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REVIEW

An Overview on the Impact of Microbiota on Malaria Transmission and Severity: *Plasmodium*–Vector–Host Axis

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Abstract

Purpose Malaria, which is a vector-borne disease caused by *Plasmodium* sp., continue to become a serious threat, causing more than 600,000 deaths annually, especially in developing countries. Due to the lack of a long-term, and effective vaccine, and an increasing resistance to antimalarials, new strategies are needed for prevention and treatment of malaria. Recently, the impact of microbiota on development and transmission of *Plasmodium*, and the severity of malaria has only begun to emerge, although its contribution to homeostasis and a wide variety of disorders is well-understood. Further evidence has shown that microbiota of both mosquito and human host play important roles in transmission, progression, and clearance of *Plasmodium* infection. Furthermore, *Plasmodium* can cause significant alterations in the host and mosquito gut microbiota, affecting the clinical outcome of malaria.

Methodology In this review, we attempt to summarize results from published studies on the influence of the host microbiota on the outcome of *Plasmodium* infections in both arthropods and mammalian hosts.

Conclusion Modifications of microbiota may be an important potential strategy in blocking *Plasmodium* transmission in vectors and in the diagnosis, treatment, and prevention of malaria in humans in the future.

Keywords Parasite–microbiota interactions · Malaria · *Plasmodium* · *Anopheles* · Microbiota

Introduction

Vector-borne diseases (VBD) are a major cause of morbidity and mortality worldwide especially in the tropical and subtropical regions. In fact, according to a World Health Organization (WHO) report, approximately 80% of the world's population is at risk of one or more vector-borne disease. Annually, over 700, 000 deaths are reported from VBDs, such as malaria, leishmaniasis, chagas disease, yellow fever, human African trypanosomiasis, dengue, Japanese encephalitis, and onchocerciasis [1]. Due to global warming, there has been increased distribution of vectors to regions that were previously inhabitable, thus increasing the risk of VBDs. This has increased a sense of urgency in the research and public health communities to develop novel effective vector control strategies to prevent a potential future vector-borne disease outbreak. One of vector control strategy is the symbiotic control, which employs the identification of suitable microbes that can spread among vector populations. Through paratransgenesis, these microbes are bioengineered to reduce or induce the fecundity and vector competence of insects [2–4]. Paratransgenic microbes can also be applied in development of transmission-blocking strategies, thus reducing disease transmissions while maintaining disease-free arthropods that maybe important in ecosystem food chains [5]. Identification of best candidates for paratransgenesis requires better understanding of composition, diversity, function, and role of host microbiota in vector–parasite interactions.

Many protozoan parasites that cause important human VBDs are heteroxenous i.e., during one stage of their lifecycle they live in the vertebrates and during other stages they live in the gut of bloodsucking insects. Therefore, there is a

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high possibility of interactions between parasite and microbiota of the host. Microbiota refers to the collective microorganisms (bacteria, archaea, viruses, fungi, and protozoans) that inhabit a host, while microbiome describes the entire genomic elements of microbiota [6]. Microbiota of important VBD vectors such as mosquitoes, sand fly, tsetse fly, triatomines and ticks have been previously well reviewed [3, 7–9]. Microbiota influence vector's innate immunity, reproduction, feeding and behavior, ultimately affecting vectorial capacity of various arthropod vectors. Furthermore, microbiota is also significant in determining the pathogenicity and virulence of parasites such as *Plasmodium* sp., *Leishmania* sp., and *Trypanosoma cruzi* in mammalian hosts [10–12]. However, the mechanisms of interactions between parasite and microbiota, which play a role in the development and transmission of infections, are not fully clear.

To understand the effects and possible mechanisms of parasite-microbiota interactions on the development and transmission of malaria, we reviewed published articles on *Plasmodium* and microbiota interactions in both mammalian and mosquito hosts.

Vector-Borne Parasite and Microbiota Interactions

Humans and arthropods are colonized with a variety of bacteria, fungi, viruses, and protozoans that play important role in homeostatic balance of the host. Now, it has been revealed that the formation and severity of diseases are associated with microbial diversity in humans, as well as the roles of microbiota in food digestion, vitamin production and regulation of the immune system [13]. Several studies have reported the influence of gut microbiota in vector competence of mosquito, tsetse fly, and sand fly [14–16]. Regarding *Anopheles* mosquitoes, presence of some bacterial species increases the chances of *Plasmodium* to develop into transmissible stages while others are associated with negative growth of parasites in arthropod hosts [17–19].

