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Editorial

Mosquito Microbiota and Metagenomics, and its Relevance to Disease Transmission

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Abstract

Application of high throughput sequencing to infer microbial diversity in environmental as well as animal and plant samples is the central theme of metagenomics. This is an immerging area of modern biology that has huge potential to uncover the forms of life we would have never imagined, for example the diversity of microorganisms living within a tiny insect. Metagenomics analyses of disease spreading insects will open up new avenues for better understanding the role of gut microbiota of insect vectors, such as mosquitoes, in ability of these vectors to spread deadly human diseases. The aim of this editorial is to provide the current state of our knowledge on identification of microbial communities in mosquitoes, but more importantly, to give a wakeup call to the vector biology community that it is time to take a good look on the guts of these disease-spreading insects.

Keywords
Vector-borne disease, disease transmission, gut, mosquito, microbiota and metagenomics

Understanding pathobiology of mosquitoes is an important component of understanding vector-borne diseases. Significant efforts have been made in understanding vector competence of mosquitoes that transmit major pathogens such as malaria, dengue and West Nile Virus and other disease-causing pathogens [1-6]. Study suggests that zoonotic mosquito-borne flaviviruses are potential candidates of future emerging diseases because of their worldwide presence and also due to proven pathogenicity to humans [7]. Identification of influential factors that drive vectorial ability of mosquitoes to spread diseases is an urgent need. The genomic and metagenomic approaches have shown huge potential in this direction. Genomes of major pathogens that are transmitted by mosquitoes have been sequenced by now. Analysis of these sequences has revealed forces that shape pathogen evolution and their influence on mosquito populations. It is imperative that more insights should be gained based on genome-wide effects of pathogen on vector populations in order to better understand the evolutionary dynamics of vector-pathogen interactions. Here, I briefly describe some of the progresses made in the areas of pathobiology, endosymbioants as well as cultured and uncultured bacterial populations in mosquitoes and emphasize on the metagenomic approach for a comprehensive analysis of role of microbiota in vectorial ability of mosquitoes to disease transmission.

Several bacterial endosymbioants have been identified in mosquitoes that either permanently reside within specific species/ strains or present as a predominant component of the entire microbiota of related mosquito species [8,9]. *Wolbachia* is a well known endosymbiont bacteria of mosquitoes [10,11]. Because of their stable association and peculiar effect on the host organism (effect on age), *Wolbachia* has been described as a potential tool for suppressing vectorial ability of mosquitoes to disease transmission [12-15]. In *Anopheles stephensi*, Asaia bacteria were the dominant component of the whole microbiota of these mosquitoes, particularly in the female gut and in the male reproductive tract [16]. Further experimental evidences from this study also indicated that the Asaia bacteria are stably associated with the female guts and salivary glands, sites that are crucial for Plasmodium sp. development and transmission. In *A. gambiae* mosquitoes also, the Asaia bacteria are primarily localized in the midgut, salivary glands and reproductive organs [17]. Using fluorescent in situ hybridization on the reproductive tract of females of *A. gambiae*, this study has further shown that the density of Asaia is relatively high at the very periphery of the eggs, suggesting that transmission of Asaia from mother to offspring is likely mediated by a mechanism of egg-smearing. Furthermore, molecular studies have shown that different Asaia strains are present in different mosquito populations, and even in single individuals suggesting that multiple infections of Asaia bacterial symbionts may have occurred in these mosquito species.

Several studies have been performed in laboratory-raised and filed-collected mosquitoes to survey bacterial diversity, mostly in the midgut. The culture dependent and culture independent methods are particularly useful approaches in this effort to make a comprehensive assessment of the bacterial species in mosquitoes [18]. Using this approach in *Anopheles stephensi* mosquitoes, it was found that the field-caught adult males were predominantly infected with uncultured *Paenibacillaceae* where as the female and larvae samples had *Serratia marcescens* as major source of infections. In contrast to the field-collected samples, the lab-reared mosquitoes were mostly inhabited

with *Serratia marcescens* and *Cryseobacterium meningosepticum* bacteria. Using similar approaches in *Culex quinquefasciatus*, Pidiyar *et al.* (2004) [19] determined that the majority of the cultured isolates and the 16S rRNA gene library clones generated from midgut samples belonged to the gamma-proteobacteria class. The study also found that about 46% of all bacteria identified from rRNA sequences were classified as unidentified and uncultured. Recently, the microbiota associated with four mosquito species, *Anopheles stephensi*, *Anopheles gambiae*, *Aedes aegypti*, and *Aedes albopictus* have been compared [20]. The results revealed the presence of several bacterial taxa in these mosquitoes, among which Asaia sequences were dominant in most of the samples. Analysis of field-collected *Aedes albopictus* and *Aedes aegypti* from Madagascar, however, reveals that Proteobacteria and Firmicutes are the major phyla in these mosquitoes [21].

The major limitation of comprehensive survey of mosquito microbiota stems from the presence of large proportion of uncultured bacterial species [22]. Metagenomics is the application of modern genomics techniques to the study communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species. It allows us to identify and determine DNA sequences of species that are difficult to grow in laboratory culture. In a large scale metagenomic study, Dinsdale *et al.* (2008) [23] also included mosquito samples collected from California (Mission Valley and Buena Vista Lagoon) to understand microbial diversity within mosquitoes. Now, several studies have shown compelling evidences that supports role of resident microbiota on mosquito's ability to spread disease causing pathogens [24-27]. The metagenome sequencing has identified hundreds and thousands of microbial species from various environmental samples as well as from fossils, living animals and plants, and the human gut [28-31]. It is needless to argue that metagenomics has huge potential to uncover 'life-within-life' that we would have never thought in the pre-genomic era. Hence, it is not only essential but also timely that we exploit the tools and technologies (large number of computational tools are also available) towards systematic investigation on role of gut microbiota of vector mosquitoes in disease transmission.

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