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Malaria and the Microbiome: A Systematic Review

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Background. The microbiome influences malaria parasite fitness and transmission efficiency in mosquitoes and appears to affect malaria dynamics in mammalian hosts as well. Nascent research examining the interrelationship of malaria and the mammalian microbiome has yielded interesting insights inviting further study.

Methods. We conducted a systematic review of the literature examining associations between the microbiome and malaria in mammalian hosts. An electronic search algorithm was adapted to PubMed, MEDLINE, Scopus, Embase, and Web of Science, and reference lists of relevant sources were manually searched. Identified studies were screened and assessed independently by 2 authors, and results were compiled in a qualitative synthesis of the evidence.

Results. Ten relevant studies were identified. They demonstrate associations between certain intestinal communities and protection against *Plasmodium* infection and modulation of disease severity. *Plasmodium* infection acutely and reversibly reshapes gut microbial composition in mice. The makeup of human skin microbial communities may influence mosquito attraction and thus disease transmission.

Conclusions. Early research supports a relationship between malaria and the microbiome. The evidence is incomplete, but the observed associations are evocative and signal a promising avenue of inquiry. Microbiome-based studies of malaria can be readily integrated into field-based research.

Keywords. malaria; *Plasmodium*; microbiome; systematic review.

The malaria parasite *Plasmodium* has coevolved with its insect and vertebrate hosts over millennia. It has exerted a Darwinian influence on populations within which it shape-shifts through a complex lifecycle and propagates with superb efficiency. It imposed itself indelibly on the human genome [1–5], but how it may interact with our "second genome"—the microbiome—remains largely unexplored.

Malaria persists as the leading parasitic cause of death worldwide. Its burden is felt foremost in sub-Saharan Africa where it takes the lives of >1200 children each day [6]. The predominant species is also the most lethal; within hours of the first sign of infection, *Plasmodium falciparum* can progress to fatal cerebral edema, profound anemia, and organ failure. In regions of high malaria endemicity, subclinical infections in partially immune individuals contribute to sustained transmission. Study of malaria's pathophysiology is often performed in animal models; rodent *Plasmodium* spp. exhibit similar disease phenotypes, and their laboratory use has led to important preclinical discoveries.

A microbiota is a community of microorganisms (bacteria, viruses, fungi, eukaryotes, and archaea) common to an environmental or corporeal niche, and the term microbiome subsumes the microbiota and its collective genetic information. The human microbiome encompasses diverse ecosystems of individual microbial communities that inhabit different body areas (eg, intestine, skin) and differ in species composition. Advances in genomic sequencing and biocomputational analysis have propelled culture-independent investigations of the microbiome and disease, and these early endeavors have yielded novel insights [7]. Human and animal studies have demonstrated associations with enteric infections, diabetes mellitus, cardiovascular disease, colorectal cancer, reactive airway disease, and mood disorders [8–13]. Within the field of malaria research, a robust body of literature already describes the influence of the mosquito microbiome on malaria. Mosquito midgut microbes appear to impact parasite fitness and transmission efficiency [14–17], and the influence is modifiable [18], wherefore practicable vector-control interventions seem possible.

Less well described is the interrelationship between malaria and the mammalian microbiome. Early work suggests bidirectional associations that relate to disease phenotype, infection risk, and intestinal dysbiosis (disruptions of gut microbe communities) [19]. Here we present what is, to the best of our knowledge, the first systematic review of the literature examining the malaria-microbiome interface in mammalian hosts, with the intention of informing future inquiries, especially field-based, in this emerging area of malaria research.

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METHODS

Eligibility Criteria

All studies, published and unpublished in any language at any time, were considered for inclusion. Eligible studies were those that examined interactions of malaria (any *Plasmodium* spp. or vector mosquito) and the microbiome (eg, intestinal, skin) in mammalian hosts. Outcomes included any aspect of malaria transmission, disease, or immunity and characterization of the host microbiome using molecular-genetic methods and/or culture.