Mosquitoes, sand flies, tsetse flies and triatomines have different biology, behavior, and ecologies, therefore, they have different core microbiota. Due to this microbial community, the interactions among microbiota, parasite, and host systems can significantly vary. However, outcomes of parasite- microbiota interactions can be summarized into three types: (i) perturbation of gut microbiota composition reduce\increases the survival of parasite and severity of disease in host; (ii) parasite infection reshapes microbial composition in the host; and (iii) microbiota and parasite influence on host's metabolism and immune system (Table 1). These outcomes can result from competition in the use of resources, production of antimicrobial and antiparasitic metabolites, and modulation of host's immune system by both the microbiota and parasite (Fig. 1).

Microbiota Analyzes

Accurate identification of host microbiota is the basis for investigating possible symbiont candidates for paratransgenesis and mechanisms involved in parasite-microbiota interaction. Currently culture-dependent and\or culture-independent techniques have been used in host microbiota studies [20, 21]. Although culture-dependent techniques are widely applied in microbial studies, they are limited to identification of culturable microbes. Consequently, various culture-independent methods such as gene sequencing have been developed for identification of all microorganisms. Recently, with the further development of sequencing technologies and data analysis methods, the composition and functions of the microbiota has been successfully revealed [21–23].

The most widely used method in microbiome analysis is marker gene (16 s rRNA) sequencing [20, 23]. Sequencing of the hypervariable regions of the 16 s rRNA gene, which is found in all bacteria and is highly conserved, enables the identification of bacterial species and taxonomic prediction [21, 24]. However, this technique can cause inaccuracies in species-level classification and only analyzes for bacterial species, ignoring other members of the community [20–22].

Another method, whole metagenome sequencing, examines unbiasedly at the full genomic content of the microbiota, rather than at a single gene in bacteria [22, 23], and functionally evaluates the genetic contribution of each member of the community [23, 25]. For this, shotgun metagenomic sequencing, in which long DNA molecules are randomly fragmented and then sequenced, is the most suitable strategy [23]. It allows for the identification of the full genome as well as a more accurate of species-level classification, and detection of less abundant taxa [22, 23]. It can also reveal the metabolic potential of these communities by providing information about functional genes [22, 26].

Plasmodium sp.–Microbiota Interactions

Plasmodium spp. are apicomplexan protozoan parasites that are transmitted by anopheline mosquitoes, causing malaria. WHO estimates that there are 219 million cases of malaria globally and more than 400, 000 deaths from the disease reported every year. Severity of malaria infection is dependent on many factors including *Plasmodium* species, age, genetic factors, nutrition status, immune status, duration of exposure, and history of previous exposure [27, 28]. In addition, recently, the role of composition of human gut microbiota in severity of malaria has been investigated.

Table 1 The interactions *of Plasmodium* and microbiota in mammalian/arthropod host

