klebsiella</i> , <i>Rahnella</i> , <i>Comamonas</i> , <i>Pseudomonas</i> , <i>Serratia</i> , <i>Actinobacter</i> , <i>Enterobacter</i> , <i>Escherichia</i> , <i>Shigella</i>	Cameroon, Africa	Tchioffo et al., 2013
12	<i>Anopheles Culicifacies</i>	<i>Acinetobacter</i> , <i>Staphylococcus</i> , <i>Aeromonas</i> , <i>Bacillus</i> , <i>Exiguobacterium</i> , <i>Pseudomonas</i> , <i>Variovorax</i> <i>Chryseobacterium</i> , <i>Thorsellia</i> , <i>Bacillus</i> , <i>Kurthia</i> , <i>Microbacterium</i> , <i>Acinetobacter</i> , <i>Brachybacterium</i>	Iran	Chavshin et al., 2014
13	<i>Anopheles barbumbosas</i>	<i>Acinetobacter</i> sp., <i>Asaia</i> sp., <i>Enhydrobacter</i> sp, <i>Klebsiella pneumonia</i> , <i>Serratia</i> sp., <i>Thorsellia anopheles</i> , <i>Yersinia</i> sp, <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus pasteurii</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus warneri</i> , <i>Enterococcus faecium</i> , <i>Brachybacterium</i> spp., <i>Brevibacterium</i> sp.	Dak Nong Province, Vietnam	Ngo et al., 2015

14	<i>Anopheles crawfordi</i>	<i>Acinetobacter</i> sp., <i>Diplorickettsia massiliensis</i> , <i>Cellvibrio ostravienis</i> , <i>Enterobacter aerogenes</i> , <i>Hafniaparalvei</i> , <i>Tayumella</i> sp., <i>Moraxella osloensis</i> , <i>Novosphingobium</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Sphingobium</i> spp, <i>Stenotrophomonas</i> sp.	Dak Nong Province, Vietnam	Ngo et al., 2015
15	<i>Anopheles diras</i>	<i>Acinetobacter</i> sp., <i>Bartonella</i> sp., <i>Thorsellia anopheles</i> , <i>Sphingomonas</i> spp, <i>Staphylococcus pasteurii</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus warner</i> , <i>Bacillus</i> sp.	Dak Nong Province, Vietnam	Ngo et al., 2015
16	<i>Anopheles gigas</i>	<i>Acinetobacter</i> sp., <i>Bartonella</i> sp., <i>Thorsellia anopheles</i> , <i>Sphingomonas</i> spp., <i>Staphylococcus pasteurii</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus warner</i> , <i>Bacillus</i> sp.	Dak Nong Province, Vietnam	Ngo et al., 2015
17	<i>Anopheles maculatus</i>	<i>Acinetobacter</i> sp., <i>Enhydrobacter</i> sp., <i>Novosphingobium</i> sp., <i>Staphylococcus pasteurii</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus warner</i> , <i>Janibacter</i> sp.	Dak Nong Province, Vietnam	Ngo et al., 2015
18	<i>Anopheles subpictus</i> Grassi	<i>Bacillus cereus</i>	Sundarban, West Bengal, India	Mukhopadhyay et al., 2016

some bacteria colonize in the reproductive organs of insects which allow them to manipulate reproductive ability. Some mosquito species depends on *Wolbachia* in order to produce viable progeny. This bacterial genus is able to control the mating of mosquito by a phenomenon called cytoplasmic incompatibility. In this process, mating between an infected male and uninfected female or female infected with incompatible *Wolbachia* strain is unable to produce viable progeny. Other than *Wolbachia*, there are some bacteria such as the *Bacillus* and *Staphylococcus*, which are supposed to play a role during reproduction of insects, and affect the fertility of several mosquito species, although the mechanisms is not fully understood (Fouda et al., 2001).

**Development:** The gut bacteria are known to release various compounds which are useful for the development of mosquito larvae. Several studies have showed that removal or killing of these bacterial strains would lead to the inhibition of mosquito development, such as elimination of gut bacterial strains by application of antibiotics lead to the inhibition of larval development and survival of *Culex vishnui*, a vector of Japanese encephalitis in tropical regions (Roy et al., 2010). Malarial vector *Anopheles subpictus* of Sundarban areas of West Bengal also depends on symbiotic gut bacterial strain *Bacillus cereus* for the larval development and survival (Mukhopadhyay et al., 2016)

## Conclusions

Malaria is still a major health problem in several

regions of the world. An estimated 219 million malarial cases were reported from 87 countries throughout the world with approximate 43,500 deaths (WHO, 2018). Currently several vector controlling strategies and anti-malarial drugs have been used to prevent the spread of malarial diseases. For the control of malaria, in 1953 National Malaria Control Programme (NMCP) was launched, and then in 1958 the National Malaria Eradication Programme (NMEP) was initiated. Through these programmes, DDT, HCH and residual organochlorine insecticides were used widely (Subbarao, 1988). But continuous use of these insecticides led to the insecticidal resistance of vector (Pluess et al., 2010) and this resistance may be of different kinds like cuticular, metabolic, behavioral (Ranson et al, 2011), even resistance to one insecticide may cause resistance to another insecticide by a phenomenon called cross resistance. In addition, drug resistance of the malarial parasite has been considered as a rising problem in the disease management (Parija et al., 2011). So, now a days more novel vector management strategy is required in order to check the reemergence of malaria and other vector-borne diseases.

A wide variety of microbial symbionts is present in the gut environment of these vector insects as a result of continuous exposure to the external environment and also for the phenomenon of transovarial transmission (Dillon and Dillon 2004; Rani et al., 2009). Since microbial symbionts of insects play several important roles starting from reproduction to development

and nutrition and also mediate adaptation to several environmental fluctuations, so better knowledge about these mosquito associated bacterial communities is an important aspect to understand insect-bacteria interactions and further control of these symbiotic bacteria will elucidate an alternative strategy of mosquito control and thereby preventing the disease outbreaks.

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