Search Strategy

The review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses Statement [20]. We systematically searched PubMed (including MEDLINE), Scopus, Embase, and Web of Science from database inception to 3 May 2017. The electronic search algorithm consisted of terms relating to key concepts of "malaria" and "microbiome" (Supplementary Table 1). Reference lists of included articles, related reviews, and other relevant sources were manually searched. The study protocol was registered in the PROSPERO database of systematic reviews (registration no. CRD42017075670).

Eligibility Assessment and Data Extraction

Two authors (M. I. and J. D.) independently screened titles and abstracts and performed full-text assessments. Two other authors (N. S. and C. L.) resolved discrepancies. M. I. and J. D. independently extracted characteristics of each eligible study: study design, research objectives, setting (laboratory, field), experimental and control subjects/conditions, host type (and source, for animal subjects), *Plasmodium* spp., vector species (for transmission studies), microbial community (eg, intestinal, skin), method of characterization of the microbiome, phylotype and/or other relevant features of the microbiome, and study findings.

Data Synthesis

Results of included studies were organized in a qualitative synthesis according to the general direction of association—the influence of malaria on the microbiome and the influence of the microbiome on malaria—with subsections derived from the availability of study findings.

Risk-of-Bias Assessment

Risk of bias was assessed using the Systematic Review Center for Laboratory Animal Experimentation risk of bias tool for animal studies [21] and the Newcastle-Ottawa Scale for human cohort studies [22].

RESULTS

We identified and assessed 4177 records, of which 10 were eligible for inclusion (Figure 1). Studies examined an array of outcomes: incidence of *Plasmodium* infection, severity of disease, immune response, variations in gut microbial communities between infected and noninfected hosts, and mosquito attraction (Table 1). Most were laboratory-based experiments in mouse models of malaria. Two studies included embedded experiments using human specimens, one of which characterized the human intestinal microbiome; the other assessed antibodies implicated in microbiome-mediated protection against *P. falciparum* infection. Risk of bias was generally low or unclear (Table 2, Supplementary Table 2). One study of malaria and the intestinal microbiome had potential risk of attrition bias due to data exclusion [23], and a study of the skin microbiota and mosquito attraction had risks of selection bias due to nonrandomized experimental design and misclassification bias due to possible contamination [24].

The qualitative synthesis that follows is organized into 4 sections. The first section examines evidence of the influence of *Plasmodium* infection on the host microbiota, the next 2 review the evidence for effects of the microbiome on malaria immunopathophysiology, and the fourth presents findings related to the role of skin microbiota in vector mosquito attraction (Table 3, Figure 2).

Plasmodium Infection and Alterations to the Intestinal Microbiota

Mooney et al showed that infection with *Plasmodium yoelii* in Swiss Weber and C57BL/6 (B6) mice was associated with a transient dysbiosis not observed in control mice inoculated with uninfected blood [25]. Analysis of 16S rRNA amplicon sequences from fecal pellets of infected mice demonstrated a reduction in the Firmicutes/Bacteroidetes ratio and a reduction in Proteobacteria abundance 10 days after infection, which reverted to baseline by 30 days. These mice were also found to be more susceptible to gut colonization by the Proteobacteria *Escherichia coli* and nontyphoidal *Salmonella* spp., a finding theorized by the authors to relate to the clinical observation of increased prevalence of disseminated *Salmonella* in children with malaria [25]. In a separate group of experiments by Taniguchi and colleagues, *Plasmodium berghei* infection reduced Firmicutes in both B6 and BALB/c and increased Proteobacteria and Verrucomicrobia in B6 mice over a period of 9 days [26]. The dysbiosis that developed in the B6 mice was more heterogeneous and occurred earlier than in the BALB/c mice and was associated with more severe cerebral and intestinal pathologies [26].