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. berghei	*Serratia marcescens*	*An.sinensis* (China)[b]	Midgut	16S rRNA, culturing	*An. stephensi*	Presence of the bacteria activated the host immune system	[49]
P.falciparum, P. berghei	*Penicillium chrysogenum*	*Anopheles* mosquitoes (Puerto Rico)[b]	Midgut	ITS r18S DNA, culturing	*An. gambiae*	Presence of the bacteria compromised the host immune defenses	[56]
P. berghei	*Wickerhamomyces anomalus*	*An. Stephensi* (Italy)[b]	Midgut	Culturing	*In vitro*	The fungus showed *in vitro* strong anti-plasmodial effect	[41]
P. yoelii	Unidentified host microbiota	*An. Dirus* (China)[a]	Midgut	16SrDNA	*An. dirus*	Microbiota was associated with up regulation of expression of TEP1 in the host	[53]
P. falciparum	*Escherichia coli* (strains 351 & 444)	*An. Gambiae* (Cameroon)[b]	Midgut	Culturing, 16S rRNA	*An. gambiae*	Both bacteria strains reduced parasite infection	[58]
P. falciparum, P. berghei	*S. aureus, E. coli, Enterobacter cloacae*	*An. Gambiae* (UK)[a]	Midgut	Culturing, 16S rDNA	*An. gambiae*	Presence of the bacteria was associated with activation of PGN Recognition Protein LC (PGRP-LC)	[59]
P. berghei	*Asaia* (SF2.1 strain)	*An. Stephensi* (Italy)[a]	Midgut	Culturing, 16S rRNA	*An. stephensi*	Presence of the bacteria up-regulated CLT4 and TEP1 production in the host	(55]
P. berghei	*Asaia* (SF2.1 strain)	*An. Stephensi* (Italy)[a]	Midgut	Culturing, 16S rRNA	*An. gambiae*	Presence of the bacteria did not activate TEP1 in the host	[55]
P. vivax	*En. amnigenus*2, *En. cloacae, S. marcescens*	*An. Albimanus* (South Mexico) [b]	Midgut	Culturing	*An. albimanus*	Presence of these bacteria reduced parasite infection	[35]
P. yoelii, P. chabaudi, P. berghei	*Lactobacillus, Bifidobacterium*	Mice (USA)[a]	Small intestine, cecum, colon	16S rRNA	mice	Presence of the bacteria was associated with decreased parasitemia and severity of malaria	[31]
P. falciparum	*Serratia* strains (AS1 strain)	Lab strain	Ovaries	Culturing, 16S rRNA	*An. gambiae*	Produce anti-*Plasmodium* effector molecules	[60]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. berghei, *P. falciparum*	*Chryseobacterium, Asaia, Elizabethkingia, Cedecea, Enterobacter, Ewingella, Serratia, Aeromonas, Comamonas, Burkholderia,*	*An. Gambiae* (UK)[a]	Midgut	16S rDNA	*An. gambiae*	Antibiotic treatment increases mosquito survival and fecundity, but reduced resistant to parasite infection	[46]
P. falciparum	*Pseudomonas cepacia, Enterobacter agglomerans, Flavobacterium*	*An. gambiae, An. stephensi, An albimanus* (USA)[a]	Midgut	Culturing	*An. stephensi, An. Gambiae An. albimanus*	Presence of the bacteria reduced parasite infection in the host	[61]
P. falciparum	*Chromobacterium* sp. (isolate Csp_P)	*Aedes aegypti* (USA)[a]	Midguts	Culturing, 16 s rDNA	*An. gambiae*	Presence of the bacteria strain inhibited of *P. falciparum* infections	[39]
P. falciparum	*Sphingomonas, Brevundimonas, Ensifer, Ramlibacter, Bergeyella, Bacillus, Corynebacterium, Microbacterium, Escherichia- Shigella*, *Asaia*, *Delftia*, *Gluconacetobacter*, *Cedecea*, *Enterobacter, Burkholderia*, *Acinetobacter*, *Elizabethkingia*, *Streptococcus*, *Micrococcus, Aeromonas*, *Pseudomonas*, *Staphylococcus, Bacteroides*, *Chryseobacterium*, *Devesio*, *Rhyzobium*, *Citrobacter*, *Nocardioides*, *Brevibacterium*, *Comamonas, Ensifer*, *Intrasporangiceae-Y, Serratia*, *Methylobacterium*, *Sphingobium, Ralstonia*, *Propionibacterium*	*Anopheles* mosquitoes (Cameroon)[b]	Salivary glands, ovaries, midgut	16S rDNA	*An. gambiae, An. coluzzi*	Increase abundance of *Serratia* sp., and *Methylobacteria* sp. in *P. falciparum* infected mosquitoes	[57]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
avian *Plasmodium*	*Microbacterium, Renibacterium, Odoribacter, Paraprevotella, Pedobacter, Exiguobacterium, Streptococcus, Anaerococcus, Blautia, Dorea, Oscillospira, Dialister, Phascolarctobacterium, Paracoccus, Ralstonia, Micrococcus, Succinivibrio, Corynebacteria, Propionibacterium, Butyricimonas, Bacteroides, Prevotella, Staphylococcus, Lachnospira, Faecalibacterium, Ruminococcus, Methylobacterium, Sphingomonas, Sutterella, Cupriavidus, Bilophila, Acinetobacter, Pseudomonas, Agrobacterium, Enhydrobacter*	*Culex pipiens* (Spain)[a]	Midgut	16S rRNA	*Culex pipiens*	Antibiotic treatment increased prevalence of *Plasmodium* in saliva of *Plasmodium*-infected mosquitoes. Antibiotic treatment increased survival of *Plasmodium*-infected mosquitoes compared to controls	[62]
