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MODULAÇÃO DA MICROBIOTA DO INTESTINO MÉDIO DE *AEDES* PELOS SOROTIPOS DO VÍRUS DA DENGUE: UMA REVISÃO DAS INTERAÇÕES HOSPEDEIRO-PATÓGENO-MICROBIOMA

MODULATION OF AEDES MIDGUT MICROBIOTA BY DENGUE VIRUS SEROTYPES: A REVIEW OF HOST-PATHOGEN-MICROBIOME INTERACTIONS

MODULACIÓN DE LA MICROBIOTA DEL INTESTINO MEDIO DE AEDES POR LOS SEROTIPOS DEL VIRUS DEL DENGUE: UNA REVISIÓN DE LAS INTERACCIONES HOSPEDADOR-PATÓGENO-MICROBIOMA

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RESUMO

A microbiota do intestino médio de *Aedes aegypti* atua como um determinante crítico da competência vetorial, modulando respostas imunes e metabólicas ao vírus da dengue (DENV). A infecção viral provoca alterações ecológicas distintas nesse ambiente, desafiando a estabilidade do "núcleo" bacteriano diante da disbiose induzida pela alimentação sanguínea. Essas dinâmicas microbianas são moldadas por uma interação complexa entre os sorotipos virais, o tempo pós-infecção, o estado fisiológico do mosquito, a fonte do repasto sanguíneo e as condições experimentais. Embora o núcleo basal possa persistir, famílias bacterianas específicas têm sido relatadas como apresentando flutuações associadas ao sorotipo e podem representar correlatos candidatos de suscetibilidade ou resistência viral. Elucidar essas interações hospedeiro-patógeno-microbioma é essencial para definir barreiras biológicas à transmissão. Esta revisão narrativa sintetiza as evidências atuais sobre a modulação do microbioma do mosquito pelos sorotipos 1–4 do DENV, destacando nuances ecológicas e identificando táxons e mecanismos bacterianos que merecem investigação adicional como potenciais alvos de estratégias de controle vetorial baseadas no microbioma.

Palavras-chave: *Aedes aegypti;* vírus da dengue; microbiota; disbiose do intestino médio; competência vetorial.

ABSTRACT

The *Aedes aegypti* midgut microbiota serves as a critical determinant of vector competence, modulating immune and metabolic responses to Dengue virus (DENV). Viral infection precipitates distinct ecological shifts within this environment, challenging the stability of the bacterial "core" against blood-meal induced dysbiosis. These microbial dynamics are shaped by a complex interplay between viral serotypes, time post-infection, mosquito physiological status, blood-meal source, and experimental conditions. While the basal core often persists, specific bacterial families have been reported to show serotype-associated fluctuations and may represent candidate correlates of viral susceptibility or resistance. Elucidating these host-pathogen-microbiome interactions is essential for defining biological barriers to transmission. This narrative

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review synthesizes current evidence on the modulation of the mosquito microbiome by DENV serotypes 1-4, highlighting ecological nuances and identifying bacterial taxa and mechanisms that warrant further investigation as potential targets for microbiome-based vector control.

Keywords *Aedes aegypti*; Dengue virus; Microbiota; Midgut dysbiosis; Vector competence.

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1. Introduction

Vector-borne diseases account for more than 17% of all infectious diseases globally. In this context, dengue fever stands out as the most prevalent viral infection, resulting in fourteen million cases, more than half of which were laboratory-confirmed (1). The disease is caused by the Dengue virus (DENV), a member of the Flavivirus genus within the Flaviviridae family, it's characterized by a spherical virion with a diameter of approximately 44–52 nm, encapsulating a host-derived lipid bilayer (2,3). Its genetic material consists of a single-stranded, positive-sense RNA genome of approximately 11 kilobases in length (3,2,4,5). Based on the genetic divergence accumulated within this genome, the virus is classified into four medically significant, antigenically distinct yet phylogenetically related serotypes. These four established serotypes share approximately 65% to 70% amino acid sequence homology. The remaining non-homologous genetic divergence is highly concentrated in the genes encoding structural components, particularly the envelope (E) protein (2,3,6). These variations are the evolutionary consequence of centuries of independent genetic drift, host-adaptation, and geographic isolation following their divergence from a common sylvatic ancestor (6). Because the E protein harbors the primary epitopes responsible for viral entry and immune recognition, these structural polymorphisms endow each serotype with a unique antigenic signature (6,2). Consequently, the host's immune system perceives DENV 1, 2, 3, and 4 (DENV 1-4) as distinctly different immunological entities, which explains why neutralizing antibodies raised against one serotype offer only transient, suboptimal cross-protection against the others (2,3). Furthermore, these serotypes exhibit distinct epidemiological and evolutionary dynamics. A putative fifth dengue virus serotype (DENV-5) has also been described, but its reported sylvatic context places it outside the scope of the present review; therefore, it is mentioned only as a potential emerging issue rather than as part of the DENV-1–4 evidence base (7).

Regardless of the specific circulating serotype, the continuous emergence and re-emergence of this disease are driven by a complex interplay among the pathogen, the



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host, and environmental factors (8). In tropical regions, climatic conditions coupled with non-climatic variables such as rapid urbanization, high population density, socioeconomic determinants, and shifting land-use patterns, significantly amplify the virus's transmission by creating highly favorable ecological niches for vector proliferation, sustaining a year-round life cycle of the primary DENV vector, *Aedes aegypti* (*Ae. aegypti*) (9,10,11) and although macroscopic environmental factors dictate vector distribution and human exposure, it is the microscopic host-pathogen interplay within the insect that ultimately governs vector competence. Following the ingestion of a viremic blood meal, DENV must sequentially overcome critical anatomical barriers to ensure successful transmission. The virus must first establish infection within the midgut epithelium, bypass the midgut escape barrier to disseminate into the hemocoel, and ultimately invade the salivary glands (4,10).

Ae. aegypti midgut is far from being merely a digestive tube; but a highly dynamic ecological niche, shaped by the mosquito's continuous exposure to a diverse array of environmental microorganisms throughout its life cycle, many of which have coevolved to establish enduring symbiotic or commensal relationships within the insect, culminating in a complex resident microbiota and current strategies to mitigate DENV transmission face mounting challenges (11). The variable efficacy of licensed vaccines across the four viral serotypes and their restricted clinical indications limit their immediate capacity to curb escalating global outbreaks, as traditional vector control campaigns, heavily reliant on chemical insecticides, are being progressively compromised by the widespread evolution of physiological and behavioral resistance in natural *Ae. Aegypti* populations (12,13,14). This epidemiological bottleneck drives an urgent need for novel, environmentally sustainable alternatives by disrupting the viral transmission cycle directly within the mosquito has emerged as a paramount public health priority, in this context, exploiting the resident microbiota offers an innovative biological control paradigm (15). By leveraging these symbiotic communities as an immunometabolic shield, it can become possible to modulate the vector's physiological homeostasis, actively antagonize viral replication, and drastically reduce vector competence, thereby paving the way for next-generation, microbiome-targeted interventions.

