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FAUNAL DIVERSITY AND RECENT TRENDS in **ANIMAL TAXONOMY**

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MICROBIAL ANALYSIS OF LARVAL GUT OF *Aedes Aegypti*

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ABSTRACT

Mosquito larval gut remains as an ecosystem that accommodates a wide array of microbes. These microbes play a significant role in nutrition, growth, reproduction and vector competence of mosquitoes. The host feeding and prevailing environmental conditions are important parameters that shape gut biome. The gut microbial community is mainly dominated by bacterial species. It also harbors viral and fungal population. The presence of gram positive and gram negative bacterial inmates has a potential role on disease transmission. *Aedes aegypti*, flavi virus vector of many diseases like Dengue virus, Zika virus, Yellow fever virus and Chikungunya virus draw special attention in this regard. Lack of vaccination in man and insecticide resistance among mosquitoes leads to rise of mosquito borne diseases. Several control strategies were being employed to control these vector borne diseases. A better understanding of gut microbial community and its relationship with host can be explored as effective measure to check these vector mosquitoes. This approach can reduce adverse effect of many insecticides on non target species including humans, environment as well as the development of mosquito resistance against insecticides.

Key words: Diversity, microbes, gut analysis, mosquito

INTRODUCTION

Mosquitoes are holometabolus insects having four different stages from egg to adult through two intermediates, larval and pupal stages respectively. Among them larval stage is the active form in its life cycle. During the larval stage the feeding depends on the environmental conditions prevailing in its habitat. Recent studies by Mang et al., 2017 indicate that the gut microbiota of larva has an impact on transmission of diseases. Microbial content of *Aedes aegypti* draw special attentation in this regard as it remain as the vector of many dreaded arboviral diseases like Dengue, Yellow Fever, Zika and Chikungunya virus. This vector is prevalent in Indian urban areas. The presence non degradable tyres, long lasting plastics, inappropriate waste water management create ideal breeding place for these mosquitoes. The population of *Aedes aegypti* varies with rainfall and humidity. The life cycle can be categorized into two phases, an aquatic phase (egg, larvae, and pupae) and a terrestrial phase having an aerial adult form. Under the optimal conditions the life cycle of aquatic stage of the *Aedes aegypti* (hatching to adult emergence) can be as short as seven days. At low temperatures, it may take several weeks for adult emergence. During

the rainy season, as the survival is longer, the risk of virus transmission is greater.

RESULT

Histology of Larval Midgut

The basic structure of digestive system is similar in all insects with slight modification to adapt their respective feeding behaviour (Wang et al. 2011). In mosquitoes a prominent digestive system began to appear from larval stage onwards. The digestive tract is divided into three regions mainly foregut, midgut and hindgut. Among these regions midgut plays a vital role in digestive process. The midgut of larval mosquitoes is divided into four regions. Within the thorax region lies the cardia which is followed by eight ovate globular extensions of the midgut called gastric caecae. Extending through the abdomen from the gastric caecae to the hindgut is the 'stomach', which is divided into histologically distinct anterior and posterior regions (Clements, 1992). Studies by Thomas et al., 1999 revealed the difference that distinguish between anterior and posterior by the appearance and larger diameter of the posterior stomach relative to the anterior stomach and by the presence of Malpighian tubules or gastric caecae at either end of the midgut.

Influence of microbes and larval existence

Microbial communities were indispensable parts of larval life. Innate microbial presence was mainly found in the gut region, salivary gland, reproductive organ and hemolymph in adult. Moreover breeding water also contributes to these microbial colonies. The gut microbes were mainly dominated by bacteria followed by fungi and viruses.

Karima Zouache and coworkers 2010 explored the composition and diversity of mosquito associated bacteria in relation with mosquito habitats from different geographical regions of Madagascar on wild *Aedes aegypti* and *Aedes albopictus* by using the traditional culturing method and by denaturing gradient gel electrophoresis (DGGE) and sequencing of rrs amplicons. 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 laboratory reared and wild targeted population. Wild *Aedes* gut generally has a bacterial profile consist of *Serratia marcescens*, *Klebsiella*, *Ozaenae*, *Pseudomonas aeruginosa*, *Enterobacter* spp, *Proteobacteria*, *Flavobacteriaceae* depending upon the stages of development. Indeed it was shown that field caught *Aedes triseriatus* harbor a greater bacterial diversity than laboratory reared ones (Rani et al. 2009).

In India, attempts to survey the midgut microflora has remained mainly focused on two genus, *Culex*, and *Anopheles* mosquitoes, which act as vectors for Japanese encephalitis, Filariasis, and Malaria (Chandel et al., 2015; Pai et al., 2014) In spite being the major vector for Dengue, midgut microbial diversity studies in different species of *Aedes* mosquitoes are rare, especially from India. So, one such attempt to characterize the genus *Aedes* was done on the midgut microbiota of *Aedes aegypti* and *Aedes albopictus* from Arunachal Pradesh. 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 only be used for further applications in the management of disease transmission such as paratransgenesis. The result 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 a number of studies have been done and this species of Enterobacter has been found to block the development of *Plasmodium falciparum* in *Anopheles gambiae* and sporogonic development of *Plasmodium vivax* in *Anopheles albimanus* (Cirimotich et al., 2011; Gonazalez et al., 2003) by inducing the immune response.

Larval mycobiome shows symbiosis, commensalism and parasitism within vector body. Most of them reside in the midgut region a few reside in hind gut. Filamentous fungi and yeast are the common fungal isolates normally present in the midgut and other tissues of mosquitoes. The filamentous fungus includes some species of *Aspergillus* and *Penicillium* as pathogenic forms and some genera of fungi like *Beauveria* and *Metarhizium* as entomopathogenic forms. Different genera of yeast like *Candida*, *Pichia* and *Wickerhamomyces* have been identified in *Aedes* and *Anopheles* mosquitoes through culture dependent and culture independent method. Earlier research in mosquito mycodiversity was based on these types of the culture-dependent method (Gusmao et al., 2010). Mosquito act as an exclusive host for a large group of virus which is insect specific (Bolling et al., 2015). The metagenomic approach was used to evaluate viral load by (Mang et al., 2017) in two genera of mosquitoes *Aedes* and *Culex*. The comparison presented a striking difference in the virome of mosquitoes, in which genus *Aedes* represent a low viral diversity and less abundance than *Culex*.

DISCUSSION

Vector Borne Diseases (VBD) is spreading at an alarming rate. It is responsible for worldwide morbidity and mortality in humans. The traditional control strategies had made a great progress in Malaria control, but the incidence of Arboviral diseases are on rise. The insecticide resistances among vectors, lack of vaccination in humans are the two important reasons for this pandemic (Naqqash et al., 2016). 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 VBD. Paratransgenesis technique is employed to eliminate the pathogen from vector through the transgenesis of vector symbiont. This vector symbiont is maternally inherited or by coprophagy across an insect population. (Irene Ricci et al., 2012). This approach can reduce the adverse effects of many insecticides on non-target species, including humans, environment, soil and water contamination and the development of mosquito resistance to insecticides (Dorta et al., 1993). The use of entomopathogenic fungi as novel biological control agents of

adult mosquitoes appears promising under laboratory and in simulated field conditions.

CONCLUSION

The mosquito gut is inhabited by a large number of microbes. Understanding the symbiotic relationship between the gut microbiome and the host mosquito will perhaps facilitate novel intervention strategies for mosquito vector control, as a preventive measure of disease transmission. New approaches like paratransgenesis technique and use of entomopathogenic fungi are now being employed in this regard.

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