P. berghei, *P. falciparum*	*E. cloacae*	*An. Stephensi* (USA)[a]	Midgut	Culturing	*An. stephensi*	*E. cloacae* strongly induced A*s*SRPN6 expression which downregulated *P. falciparum* development	[38]
P. falciparum	*Pseudomonas putida, S. marcescens, Pantoea, Comamonas, Acinetobacter, P. rhodesiae, Elizabethkingia anophelis*	*An. arabiensis* (Zambia)[b] *An. gambiae* (USA)[a]	Midgut	Culturing	*An. gambiae*	*P. putida*, *Pantoea* sp., and *S. marcescens* had strong inhibition of *P. falciparum* infection in mosquitoes. *Pantoea* sp., *P. rhodesiae*, and *S. marcescens* had strong *in vitro* inhibition of *P. falciparum* development	[17]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. falciparum	*Knoellia, Acinetobacter, Bacillus, Pseudomonas, Exiguobacterium, Kocuria, Pantoea, Pseudomonas, Staphylococcus, Arthrobacter, Comamonas, Enterobacter* bacterium *Esp_Z*	*An. Arabiensis* (Zambia)[b]	Midgut	Culturing, 16S rRNA	*An. gambiae*	Microbiota presence inhibited *Plasmodium* sporogonic development via Reactive oxygen species (ROS) mechanism	[19]
P. berghei	*Asaia bogorensi*	*An. Stephensi* (China)[a]	Midgut	Culturing, 16S rRNA	*An. stephensi*	Presence of the bacteria was associated with changes in glucose metabolism and increases in pH for enhanced *Plasmodium* development and infection	[63]
P. vinckei petteri	Unidentified host microbiota	*An. Stephensi* (India)[a]	Midgut	Culturing	*An. stephensi*	Antibiotic treatment increased mosquito susceptibility to *Plasmodium* infection	[45]
P. yoelii, *P. berghei*	Unidentified host microbiota	Mice (USA)	Cecum	N/A	Mice	Microbiota enhanced germinal centre-associated immune cells and *Plasmodium*-specific antibody. Microbiota maintained a long *Plasmodium* resistance in the host	[64]
P. berghei	*E. coli* O86:B7	Stockpile	Gut	N/A	Mice	Enhanced production of protective anti-α-gal Abs that target *Plasmodium* sporozoites	[29]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. berghei	Unidentified host microbiota	*An. Stephensi* (China)[a]	Midgut	Culturing	*An. stephensi*	Antibiotic treatment of the mosquitoes resulted to no activation of PGRP-LA that participates in antiparasitic immune defenses. Gut microbiota stimulated synthesis of peritrophic matrix which inhibits *Plasmodium* development	[48]
P. berghei, *P. falciparum*	Unidentified host microbiota	*An. Gambiae* (USA)[a]	Midgut	16S rRNA	*An. gambiae*	Presence of midgut microbiota reduced *Plasmodium* survival upon re-infection to similar *Plasmodium* sp.	[65]
P. berghei	*Asaia*, *Cedecea, Elizabethkingia*, *Gibbsiella*, *Klebsiella*, *Rahnella*, Serratia, *Chryseobacterium*, Enterobacter, *Ewingella*, Aeromonas, *Comamonas*, *Burkholderia*,	*An. coluzzii*, *An. arabiensi*, *An. Quadriannulatus* (UK)[a]	Midgut	16S rRNA	*An. coluzzii*, *An. arabiensi*, *An. quadriannulatus*	In *An quadriannulatus* mosquitoes there was a higher tolerance to gut microbiota post bloodmeal consequently lower tolerance to *Plasmodium* infection	[66]
P. falciparum	*Enterobacter asburiae*, *Microbacterium*, *Sphingomonas*, *Serratia*, *Chryseobacterium meningosepticum, Asaia bogorensis, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Herbaspirillum, Pantoea agglomerans, Pseudomonas fluorescens, Pseudomonas straminea, Phytobacter diazotrophicus, Serratia marcescens*	An. Gambiae (Italy)[a]	Midgut	Culturing, 16S rDNA	*An. gambiae*	Antibiotic-treated mosquitoes had a greater number of oocysts compared to untreated mosquitoes upon blood feeding. Indirect microbiota anti-*plasmodium* activity was reported	[44]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. berghei	*Asaia* SF2.1 strain	An. Stephensi (Lab strain)[a]	Midgut	Culturing	*An. stephensi*	Paratransgenic Asaia strains reduced the prevalence of P. berghei in An. stephensi females	[40]
P. berghei	*Wickerhamomyces anomalus* (WaF17.12 strain)	*An. Stephensi* (Lab strain) [a]	Midgut	Culturing	*An. stephensi*	*in vitro* anti-sporogonic action of WaF17.12. *in vivo* WaF17.12 interferes with ookinete development in mosquito gut	[43]