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2. Baseline midgut microbiota composition in *Ae. aegypti*

The basal microbiota composition of the *Ae. aegypti* midgut exerts a fundamental influence on host survival, facilitating nutrient assimilation and modulating vector competence, the taxonomic diversity within the midgut is dynamic, and influenced notably by geolocation, dietary intake, and infection status. *Ae. aegypti* can acquire its microbiota community through vertical inheritance, environmental acquisition, and transstadial transmission, whereby a portion of the larval microbiota is successfully retained into the adult stage, nevertheless, the mosquito undergoes a significant reduction in microbial load, often described as a selective bottleneck, mediated by bactericidal mechanisms within the lumen during the transition from the larval to the adult stage (16). This process facilitates the selective colonization of the gut by specific taxa. In this review, the term “core microbiota” should be interpreted according to the prevalence or abundance threshold applied by each study (17).

A core community identified in a specific mosquito population or environment should not automatically be considered a universal *Ae. aegypti* core. Recent evidence suggests that environmental factors dominate over genetics in shaping these communities, indicating that mosquitoes may not host a strict ‘core’ microbiota across their entire geographic range (17,18). The microbial community is primarily composed of Gram-negative aerobes or facultative anaerobes, a profile typically observed in arthropods that recruit their microbiota from the surrounding environment (18). In accordance with previous taxonomic surveys conducted by Yadav (2015), approximately 14 distinct taxa have been identified in the *Ae. aegypti* midgut, with a predominance of four main phyla: Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes. Within the Proteobacteria lineage, Enterobacteriaceae and Acetobacteraceae represent the principal families; notably, other taxa frequently reported in the *Ae. aegypti* microbial profile include Pseudomonadaceae, Aeromonadaceae, Bacillaceae, Staphylococcaceae, and Weeksellaceae (16). Building upon this baseline diversity, specific dietary inputs, such as the host blood-meal source, can alter the microbial composition, and the ingestion of a

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blood meal containing an arboviral infection can significantly affect and alter the microbiota profile. Studies have shown that bacteria belonging to the Enterobacteriaceae family and the genus Gluconobacter become more abundant in the microbiota of the midgut of mosquitoes infected with the Chikungunya virus, whereas the genus Pseudomonas (family Pseudomonadaceae) and Cellulosimicrobium (family Promicromonosporaceae) are specifically isolated in conditions of Zika virus infection (19).

Regarding other biological conditions that shape the microbial core, the presence of the endosymbiotic bacterium Wolbachia pipientis acts as a major modulator; it alters the host's energy metabolism and immune pathways, which consequently creates a distinct physiological environment that can reshape the resident microbiota (20,21). The co-occurrence of native gut bacteria, such as Lysinibacillus and Serratia, during arboviral infection has been shown to interfere with viral replication and salivation, highlighting the dynamic nature of gut microbial interactions and their potential influence on vector competence (22). The meal type also exerts a crucial role in modulating the microbial core. Regarding taxonomic families, Enterobacteriaceae and Weeksellaceae are reported in the gut of blood-fed mosquitoes, irrespective of the host (19,22). Specifically, Enterobacteriaceae is frequently found in mosquitoes fed on human blood, while Weeksellaceae is more associated with feeding on chicken and rabbit blood. Other families such as Microbacteriaceae are characteristic of newly emerged insects, while Acetobacteriaceae is likely to be found in sugar-fed individuals. The prevalence of Enterobacteriaceae in blood-fed mosquitoes has been hypothesized to be related to hemolytic activity; however, taxonomic enrichment alone does not demonstrate this function, and functional inference should be restricted to studies that directly measured hemolysis or related phenotypes (23).

Ultimately, these observations reinforce that the establishment of a so-called microbial core is not static; rather, it is highly dependent on the multitude of overlapping factors previously cited, including geolocation, diet, environment, and infection status, as schematically represented in **(Figure 1)**. It is important to note that different studies

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frequently yield contrasting results regarding the precise taxonomic composition of the midgut, reflecting the inherent variability of the mosquito microbiome, but collectively, these studies provide a better understanding of the ecological and physiological function of the microbiota and how it can be altered due to these diverse factors, carrying important implications for modulating vector competence.

3. DENV-induced alterations in the midgut microbiome

As established, the basal gut microbiota acts not only as a digestive aid, but also as an ecosystem that helps regulate mosquito physiological homeostasis in a complex manner, influencing nutrition, longevity, fecundity, and innate immunity. From this perspective, the introduction of DENV into this environment establishes a bidirectional relationship between the virus and the microbiome. While the resident microbiota can modulate innate immune responses that restrict DENV infection and affect vector competence, DENV infection itself may reshape the composition, diversity, and functional organization of the gut microbiota (24,25). This interaction is strongly influenced by the physiological status of the mosquito and is continuously shaped by development, diet, immune activation, gut integrity, and the selective pressures imposed by viral replication (17).

3.1 Physiological Remodeling of the Midgut Microbiota

One of the earliest determinants of microbiome structure occurs during the transition from the aquatic larval stage to the adult mosquito. In larvae, microbiota is mainly acquired from the environment and is usually highly diverse, with many bacterial taxa associated with dissolved organic matter and detritus-rich aquatic habitats. During metamorphosis, however, the gut undergoes extensive remodeling, including epithelial apoptosis, cell renewal, and clearance of intestinal contents. Current literature states that this process can create a microbial bottleneck, linked to the formation of the meconial peritrophic matrix and the subsequent elimination of the meconium after adult emergence (26,27). As a result, most larva-associated bacteria are physically removed, and the taxa that persist tend to share traits such as metabolic plasticity, tolerance to nutrient limitation and oxidative stress, the ability to adhere to the gut epithelium, and rapid proliferative

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potential, features that may favor groups such as Proteobacteria, and this transition corresponds to a major bottleneck **(Figure 1)**, in which meconium elimination separates the larval, aquatic-associated community from the selectively colonized adult midgut (27).

After emergence, diet becomes an important driver of microbial remodeling. In mosquitoes maintained exclusively on sugar, the midgut microbiota generally reflects a comparatively less disturbed nutritional environment, supporting a more balanced and diverse bacterial community in which relative abundance reflects competition for simple nutrients and basal immune regulation and blood feeding, by contrast, represents a major physiological disturbance (22). A blood meal introduces large amounts of proteins, lipids, and iron into the midgut, abruptly increasing nutrient availability and favoring bacteria with high proliferative capacity and versatile metabolism **(Figure 1)**. At the same time, blood digestion releases heme and free iron, which catalyzes the production of reactive oxygen species (ROS) and this oxidative environment can selectively suppress bacteria that are sensitive to redox stress, while favoring taxa equipped with antioxidant systems, including catalases and superoxide dismutases. Thus, even before viral infection is considered, the shift from sugar feeding to blood feeding imposes a strong ecological filter on the midgut microbiota (28).

3.2 Blood Meal-Induced Dysbiosis and Microbiota-DENV Modulation

Blood ingestion also changes the intestinal pH and, as a consequence, remodels the structure of the midgut through the formation and thickening of the peritrophic matrix. This matrix acts as a physical barrier between the luminal contents and the epithelial surface, limiting direct bacterial access to enterocytes and favoring microorganisms able to persist in the lumen or adhere efficiently to intestinal interfaces. At the same time, blood feeding activates immune pathways such as Toll, IMD, and JAK-STAT, leading to the production of antimicrobial peptides (AMPs) (29). These immune effectors impose selective pressure on the bacterial community, eliminating more susceptible taxa while allowing the persistence or expansion of bacteria with resistance, tolerance, or immune-evasion traits.

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Together, nutrient enrichment, oxidative stress, pH variation, epithelial remodeling, and immune activation can induce transient changes in the microbiota, including shifts in diversity or the relative abundance of stress-adapted taxa, depending on blood-meal source and experimental conditions and these local intestinal changes are accompanied by systemic immune responses. Blood feeding can increase the number of hemocytes and alter their size and granularity, reflecting the activation of cellular immunity (29,30,31). It may also transiently compromise the integrity of the midgut basal lamina, including damage to collagen IV, which can facilitate viral dissemination before tissue repair is complete (32). These changes may reshape the spatial and functional relationships among the microbiota, the intestinal epithelium, and DENV, ultimately influencing the likelihood that the virus will establish infection, escape the midgut barrier, and disseminate through the hemolymph.

The relationship between the microbiota and DENV involves both direct and indirect mechanisms. A functional gut microbiota contributes to the maintenance of basal immune activity and may contribute to immune priming; these concepts should be distinguished because persistent basal signaling does not necessarily imply an induced or memory-like primed state. Ramirez (2012) showed that reducing the bacterial load with antibiotics increases mosquito susceptibility to DENV, highlighting the role of the microbiota in limiting vector competence (33). The reintroduction of specific bacterial taxa can reduce viral replication, suggesting that selected members of the microbiota may act as biological modulators of infection and this effect, depends on bacterial colonization capacity, interaction with host tissues, and metabolic profile (33,34). This helps explain why some taxa restrict viral replication, whereas others have little effect or may even favor infection. In addition to environmental acquisition, microbial persistence may be shaped by transstadial transmission, allowing bacteria acquired during larval development to survive into adulthood if they pass through the metamorphic bottleneck (35,36). Vertical transmission may also allow selected bacteria to colonize eggs and persist across generations, favoring microorganisms capable of crossing tissue barriers and maintaining stable associations with the host (32). A critical implication of this functional heterogeneity

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is that the mosquito microbiota should not be viewed as uniformly antiviral. Rather, the same microbial community may restrict or facilitate DENV infection depending on its nutritional, metabolic, and taxonomic context. Harrison et al. (2023) showed that axenic *Ae. aegypti* adults had reduced vector competence for DENV-2, whereas vitamin B supplementation or recolonization with vitamin B–prototrophic bacteria restored viral permissiveness to levels comparable to those observed in conventionally colonized mosquitoes. These findings indicate that some members of the microbiota may enhance DENV transmission not by directly increasing the virus itself, but by supplying metabolic cofactors that support mosquito physiology and the host-cell metabolic activity required for efficient viral replication (37).

In this ecological context, Wolbachia pipientis represents a key modulator of the mosquito internal environment. This obligate intracellular bacterium colonizes mosquito cells, including reproductive tissues, and is vertically transmitted through cytoplasmic inheritance (38,39,40). Wild *Ae. aegypti* mosquitoes do not naturally harbor *Wolbachia*, instead, its establishment in modern vector populations results from laboratory introgression, initially achieved through embryonic microinjection of strains such as wMel from *Drosophila melanogaster*, and once stably introduced, Wolbachia spreads through mosquito populations by cytoplasmic incompatibility, which gives infected females a reproductive advantage (41,42,43). Unlike environmentally acquired bacteria, Wolbachia is not transmitted through feeding or direct contact, since it does not survive outside host cells (44). Its presence has been associated with changes in microbial diversity and ecological interactions among bacterial taxa in some experimental or field contexts, in addition to metabolic and immune modulation; these effects should not be assumed to be uniform across mosquito populations. These effects help explain the pathogen-blocking phenotype that supports current population replacement strategies aimed at reducing the transmission of DENV and other arboviruses **(Figure 1)** (44,45,46).

3.3 Reported Serotype-Associated Microbiome Patterns and Vector Competence

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Vector competence in *Ae. aegypti* should not be treated as an intrinsic, fixed property of a DENV serotype, but instead, each serotype may interact with the mosquito internal environment in a distinct way, producing different ecological and physiological outcomes in the midgut, therefore, serotype-associated microbiome patterns should be described as findings reported under specific experimental conditions. Reported outcomes of DENV-2 for example infection has often been associated with high transmission potential in experimental settings, a pattern that may be favored by a more pronounced virus-induced dysbiosis (17). Studies involving DENV1-4 may show differences in microbial composition, diversity, or vector competence, but these observations do not by themselves establish universal profiles for each serotype (25). These observations may suggest that DENV-associated microbiome changes arise from multiple biological and experimental influences acting simultaneously. Accordingly, community-level measures, including beta diversity, may provide a broader description of structural changes than isolated taxonomic shifts. Any serotype-associated interpretation should therefore be considered in relation to the metric used, the baseline microbiome, and the experimental context, rather than interpreted as a consistent ecological pattern across studies. The interpretations discussed above require caution as the available evidence remains fragmented, and current studies differ substantially in mosquito population origin, viral strain, infectious dose, blood-meal source, laboratory rearing conditions, sequencing platform, taxonomic resolution, and post-infection sampling time, for this reason, the microbial signatures attributed to DENV-1 through DENV-4 should not be treated as definitive or universally reproducible profiles, they are better understood as preliminary observations derived from specific experimental conditions. Divergent results among studies may reflect true biological variation, but they may also arise from methodological heterogeneity and limited standardization in microbiota analysis. Therefore, these data should be considered hypothesis-generating rather than conclusive. With these limitations in mind, the table below summarizes the main serotype-associated patterns discussed in this section.

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Table 1. Reported serotype-associated microbiome patterns regarding DENV midgut microbiome variation

Serotypes	Impact on intestinal microbiota	Potential functional implications	Taxonomic Important Signatures
DENV-1	Relative increase of proteobacteria; partial maintenance of alpha diversity	Possible modification of the intestinal environment associated with viral establishment; causal effects remain unresolved	Proteobacteria Firmicutes
DENV-2	Marked dysbiosis with increase in Enterobacteriaceae and reduction of *Lactobacillus-related spp*.; reduced alpha diversity	Potential changes in taxa previously associated with antiviral or permissive intestinal states	Enterobacteriaceae *Lactobacillus* and phylogenetically related species
DENV-3	Higher proportion of facultative anaerobes; less reduction of general bacterial groups	Potential modification of immune/metabolic interactions within the midgut, but direct effects on transmission remain uncertain	Facultative anaerobic consortia
DENV-4	Greater microbial stability after infection; rapid recovery approximately 96 h post-infection in laboratory studies	Suggests a distinct ecological response to infection	Basal microbial core

Source: Authors' synthesis based on the available experimental and review evidence (17, 25, 33, 34, 37).