P. falciparum	*Asaia*, *Burkholderia*, *Serratia*, *Ralstonia*, *Acinetobacter*, *Pseudomonas*, *Sphingomonas*, *Staphylococcus*, *Streptococcus*, and *Escherichia/Shigella* generas	*An. Gambiae* (Cameroon)[a]	Midgut	16S rDNA	*An. gambiae*	A positive correlation between relative abundance of *Enterobacteriaceae* in the midgut and *Plasmodium* infection	[18]
P. falciparum	*Delftia, Comamonas, Acinetobacter, Acinetobacter septicus, Aeromonas* sp., *Aeromonas hydrophila, Aeromonas veronii, Aeromonas caviae, Pseudomonas stutzeri, Pseudomonas stutzeri, Pseudomonas Mendocina, Enterobacter cloacae subsp, Enterobacter, Klebsiella., Klebsiella, Salmonella, Escherichia coli, Escherichia-Shigella, Shigella flexneri, Rahnella aquatilis, Serratia marcescens, Serratia*	*An. Gambiae* (Cameroon)[a]	Midgut	Culturing, 16S rRNA	*An. gambiae*	*Escherichia coli*, *Serratia marcescens*, *Pseudomonas stutzeri*, *Enterobacter spp.*, *Acinetobacter septicus*, *Comamonas spp.* and *Bacillus pumilus* had significant impact on oocyst numbers and infection prevalence	[34]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. falciparum, P. yoelii	*Pseudomonas aeruginosa*, *Ps. Vesicularis, Cedecea lapagei*	*An. Gambiae* (Namibia)[a]	Midgut	Culturing	*An. gambiae*	Mosquitoes fed on immunized infected sera had high number *P. falciparum* oocysts. Feeding on immunized sera had no effect on oocyst development in *P. yoelii*	[67]
P. berghei	Unidentified host microbiota	*An. Gambiae* (USA)[a]	Midgut	Culturing	*An. gambiae*	In presence of gut microbiota mosquitoes produced high levels of prostaglandin upon feeding on *P. berghei*-infected blood compared to antibiotic treated mosquitoes	[68]
P. falciparum	*Acinetobacter, Baciullus pumilus, Bacillus, Enterobacter, Pseudomonas putida, Bacillus cereus, Exiguobacterium mexicanum, Kocuria turanensis, Pantoea, Pseudomonas rhodesiae, Staphylococcus, Arthrobacter, Comamonas, Knoellia*	*An. Arabiensis* (Zambia)[b]	Midgut	16S rDNA	*An. gambiae, An. stephensi*	*Enterobacter sp.* (*Esp_Z*) bacterium inhibited ookinete, oocyst, and sporozoite development of Plasmodium via generation of reactive oxygen species (ROS)	[36]
P. berghei	*Massilia, Herbaspirillum, Moraxella, Acetobacter, Brevundimonas, Gluconobacter, Enterobacter, Pseudomonas, Asaia, Acinetobacter*	*An. Stephensi* (China)[a]	Midgut	Culturing, 16S rRNA	*An. stephensi*	Presence of gut microbes is essential to maintain the structural integrity of the PM that influences *Plasmodium* infection. Recolonization of gut with *Enterobacter sp*. led to reduced susceptibility to *Plasmodium* infection in antibiotic treated mosquitoes	[47]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. falciparum	*Ewingella americana, Seratia marscens, E.coli* H243, *E.coli* HB101, *Pseudomonas aeruginos, S. auerus, S.epidermis*	*An. stephensi,* culture stock (USA)[a]	Midgut	Culturing	*An. stephensi*	Concomitant infections of mosquitoes with gram negative bacteria had significant reduction in oocyst numbers	[69]
P. falciparum	*Bifidobacterium, Streptococcus, Prevotella, Clostridiaceae, Erysipelotrichaceae, Frimucutes, Turicibacter, Ruminococcus, Dialister, Collinsella, Enterobacteriaceae, Ruminococcaceae, Bacteroides, Lachnospiraceae, Faecalibacterium*	Human (Mali)	Stool sample	16S rRNA	Human	Significant association between gut microbiota and prospective risk of *Plasmodium* infection	[10]
P. yoelii	*Bacteroides, Provetella, Turicibacter, Stomobaculum, Romboustia, Alistipes, Butyricimonas, Lactobacillus, Parasutterella, Akkermasia, Parabacteriodes, Blautia, Flavonifractor,*	Mice	Fecal pellets	16S rRNA	Mice	Shift in gut microbiota composition caused significant alterations in the severity of malaria in mice	[30]
P. berghei	*Wolbachia* wAlbB strain	*Ae. Albopictus* (USA)[a]	Midguts, salivary glands, fat bodies, ovaries	RNA	*An. stephensi*	Wolbachia wAlbB infection reduced oocyst numbers and induced anti-plasmodium immune genes in moquitoes	[42]
P. berghei	*E. coli*	Lab strain		Culturing, 16S rRNA	*An. stephensi*	Recombinant *E. coli* strains produced anti-plasmodium peptides	[70]
P. falciparum	*Wolbachia* wMelPop and wAlbB strain	*Anopheles* cells (USA)[a]	Fat body, head, sensory organs, other tissues excluding ovaries, midgut	DNA or RNA	*An. gambiae*	*P. falciparum* oocyst development was significantly reduced in the presence of bacteria strains	[71]