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4. Microbiota as a modulator of vector competence and the molecular mechanisms involved

The continuous presence of symbiotic bacteria in the *Ae. aegypti* midgut helps maintain a persistent basal activation of the mosquito innate immune system, a physiological state commonly described as immune priming (17,47). This homeostatic communication is mediated by host pattern-recognition receptors (PRRs), which constantly survey the epithelial interface for microbe-associated molecular patterns (MAMPs), especially peptidoglycans and lipopolysaccharides (18). Recognition of these microbial signatures activates conserved signaling cascades. The Toll pathway responds mainly to Gram-positive bacteria, whereas the immune deficiency pathway (IMD) is primarily activated by Gram-negative bacteria and viral agents. Together with the JAK/STAT axis, these pathways promote the transcription of antimicrobial peptides (AMPs), including cecropins and defensins, as well as the localized production of reactive oxygen species (ROS) through the epithelial dual oxidase system (DUOX) (18,17). Recent studies also point to the involvement of non-canonical immune mechanisms, such as the increased expression of ATP-binding cassette (ABC) transporters in the midgut epithelium. These transporters contribute to active detoxification and defense against both local and systemic bacterial stressors, helping to maintain a restrictive microenvironment that may limit early arboviral replication (47,48). These processes are also important for intestinal stem cell (ISC) proliferation and for preserving epithelial barrier integrity. Blood ingestion, however, disrupts this balance. The sudden influx of proteins, together with the thermal and oxidative stress associated with blood digestion, induces a rapid but transient dysbiosis.

This post-blood-meal shift reshapes gut bacterial communities in a manner that depends strongly on the vertebrate host source (22). It also changes competition for lipids and essential metabolites and may alter microbial nutrient-synthesis pathways, sometimes providing biochemical substrates that support viral replication. The intestinal ecosystem is shaped by intense interspecific competition. Commensal bacteria may compete with invading viral particles for spatial niches, cell-surface receptors, and

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intracellular lipid pools, including cholesterol. This microbial blocking effect can limit viral fusion with endosomes and reduce the initiation of genome amplification (17). However, bacterial–viral interactions are not always restrictive. Some taxa can instead favor infection, as observed for species of Serratia. Opportunistic strains of Serratia marcescens secrete SmEnhancin, a metalloprotease capable of cleaving mucin networks in the peritrophic matrix. By weakening this mechanical barrier, SmEnhancin may increase the probability that virions reach the enterocytes and establish infection in the midgut (17,49,50). In contrast to these luminal interactions, the introgression of Wolbachia pipientis reshapes the mosquito internal environment from within host cells. Wolbachia competes for glucose and lipid precursors required for both embryogenesis and viral envelope formation, while also reprogramming mosquito cellular energy metabolism, as discussed above (9). Recent 16S rRNA amplicon sequencing studies in wild mosquito populations suggest that Wolbachia may also be associated with reduced midgut alpha diversity in some field contexts. This restructuring of the microbial network, combined with immune and metabolic modulation, may contribute to the pathogen-blocking phenotype that supports current population-based biocontrol strategies (44).

The *Ae. aegypti* midgut therefore acts as a dynamic ecological interface, where the balance between immune homeostasis and microbial dysbiosis can determine whether arboviral infection succeeds or fails. Understanding this host–microbiome–virus network is essential for improving targeted biocontrol strategies, but it represents only the baseline of vector competence. In hyperendemic natural settings, this crowded and highly contested niche is rarely exposed to a single pathogen in isolation. The same mechanisms that shape microbe–virus interactions, intracellular resource competition, immune activation, and physical microbial blocking, may become even more complex when multiple viral agents invade the midgut simultaneously or sequentially. Under these conditions, the ecological framework shifts from a simple host–pathogen interaction to a coinfection scenario in which different arboviruses and DENV serotypes must compete not only with the resident microbiota, but also with one another.

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5. Midgut microbiota and co-infection dynamics among DENV serotypes

Coinfection involving different viral serotypes or distinct arboviruses has become increasingly relevant to viral transmission dynamics, particularly in settings where multiple viruses share the same vector and ecological niche (51,52,53). This process may involve either simultaneous infection or sequential exposure to different viral genomes, including distinct DENV serotypes and other arboviruses such as chikungunya virus (CHIKV) and Zika virus (ZIKV). In both cases, interactions among viruses can influence viral replication, host responses, and vector competence (54,55,56). Within the *Ae. aegypti* midgut, the coexistence of multiple viruses creates favorable conditions for direct and indirect pathogen interactions mediated by cellular factors, these interactions may be synergistic or antagonistic, affecting viral load and transmission efficiency. Therefore, coinfection has become an important element for understanding the epidemiology and biology of arboviral diseases in hyperendemic settings. Variable rates of coinfection have been reported in several regions, including Brazil, Africa, and Asia, sometimes reaching significant levels in tropical areas where different viruses overlap in space and time. In addition, documented cases include simultaneous infections involving multiple DENV serotypes, as well as combinations of flaviviruses and alphaviruses (51,55,57). However, some settings show low coinfection frequencies, suggesting that biological, immunological, and ecological mechanisms may limit these interactions (52,58).

Inside the vector, acquisition of multiple viruses can occur through simultaneous coinfection during a single blood meal or through superinfection, when the mosquito feeds sequentially on different infected hosts. The latter is considered more common under natural conditions (54,59). These routes of exposure can produce different effects on viral dynamics within the midgut, which is the main site where the pathogen, host tissues, and microbiota interact (60). In simultaneous coinfections, several studies have shown that viral replication may occur independently or even synergistically. Infection rates can be similar to those observed in single infections and, in some cases, one or more of the viruses involved may reach higher viral loads (55,57). For example, experimental evidence indicates that DENV-2 and CHIKV coinfection can promote mutual facilitation

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of replication in the midgut, possibly through changes in metabolic pathways and cellular stress responses that improve viral persistence (57).

Coexposure to multiple arboviruses may lead to comparable or increased viral loads in mosquito saliva, raising the possibility of simultaneous transmission. By contrast, superinfection tends to produce stronger antagonistic effects. Compared with infection by a single serotype, sequential infection can markedly alter the balance of the gut microbiome and the interaction between the virus and the host (54,59). In this context, the primary infection may establish a cellular and immune environment that is less favorable for the entry or replication of a second virus. As a result, vector competence may be reduced, secondary viral replication may be suppressed, and transmission may even be completely blocked (59,60). These effects have been associated with the activation of antiviral responses in the mosquito, including the RNA interference pathway, increased expression of effector genes such as defensins and cecropins, and metabolic changes involving the synthesis of lipids required for viral replication.

Because the midgut contains both the resident microbiota and the main defensive barriers of the vector, these changes suggest that superinfection reorganizes the intestinal ecological niche. This reorganization can directly affect microbial composition and the availability of resources for each virus (60). The microbiota may indirectly influence coinfection dynamics by shaping the physiological and immune environment of the mosquito; however, direct evidence that the microbiota mediates superinfection exclusion remains limited. Superinfection exclusion refers to the ability of a previously established virus to prevent or limit infection by a second virus through mechanisms such as competition for cellular resources, receptor availability, inhibitory factors, or host immune responses. In alphaviruses, for instance, nonstructural proteins such as nsP2 play an important role in inhibiting the formation of new replication complexes for secondary viruses. In addition, the gut microbiota may act as an indirect modulator of this process by shaping basal mosquito immunity and contributing to either permissive or restrictive environments for viral replication (52,54). Nevertheless, competitive exclusion is not universal. Its outcome depends strongly on the infection context, including the order

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of exposure, the interval between infections, and the biological characteristics of the viruses involved (60,61). In simultaneous coinfections, the absence of strong exclusion may reflect infection of different cell populations within the midgut or early limitations in the number of infected cells, which would reduce direct competition (61). In infections involving multiple DENV serotypes, intracellular competition may influence viral replication and transmission dynamics, although the underlying mechanisms remain incompletely understood (60). Moreover, under some conditions, synergistic interactions may occur, increasing transmissibility and shortening the extrinsic incubation period. Taken together, these findings highlight the complexity of interactions among coinfecting viruses (57,58). Overall, coinfection and superinfection by multiple arboviruses should be viewed as dynamic processes capable of reshaping the *Ae. aegypti* gut microbiome, with outcomes ranging from synergism to antagonism depending on the ecological and biological context (54,57).

6. Concluding Remarks, Methodological Limitations, and Gaps in the Literature

Contemporary DENV research relies on a diverse set of experimental models, ranging from immortalized mosquito cell lines to laboratory-reared vectors and human clinical samples. These approaches have been essential for clarifying the basic virology of arboviral infections. However, they may also produce simplified views of the host–pathogen–microbiome interface, this limitation is especially relevant for studies based on *Aedes albopictus* C6/36 and *Ae. aegypti* Aag2 cell lines. Although these systems are highly tractable, they do not reproduce the three-dimensional architecture, physiological compartmentalization, epithelial polarity, microvillar organization, or peritrophic matrix of the midgut in a living mosquito (62,63,64). Importantly, these limitations are not merely structural, decades of sequential laboratory passaging have subjected insect cell lines to genetic and functional changes. For example, the widely used C6/36 cell line carries a frameshift mutation in the *Dicer-2* gene, compromising the exogenous RNA interference pathway, one of the main antiviral defenses in mosquitoes (65,66,67). As a result, viral

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replication kinetics and immune responses observed *in vitro* may not fully reflect those occurring in wild-type vectors. Similar caution is needed when extrapolating findings from laboratory mosquito colonies. These mosquitoes are usually maintained in standardized water, controlled diets, and stable insectary conditions. Such environments tend to generate cohorts with more homogeneous and less diverse microbiomes than wild *Ae. aegypti* populations, which develop in detritus-rich aquatic habitats (68,69). Because continuous exposure to environmental and symbiotic bacteria contributes to basal immune priming of pathways such as Toll and IMD, laboratory-reared mosquitoes may exhibit immune states that differ substantially from those observed in field populations (22).

6.1 Evidence baseline, ecological context, and study heterogeneity

As described by LaReau et al., understanding the interactions between DENV and the *Ae. aegypti* midgut microbiome requires consideration of the ecological, physiological, and experimental conditions preceding viral infection. Microbiota composition can naturally vary among mosquito populations and as a function of factors such as larval environment, adult age, diet, blood-meal host, *Wolbachia* status, and antibiotic exposure. In addition, methodological factors, including sample pooling strategies and variation among experimental batches, may contribute to differences in microbial communities that are not necessarily attributable to DENV serotype. Therefore, characterization of the pre-infection microbiome is essential for distinguishing alterations specifically associated with viral infection from pre-existing microbiological variability and in this context, studies using comparable pre-infection microbiomes and blood-fed, mock-infected controls provide stronger evidence for associations between DENV infection and microbiome alterations. Furthermore, because DENV and the microbiota may influence each other bidirectionally, cross-sectional analyses performed exclusively after infection are generally insufficient to establish a causal relationship between the observed alterations and viral infection (70).

6.2 Viral Isolates, Blood-Meal Protocols, and Environmental Realism

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A second major source of methodological heterogeneity lies in the viral isolates and infection systems used in vector competence studies. DENV strains that have been extensively passaged and adapted under laboratory conditions may accumulate adaptive mutations, particularly in genes encoding envelope glycoproteins. These changes can optimize replication in cell culture, while potentially altering receptor tropism and transmission-relevant phenotypes (71). In addition, many experimental designs rely on infection with a single serotype or a single pathogen delivered through artificial membrane feeders. Although this strategy improves reproducibility, it does not fully capture the epidemiological complexity of hyperendemic regions, where mosquitoes may encounter conditions that favor intracellular competition, metabolic interference, and superinfection exclusion (72,73).

The artificial blood meal itself also represents an important biological simplification. In standardized assays, mosquitoes are often fed defibrinated or mechanically processed blood supplemented with cultured viruses. Natural transmission, however, occurs when *Ae. aegypti* ingests blood from a viremic human host. This blood meal contains active host-derived components, including leukocytes, complement factors, acute-phase proteins, and inflammatory mediators, which may remain biologically active in the mosquito midgut during the early stages of infection (22,23). Moreover, dengue transmission often occurs during the febrile phase of human disease. In this context, the ingestion of warmer blood may induce heat-shock responses in the mosquito epithelium and transiently affect microbial population dynamics. These variables are further shaped by climate. Laboratory protocols usually maintain infected mosquitoes under constant temperature and humidity, whereas field populations experience daily thermal fluctuations and heterogeneous microclimates. As a result, vector competence estimates obtained under static conditions may not fully represent transmission dynamics in natural tropical and subtropical environments (58,66).

6.3 Microbiome Analysis and Functional Resolution Analytical resolution remains another major limitation in studies of DENV–microbiome interactions.

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The interpretation of DENV–microbiome interactions in *Aedes aegypti* requires careful consideration of the biological and methodological factors that may shape the mosquito gut microbiome before and during viral infection. Many studies to date have relied on 16S rRNA gene amplicon sequencing, which provides valuable taxonomic profiles of midgut bacterial communities but offers limited information about their functional activity (17). 16S-based approaches generally provide limited resolution of strain-level genetic variation and bacterial virulence factors and do not directly measure microbial gene expression or metabolic activity. Taxonomic detection alone should not be interpreted as evidence of a specific biological function. This distinction is particularly relevant because differences in microbiome composition may reflect variation in mosquito origin, rearing conditions, developmental history, or blood-meal exposure rather than direct effects of DENV infection (47,70).

16S rRNA gene sequencing data are also inherently compositional: an increase in the relative abundance of one taxon necessarily alters the relative proportions of other taxa, even when their absolute bacterial loads remain unchanged, therefore, apparent shifts in relative abundance should be interpreted cautiously and, whenever possible, complemented by measurements of total bacterial load and appropriate compositional-data analyses and comparisons across studies should also consider methodological differences, including the 16S rRNA region targeted, sequencing platform, taxonomic database, classification pipeline, and taxonomic resolution (74).

These limitations are particularly important when evaluating the potential functional consequences of microbiome changes during DENV infection. The mosquito midgut microbiome may influence viral infection through metabolic interactions, resource competition, modulation of epithelial immunity, and other host–microbe mechanisms (18,17,47). Potentially relevant processes include lipid metabolism, blood-meal-associated metabolic pathways, redox homeostasis, and microbial products capable of modulating host immune responses. However, the detection or enrichment of a bacterial taxon itself does not demonstrate that these mechanisms are active. Functional interpretations should therefore be supported by direct measurements whenever

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possible, particularly when linking specific microbial taxa or metabolic pathways to DENV replication or vector competence (74).