Table 1 (continued)

Plasmodium sp.	Microbiota	Microbiota source	Samples	Methods of microbiota characterization	Host type	Parasite-microbiota interactions outcomes	References
P. falciparum	*Wolbachia* wAlbB strain	*An. Albopictus* (USA)[a]	Ovaries, fat bodies, midguts, salivary glands	16S rDNA	*An. stephensi*	Reduction of malaria parasites in *Wolbachia* wAlbB infected mosquitoes	[37]
P. berghei	Unidentified host microbiota	*An. Gambiae* (USA)[a]	Whole body	16 s rRNA	*An. gambiae*	Microbiota presence did not influence uric acid metabolism upon *Plasmodium* infection	[72]

P Plasmodium, *An* Anopheles
[a]Colony-reared
[b]Field-caught

Yooseph *et al.* [10] performed a microbial analysis in the stool of subjects with lower and higher risk of *P. falciparum* infection and reported distinct microbiota profiles. After taking into account age and other potential co-features, the authors reported that higher proportion of *Bifidobacterium, Streptococcus, Enterobacteriaceae, Escherichia,* and *Shigell*a in lower risk subjects compared to those at higher risk of *P. falciparum* infection. These results suggest possibility of modulation of gut microbiota through probiotics to supplement microbes such as *Bifidobacterium* to reduce severity of *P. falciparum* infection. Interestingly, Yilmaz *et al.* [29] reported that anti-α-gal antibodies produced against human gut *E. coli* 0.86: B7 expressed α-gal protected humans from malaria transmission. In addition, infection of α1,3GT-deficient mice with *E. coli* 0.86: B7 induced production of anti- α-gal antibodies that targeted *Plasmodium* sporozoites inoculated by *Anopheles* mosquitoes [29]. In the future it will be interesting to investigate the association of this bacteria's α-gal expression with risk of *P. falciparum* infection in humans.

Changes in gut bacteria significantly affects severity of malaria in mammalian hosts. Mandal *et al.* [30] observed varying severity of *P. yoelii* infection in mice obtained from the same commercial vendor at different shipping times. Mice shipped in 2016 were able to clear *P. yoelii* infection faster than mice shipped in 2017, which had high malaria severity. Indeed, microbiota analysis of fecal samples from these two groups of mice showed a difference in bacteria abundance and composition. This result suggested that there was shift in microbiota in the microbiota of mice grown in the same environment over different time periods, thus, leading to a significant change in malaria severity. This result was validated by transferring fecal samples from both groups of mice to germ-free mice. Germ-free mice colonized with cecal contents from 2017 mice showed higher numbers of the parasite than those from 2016 mice [30]. Therefore, there are some difficulties in reproducing outcomes in experimental mouse models, even when using genetically identical mice ordered from the same production suite and vendor. In addition, it has been previously reported that mice from different vendors have different gut microbiota composition, thus showing significant varying levels of pathology upon *Plasmodium* infection [31]. Therefore, researchers should consider the impact of shift in gut microbiota during set up experiments and interpretation of outcomes of murine disease model systems. Particularly, researchers should include the vendor and shipping details of mice used in experiment at the time the results of publication to enable experimental reproducibility by other researchers/labs.

Mosquito first acquires its microbiota via trans-stadial transmission from the mother, and from breeding sites of its larva and pupa. Later, adult mosquitoes encounter microbes during feeding or mating. Specially, blood meal induces