A more comprehensive understanding of these interactions will require the integration of taxonomic and functional approaches. Shotgun metagenomics can provide information on the genetic repertoire and functional potential of microbial communities, whereas metatranscriptomics can help characterize changes in microbial gene expression during DENV infection. Metabolomic approaches can further identify changes in microbial and host-derived metabolites and may help connect microbiome composition with specific biochemical processes and when combined with measurements of viral load, total bacterial abundance, and mosquito immune responses, these approaches could help distinguish taxonomic associations from functionally relevant mechanisms and clarify how microbial activity changes throughout the course of DENV infection (18,17).

Another important consideration is the difference between laboratory-adapted and environmentally acquired microbiomes. Recent experimental work demonstrated that *Ae. aegypti* mosquitoes colonized with bacteria from an environmental water source exhibited distinct midgut microbial diversity, composition, community turnover, and functional traits compared with conventionally insectary-reared mosquitoes. These differences also changed over the course of blood-meal digestion, highlighting the influence of microbiome origin and experimental conditions on the observed bacterial community (70). Thus, findings obtained exclusively from laboratory colonies may not fully represent the ecological and functional complexity of microbiomes encountered by mosquitoes in natural populations.

6.4 Future Approaches for Studying DENV–Microbiome–Vector Interactions

Addressing these limitations will require a gradual shift toward experimental systems that are both mechanistically precise and ecologically realistic. Next-generation in vitro and in vivo models, including three-dimensional midgut organoids derived from intestinal stem cells and vector-derived gut-on-a-chip microfluidic platforms, represent promising tools for reproducing the physical and biological properties of the mosquito gut. These

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systems could help simulate epithelial organization, oxygen gradients, peristalsis-like flow, microbial co-culture, and barriers associated with the peritrophic matrix, allowing more representative analyses of virus–microbiota–epithelium interactions (75,76,77). When combined with single-cell RNA sequencing and spatial transcriptomics, these platforms could reveal where DENV replication bottlenecks occur, which epithelial cell populations become infected, and how adjacent bacterial microcolonies influence local immune activation. Ultimately, the field would benefit from integrating these higher-resolution tools with field-collected vectors, natural microbiota consortia, contemporary clinical DENV isolates, and realistic climate variables. Such an approach would help narrow the current gap between controlled laboratory inference and the biology of transmission in the field (44,75,76,77).

By linking mechanistic experimentation with natural transmission conditions, future studies may better define how mosquito midgut microbiota and viral serotype variation interact to shape vector competence, thereby providing a stronger basis for the future development of microbiome engineering and paratransgenesis as prospective control strategies. The available evidence supports a role for the *Ae. aegypti* midgut microbiota in modulating DENV permissiveness through interacting immune, metabolic, and structural mechanisms, while DENV infection itself can also reshape the microbial community. However, current studies remain highly heterogeneous, and the available literature does not yet support universal or reproducible microbiome profiles for DENV-1, DENV-2, DENV-3, and DENV-4. Future studies should therefore prioritize environmental conditions, well-characterized isolates of all four serotypes, mock-infected blood-fed controls, multiple post-infection time points and integrated metagenomic and metabolomic approaches alongside standardized measurements of vector competence.

7. References

[1] WORLD HEALTH ORGANIZATION. **Dengue: global situation, surveillance and progress – 2024 update**.

[2] HARAPAN, H. et al. Dengue: A Minireview. **Viruses**, v. 12, n. 8, p. 829, 30 jul. 2020.

REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[3] ROY, S. K.; BHATTACHARJEE, S. Dengue virus: epidemiology, biology, and disease aetiology. **Canadian Journal of Microbiology**, v. 67, n. 10, p. 687–702, out. 2021.

[4] SIM, S.; JUPATANAKUL, N.; DIMOPOULOS, G. Mosquito Immunity against Arboviruses. **Viruses**, v. 6, n. 11, p. 4479–4504, 19 nov. 2014.

[5] SÁNCHEZ-VARGAS, I. et al. Dengue Virus Type 2 Infections of Aedes aegypti Are Modulated by the Mosquito's RNA Interference Pathway. **PLoS Pathogens**, v. 5, n. 2, p. e1000299, 13 fev. 2009.

[6] PIERSON, T. C.; DIAMOND, M. S. The continued threat of emerging flaviviruses. **Nature Microbiology**, v. 5, n. 6, p. 796–812, 4 maio 2020.

[7] MUSTAFA, M. S. et al. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. **Medical journal, Armed Forces India**, v. 71, n. 1, p. 67–70, jan. 2015.

[8] CHALA, B.; HAMDE, F. Emerging and Re-emerging Vector-Borne Infectious Diseases and the Challenges for Control: A Review. **Frontiers in Public Health**, v. 9, 5 out. 2021.

[9] DO NASCIMENTO, R. M. et al. The influence of culture-dependent native microbiota in Zika virus infection in Aedes aegypti. **Parasites & Vectors**, v. 15, n. 1, p. 57, 17 dez. 2022.

[10] ANDRADE, A. C. et al. Are Land Use and Cover Changes and Socioeconomic Factors Associated with the Occurrence of Dengue Fever? A Case Study in Minas Gerais State, Brazil. **Resources**, v. 13, n. 3, p. 38, 8 mar. 2024.

[11] KOLIMENAKIS, A. et al. The role of urbanisation in the spread of Aedes mosquitoes and the diseases they transmit—A systematic review. **PLOS Neglected Tropical Diseases**, v. 15, n. 9, p. e0009631, 9 set. 2021.

[12] SAFIEE, A. W. M. et al. Putative Pathogenic Genes of Leptospira interrogans and Leptospira weilii Isolated from Patients with Acute Febrile Illness. **Tropical Medicine and Infectious Disease**, v. 7, n. 10, p. 284, 5 out. 2022.

[13] BISWAL, S. et al. Efficacy of a tetravalent dengue vaccine in healthy children aged 4–16 years: a randomised, placebo-controlled, phase 3 trial. **The Lancet**, v. 395, n. 10234, p. 1423–1433, maio 2020.

[14] DENG, S.-Q. et al. A Review on Dengue Vaccine Development. **Vaccines**, v. 8, n. 1, p. 63, 2 fev. 2020.

CC BY

REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[15] WANG, J.; GAO, L.; AKSOY, S. Microbiota in disease-transmitting vectors. **Nature Reviews Microbiology**, v. 21, n. 9, p. 604–618, 22 set. 2023.

[16] YADAV, K. K. et al. Molecular characterization of midgut microbiota of Aedes albopictus and Aedes aegypti from Arunachal Pradesh, India. **Parasites & Vectors**, v. 8, n. 1, p. 641, 18 dez. 2015.

[17] WORKU, A. T. et al. Microbial gatekeepers: midgut bacteria in Aedes mosquitoes as modulators of arboviral transmission and targets for sustainable vector control. **Frontiers in Microbiology**, v. 16, 9 set. 2025.