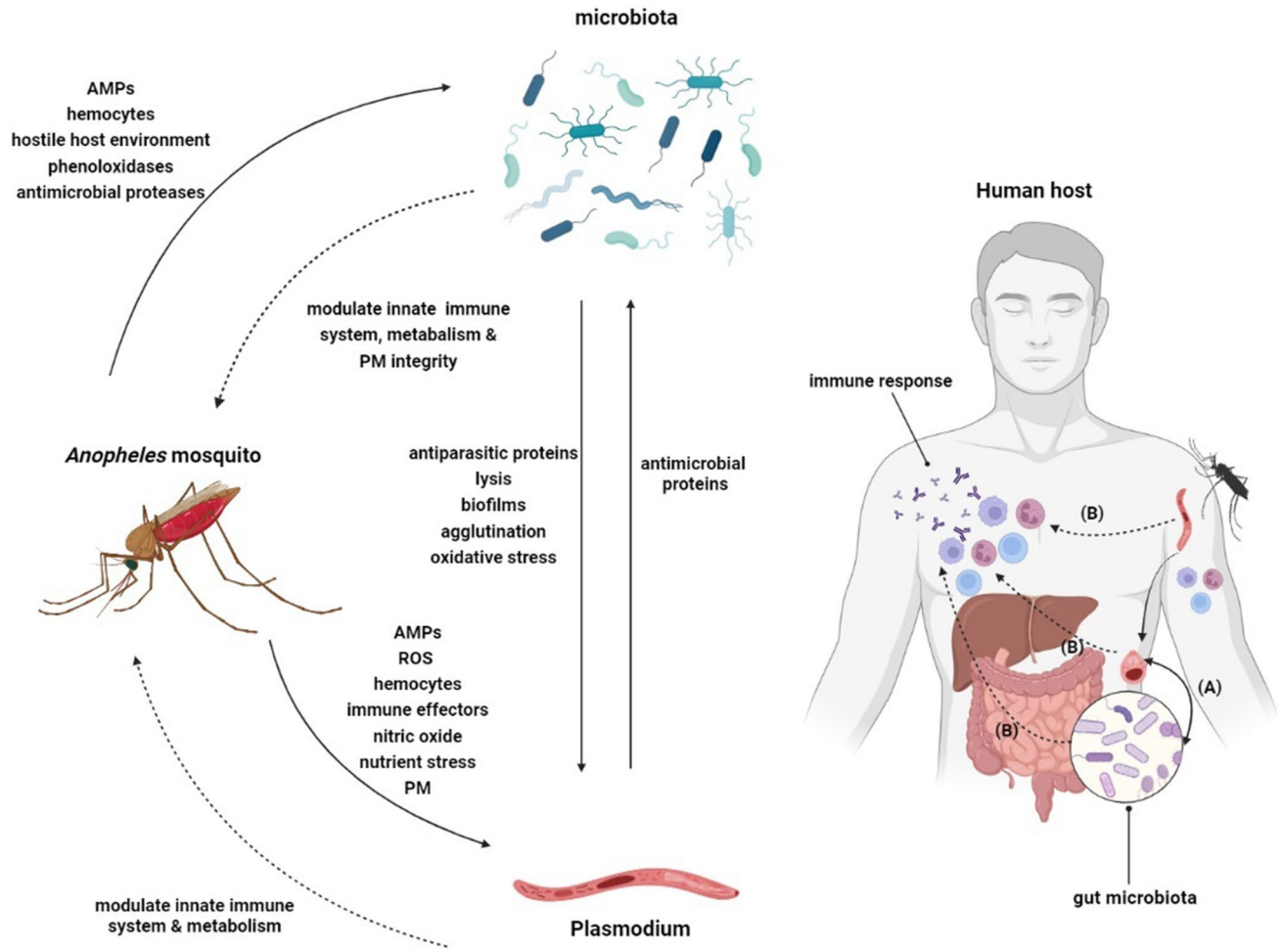

Fig. 1 Mechanisms involved in host microbiota and *Plasmodium* interactions. Microbiota and parasite can directly influence each other, independent of the host, via mechanisms that they induce individually (**A**). Furthermore, microbiota and parasite can indirectly influence each other via host modulated mechanisms such as immune response and metabolism (**B**). (Created with BioRender.com)

changes in gut microbiota community dynamics in mosquitoes [32, 33]. The shift in gut microbiota composition of the mosquito has profound effect on *Plasmodium* infection. For instance, introduction of *P. putida*, *Pantoea* sp., and *S. marcescens* resulted in strong inhibition of *P. falciparum* infection in *An. gambiae* [17]. A negative impact on numbers of *P. falciparum* oocyst has also been reported when infected *An. gambiae* mosquitoes were challenged by bacteria, such as *Escherichia coli*, *Serratia marcescens*, *Pseudomonas stutzeri*, *Enterobacter* spp., *Acinetobacter septicus*, *Comamonas* spp., and *Bacillus pumilus* [34]. *E. coli*, *S. marcescens,* and *P. stutzeri* had the highest effect on oocyst intensity. A study reported that *S. marcescens* increased mortality of *P. vivax* oocyst 13 times more than other bacteria in *An. albimanus* [35]. These studies highlight the significance of screening specific bacteria isolated from mosquitoes for anti-*Plasmodium* effects. They can be the basis of identifying bacteria candidates for vector control and transmission-blocking strategies. For instance, studies have shown that presence of *Enterobacter* bacterium renders mosquitoes 99% resistance to *P. falciparum* infection, it was then further investigated to determine specific anti-*Plasmodium* mechanism employed by the bacteria. The results show that bacteria inhibit the parasite infection via generation of reactive oxygen species (ROS) [36]. Other gut bacteria that have been investigated for inhibition of *Plasmodium* sp. infection in anopheles mosquitoes include *Wolbachia* (wAlb strain), *Wickerhamomyces anomalus* (WaF17.12 strain), paratransgenic *Asaia, E. coli* (351 and 444 strains), *E. cloacae* and *Chromobacterium* sp. (isolate Csp-P), [37–43]. Suggested mechanisms and outcomes of *Plasmodium*-microbiota interactions have been summarized in Table 1.

Experimental elimination of gut microbiota via antibiotic treatment have led to mosquitoes that are highly susceptible to *Plasmodium* spp. infection [44–48]. Antibiotic-treated mosquitoes are deprived of gut bacteria that play important

role in antiparasitic activity in the insect. For instance, Gao *et al.* [48] reported that antibiotic treatment of mosquitoes inhibited the activation of Peptidoglycan Recognition Proteins LA (PGRP-LA), which is involved in antiparasitic defenses, and that gut microbiota played important role in the formation and integrity of the peritrophic membrane (PM), which inhibits *Plasmodium* development [48]. Song *et al.* [47] also showed that when the treatment of *An. stephensi* with antibiotics led to increased vector competence. Recolonization of the gut with *Enterobacter* sp. restored PM integrity and reduced susceptibility of the mosquitoes to *P. berghei* infection [47]. These two studies suggest that gut microbiota can also influence parasite establishment in the vector via PM formation and integrity. So, it will be interesting to research in detail, which mechanisms and specific microorganisms are involved in the interaction between gut microbiota and PM for development of transmission-blocking strategies.