[18] STRAND, M. R. Composition and functional roles of the gut microbiota in mosquitoes. **Current Opinion in Insect Science**, v. 28, p. 59–65, ago. 2018.

[19] SIRIYASATIEN, P. et al. Comparative analysis of midgut bacterial communities in Chikungunya virus-infected and non-infected Aedes aegypti Thai laboratory strain mosquitoes. **Scientific Reports**, v. 14, n. 1, p. 10814, 11 maio 2024.

[20] GURUPRASAD, N. M.; JALALI, S. K.; PUTTARAJU, H. P. Wolbachia-a foe for mosquitoes. **Asian Pacific Journal of Tropical Disease**, v. 4, n. 1, p. 78–81, fev. 2014.

[21] FRASER, J. E. et al. Novel phenotype of Wolbachia strain wPip in Aedes aegypti challenges assumptions on mechanisms of Wolbachia-mediated dengue virus inhibition. **PLOS Pathogens**, v. 16, n. 7, p. e1008410, 29 jul. 2020.

[22] MUTURI, E. J. et al. Host blood meal source has a strong impact on gut microbiota of Aedes aegypti. **FEMS Microbiology Ecology**, 24 out. 2018.

[23] SARMA, D. K. et al. Influence of Host Blood Meal Source on Gut Microbiota of Wild Caught Aedes aegypti, a Dominant Arboviral Disease Vector. **Microorganisms**, v. 10, n. 2, p. 332, 1 fev. 2022.

[24] LI, M. et al. Response of the mosquito immune system and symbiotic bacteria to pathogen infection. **Parasites & Vectors**, v. 17, n. 1, p. 69, 17 fev. 2024.

[25] PEDREAÑEZ, A. et al. Role of Gut Microbiota in Dengue. **Reviews in Medical Virology**, v. 34, n. 5, 30 set. 2024.

[26] FORREST, J. R. Complex responses of insect phenology to climate change. **Current Opinion in Insect Science**, v. 17, p. 49–54, out. 2016.

[27] COON, K. L. et al. Mosquitoes rely on their gut microbiota for development. **Molecular ecology**, v. 23, n. 11, p. 2727–39, jun. 2014.



REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[28] OLIVEIRA, J. H. M. et al. Blood Meal-Derived Heme Decreases ROS Levels in the Midgut of Aedes aegypti and Allows Proliferation of Intestinal Microbiota. **PLoS Pathogens**, v. 7, n. 3, p. e1001320, 17 mar. 2011.

[29] NEPOMUCENO, D. B. et al. pH control in the midgut of *Aedes aegypti* under different nutritional conditions. **Journal of Experimental Biology**, v. 220, n. 18, p. 3355–3362, 15 set. 2017.

[30] TALYULI, O. A. C. et al. The Aedes aegypti peritrophic matrix controls arbovirus vector competence through HPx1, a heme–induced peroxidase. **PLOS Pathogens**, v. 19, n. 2, p. e1011149, 13 fev. 2023.

[31] JOHNSON, R. M. et al. Increased blood meal size and feeding frequency compromise Aedes aegypti midgut integrity and enhance dengue virus dissemination. **PLOS Neglected Tropical Diseases**, v. 17, n. 11, p. e0011703, 1 nov. 2023.

[32] BRYANT, W. B.; MICHEL, K. Blood feeding induces hemocyte proliferation and activation in the African malaria mosquito, *Anopheles gambiae* Giles. **Journal of Experimental Biology**, 1 jan. 2013.

[33] RAMIREZ, J. L. et al. Reciprocal Tripartite Interactions between the Aedes aegypti Midgut Microbiota, Innate Immune System and Dengue Virus Influences Vector Competence. **PLoS Neglected Tropical Diseases**, v. 6, n. 3, p. e1561, 6 mar. 2012.

[34] RAMIREZ, J. L. et al. Chromobacterium Csp_P Reduces Malaria and Dengue Infection in Vector Mosquitoes and Has Entomopathogenic and In Vitro Anti-pathogen Activities. **PLoS Pathogens**, v. 10, n. 10, p. e1004398, 23 out. 2014.

[35] DICKSON, L. B. et al. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. **Science Advances**, v. 3, n. 8, 4 ago. 2017.

[36] WANG, Y. et al. Dynamic Gut Microbiome across Life History of the Malaria Mosquito Anopheles gambiae in Kenya. **PLoS ONE**, v. 6, n. 9, p. e24767, 21 set. 2011.

[37] HARRISON, R. E. et al. The mosquito Aedes aegypti requires a gut microbiota for normal fecundity, longevity and vector competence. **Communications Biology**, v. 6, n. 1, p. 1154, 13 nov. 2023.

[38] PINTO, S. B. et al. Effectiveness of Wolbachia-infected mosquito deployments in reducing the incidence of dengue and other Aedes-borne diseases in Niterói, Brazil: A quasi-experimental study. **PLOS Neglected Tropical Diseases**, v. 15, n. 7, p. e0009556, 12 jul. 2021.

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REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[39] WALKER, T. et al. The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. **Nature**, v. 476, n. 7361, p. 450–453, 24 ago. 2011.

[40] MOREIRA, L. A. et al. A Wolbachia Symbiont in Aedes aegypti Limits Infection with Dengue, Chikungunya, and Plasmodium. **Cell**, v. 139, n. 7, p. 1268–1278, dez. 2009.

[41] WERREN, J. H.; BALDO, L.; CLARK, M. E. Wolbachia: master manipulators of invertebrate biology. **Nature Reviews Microbiology**, v. 6, n. 10, p. 741–751, out. 2008.

[42] KAUR, R. et al. The mechanism of cytoplasmic incompatibility is conserved in Wolbachia-infected Aedes aegypti mosquitoes deployed for arbovirus control. **PLOS Biology**, v. 22, n. 3, p. e3002573, 28 mar. 2024.

[43] BOURTZIS, K. et al. Wolbachia Infection and Cytoplasmic Incompatibility in Drosophila Species. **Genetics**, v. 144, n. 3, p. 1063–1073, 1 nov. 1996.

[44] CORRÊA-ANTÔNIO, J. et al. Midgut microbiota diversity and interactions shift when field Aedes aegypti mosquitoes carry the wMel strain of Wolbachia pipientis. **Communications Biology**, v. 8, n. 1, p. 1479, 17 out. 2025.

[45] MUSHTAQ, I. et al. Wolbachia's Secret Weapons: Decoding Metabolic Hijacking, Immune Pathways, and miRNAs of Aedes aegypti to Combat Dengue Virus. Em: [s.l: s.n.].

[46] MOLLOY, J. C. et al. Wolbachia Modulates Lipid Metabolism in Aedes albopictus Mosquito Cells. **Applied and Environmental Microbiology**, v. 82, n. 10, p. 3109–3120, 15 maio 2016.

[47] KUMAR, V. et al. Delineating the Role of Aedes aegypti ABC Transporter Gene Family during Mosquito Development and Arboviral Infection via Transcriptome Analyses. **Pathogens**, v. 10, n. 9, p. 1127, 2 set. 2021.