Microbiota-mediated parasite control may be a consequence of microbiota influence on mosquito's immune system and metabolism. Various transcriptomic analysis studies have shown activation of immune system in mosquito challenged by both parasite and microorganisms. When *An. stephensi* females were challenged with *Serratia* Y1 strain bacterium isolated from *An. sinensis*, an increase in the expression of some immunogenic genes such as C-type lectins, CLIP serine proteases, thioester-containing protein 1 (TEP1), fibrinogen immunolectin (FBN9), and leocine rich repeat protein (LRRD7), was detected [49]. These immune effectors have anti-*plasmodium* effect, as they rendered mosquitoes colonized with *Serratia* Y1 resistant to *P. berghei* infection. In addition, other immune effector molecules, such as anti-microbial peptide cecropin (CEC1), defensin (DEF1), and C-type lectin 4 (CTL4) have been reported to be involved in reducing *Plasmodium* infection in mosquitoes [50–54].

Although adaptive immunity has not been reported in insects there is a fair amount of specific immune response in mosquitoes. For instance, challenge of both *An. stephensi and An. gambiae* with *Asaia* bacterium showed upregulation of TEP1 in *An. stephensi* and not the latter [55]. Interestingly, there was a high reduction in *Plasmodium* replication in *An. stephensi* compared to *An. gambiae*. On the other hand, Bai *et al.* [49] also reported that *Serratia* J1 strain did not activate immune response in *An. stephensi* in contrast to *Serratia* Y1 strain. Notably, specific immune response in mosquitoes could be related to the ability of the bacteria to colonize the mosquito's gut with dominant symbionts showing quick and consistent activation of host's immune system [55]. Certainly, further studies are needed to confirm these hypotheses. However, it is also important to note that not all microorganisms enhance the immune response of mosquitoes to *Plasmodium* infection. For instance, Angleró-Rodríguez *et al.* [56] reported that the presence of *Penicillium chrysogenum* suppressed the immune response of *An. gambiae* rendering the mosquitoes susceptible to *Plasmodium* infections. According to the authors, the fungi secreted a heat-stable secondary metabolite that compromised mosquito defense mechanisms. Also, ingestion of *Pe. chrysogenum* resulted in upregulation of ornithine decarboxylase (ODC) gene, which limits the availability of L-arginine important for nitric oxide (NO) production [56]. Ultimately, specific microorganism-mosquito combinations need further research to identify "good" and "bad" symbionts in relation to *Plasmodium* infections. Identification of symbionts that enable parasite infections in mosquitoes may offer new opportunities to control transmission cycle via elimination of these symbionts.

In addition to feeding, the gut microbiota composition and diversity of mosquito are shaped by *Plasmodium*. Parasite infection may change the ecology of the gut via production of antimicrobials, competition for nutrients, and modification of the host physiology. As a result, some symbionts are eliminated while others gain dominance. Boissie`re *et al.* [18] reported a positive correlation between *P. falciparum* infection status and the prevalence of *Enterobacteriaceae* [18]. In addition, a study of dynamics of bacterial community in field-caught mosquitoes reported high abundance of *Serratia* and *Methylobacterium* in flies infected with *P. falciparum* compared to non-infected ones [57]. These studies suggest that the presence of certain bacteria is required for parasite development and transmission. However, within the scope of these *Plasmodium*-microorganisms cooperation, it remains unclear to decipher, which organism enables the existence of the other possible and the mechanisms involved.

Conclusion

Parasite-microbiota interactions are crucial in development, transmission, and severity of malaria. Inside the vector, the parasite must not only suppress the gut digestives enzymes and immune response, but also compete with the microbiota and protect themselves from antimicrobial effects of their metabolites. In addition, human microbiota plays a critical role in pathogenicity and virulence of the infecting parasite. Both microbiota and parasite can modulate the human immunity to control or exacerbate parasitic infections. Moreover, microbiota via production of antimicrobial metabolites, agglutination, modulation of nutrients and osmotic conditions, and formation of biofilms on parasite surfaces can kill or inhibit parasite growth. However, it remains unclear which exact mechanisms are directly employed by parasites to modulate microbiota composition and diversity. Further studies to determine the microbiota in vectors with high and low transmission capacity for malaria may provide new

data for the development of molecules that target parasite-supporting bacteria and inhibit parasite-transmission in the vector. Moreover, investigation of microbiota in human with malaria may provide data for the development of new diagnostic, therapeutic and prevention strategies for plasmodium infections.

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Declarations

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