[48] BARIAMI, V. et al. Gene Amplification, ABC Transporters and Cytochrome P450s: Unraveling the Molecular Basis of Pyrethroid Resistance in the Dengue Vector, Aedes aegypti. **PLoS Neglected Tropical Diseases**, v. 6, n. 6, p. e1692, 12 jun. 2012.

[49] APTE-DESHPANDE, A. D. et al. Serratia odorifera mediated enhancement in susceptibility of Aedes aegypti for chikungunya virus. **The Indian journal of medical research**, v. 139, n. 5, p. 762–8, maio 2014.

[50] HEU, K. et al. The Effect of Secondary Metabolites Produced by Serratia marcescens on Aedes aegypti and Its Microbiota. **Frontiers in microbiology**, v. 12, p. 645701, 2021.

CC BY

REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[51] DOS SANTOS S. MARINHO, R. et al. Detection of coinfection with Chikungunya virus and Dengue virus serotype 2 in serum samples of patients in State of Tocantins, Brazil. **Journal of Infection and Public Health**, v. 13, n. 5, p. 724–729, maio 2020.

[52] NODARI, J. Z. et al. Genomic and epidemiologic investigation of arboviral coinfections, Espírito Santo, Brazil. **American Journal of Infection Control**, v. 53, n. 11, p. 1177–1182, nov. 2025.

[53] FRITZELL, C. et al. Current challenges and implications for dengue, chikungunya and Zika seroprevalence studies worldwide: A scoping review. **PLOS Neglected Tropical Diseases**, v. 12, n. 7, p. e0006533, 16 jul. 2018.

[54] OLIVEIRA, M. E. DOS S. P. DE et al. Evaluation of the Impact of Coinfection and Superinfection on Chikungunya and Mayaro Viruses' Replication in Aedes aegypti. **Microorganisms**, v. 13, n. 9, p. 2165, 17 set. 2025.

[55] RÜCKERT, C. et al. Impact of simultaneous exposure to arboviruses on infection and transmission by Aedes aegypti mosquitoes. **Nature Communications**, v. 8, n. 1, p. 15412, 19 maio 2017.

[56] SIRISENA, P. D. N. N. et al. Concurrent dengue infections: Epidemiology & clinical implications. **Indian Journal of Medical Research**, v. 154, n. 5, p. 669–679, nov. 2021.

[57] LE COUPANEC, A. et al. Co-Infection of Mosquitoes with Chikungunya and Dengue Viruses Reveals Modulation of the Replication of Both Viruses in Midguts and Salivary Glands of Aedes aegypti Mosquitoes. **International Journal of Molecular Sciences**, v. 18, n. 8, p. 1708, 4 ago. 2017.

[58] POTEREK, M. L. et al. Interactions between seasonal temperature variation and temporal synchrony drive increased arbovirus co-infection incidence. **Royal Society Open Science**, v. 9, n. 10, 19 out. 2022.

[59] VOGELS, C. B. F. et al. Arbovirus coinfection and co-transmission: A neglected public health concern? **PLOS Biology**, v. 17, n. 1, p. e3000130, 22 jan. 2019.

[60] PENG, J. et al. Biased virus transmission following sequential coinfection of Aedes aegypti with dengue and Zika viruses. **PLOS Neglected Tropical Diseases**, v. 18, n. 4, p. e0012053, 1 abr. 2024.

[61] VISSER, T. M. et al. Effect of chikungunya, Mayaro and Una virus coinfection on vector competence of Aedes aegypti mosquitoes. **One Health**, v. 20, p. 100991, jun. 2025.

CC BY

REMUNOM
ISSN 2178-6925

Received: 19/08/2026 - **Accepted:** 01/10/2026
Vol: 13.21
DOI: 10.66104/nppkms17
Pages: 1-30

[62] AMUZU, H. E.; MCGRAW, E. A. Wolbachia-Based Dengue Virus Inhibition Is Not Tissue-Specific in Aedes aegypti. **PLOS Neglected Tropical Diseases**, v. 10, n. 11, p. e0005145, 17 nov. 2016.

[63] LU, P. et al. Wolbachia Inhibits Binding of Dengue and Zika Viruses to Mosquito Cells. **Frontiers in Microbiology**, v. 11, 4 ago. 2020.

[64] KIM, J.; KOO, B.-K.; YOON, K.-J. Modeling Host-Virus Interactions in Viral Infectious Diseases Using Stem-Cell-Derived Systems and CRISPR/Cas9 Technology. **Viruses**, v. 11, n. 2, p. 124, 30 jan. 2019.

[65] FRANZ, A. et al. Tissue Barriers to Arbovirus Infection in Mosquitoes. **Viruses**, v. 7, n. 7, p. 3741–3767, 8 jul. 2015.

[66] KRAMER, L. D.; CIOTA, A. T. Dissecting vectorial capacity for mosquito-borne viruses. **Current Opinion in Virology**, v. 15, p. 112–118, dez. 2015.

[67] FIGUEIREDO, L. T. M. Uso de células de Aedes albopictus C6/36 na propagação e classificação de arbovírus das famílias Togaviridae, Flaviviridae, Bunyaviridae e Rhabdoviridae. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 23, n. 1, p. 13–18, mar. 1990.

[68] SAVVIDOU, E. C. et al. Optimizing Aedes albopictus rearing: effects of insect- and bacteria-based larval diets on immature and adult performance. **Scientific Reports**, v. 15, n. 1, p. 21516, 1 jul. 2025.

[69] WIJEGUNAWARDANA, N. D. A. D. et al. Optimization of Aedes albopictus rearing procedures for combined sterile insect techniques (SIT) and Wolbachia-based laboratory studies in Sri Lanka. **International Journal of Tropical Insect Science**, v. 40, n. 4, p. 801–807, 13 dez. 2020.

[70] LAREAU, J. C. et al. Introducing an environmental microbiome to axenic *Aedes aegypti* mosquitoes documents bacterial responses to a blood meal. **Applied and Environmental Microbiology**, v. 89, n. 12, 21 dez. 2023.

[71] WEAVER, S. C.; VASILAKIS, N. Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. **Infection, Genetics and Evolution**, v. 9, n. 4, p. 523–540, jul. 2009.

[72] BHATT, P. et al. Current Understanding of the Pathogenesis of Dengue Virus Infection. **Current Microbiology**, v. 78, n. 1, p. 17–32, 24 jan. 2021.

[73] PEPIN, K. M.; LAMBETH, K.; HANLEY, K. A. Asymmetric competitive suppression between strains of dengue virus. **BMC Microbiology**, v. 8, n. 1, p. 28, 8 dez. 2008.



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[74] KNIGHT, R. et al. Best practices for analysing microbiomes. **Nature Reviews Microbiology**, v. 16, n. 7, p. 410–422, 23 jul. 2018.

[75] RADDI, G. et al. Mosquito cellular immunity at single-cell resolution. **Science**, v. 369, n. 6507, p. 1128–1132, 28 ago. 2020.

[76] CANSADO-UTRILLA, C. et al. The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. **Microbiome**, v. 9, n. 1, p. 111, 18 dez. 2021.

[77] LIU, T. et al. Intestinal organoid modeling: bridging the gap from experimental model to clinical translation. **Frontiers in Oncology**, v. 14, 1 mar. 2024.

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