Intestinal Microbiota and Altered Susceptibility to *Plasmodium* Infection

Animal and human studies suggest that gut microbial community composition is associated with protection against *Plasmodium* infection and mediated via microbial effects on humoral immunity [27, 28]. Sporozoites of murine and human malaria parasites (*P. berghei*, *P. yoelii*, and *P. falciparum*) display Galα1-3Galβ1-4GlcNAc-R (α-galactosyl) surface glycans

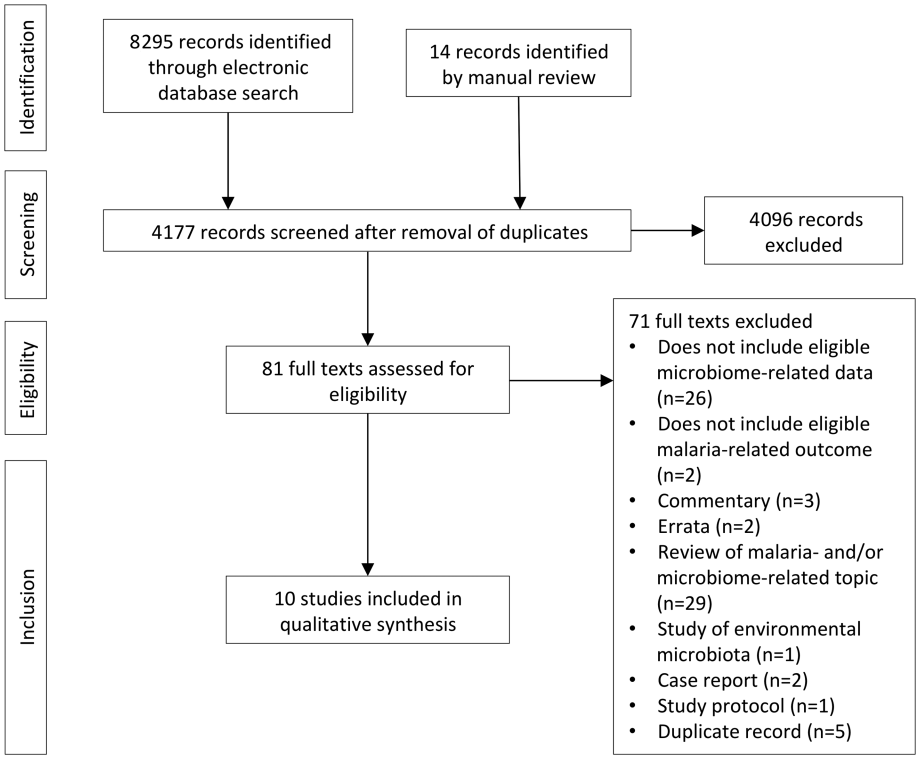

Figure 1. Selection of studies.

that are also expressed by certain Enterobactericeae, including *Klebsiella*, *Serratia*, and *Escherichia* spp. [27, 29]. In an elegant series of experiments, Yilmaz et al showed that mice experimentally colonized with the α-galactosyl–expressing gut pathobiont *E. coli* O86:B7 produced anti-α-galactosyl antibodies that cross-reacted with *Plasmodium* spp. sporozoites and conferred protection against hepatic invasion by the parasite [27]. The same study observed a possible association between anti-α-galactosyl immunoglobulin M (IgM) and incidence of *P. falciparum* infection in a cohort of 695 Malian children and adults who were followed with weekly polymerase chain reaction testing for *P. falciparum* infection over a transmission season. Baseline anti-α-galactosyl immunoglobulin G (IgG) and IgM were measured in the human cohort and analyzed against malaria incidence. Compared with children aged <2 years, as well as historical adult controls without malaria exposure, adults had more than twice the average baseline concentrations of anti-α-galactosyl IgG and IgM [27]. Children who remained uninfected had significantly higher concentrations of anti-α-galactosyl IgM ($P < .05$), but not IgG, compared with children who became infected, although time-to-event analysis failed to show an association between anti-α-galactosyl and malaria incidence [27].

Yooseph and colleagues analyzed a subset of the same Malian cohort and found an association between gut bacterial composition and protection against *P. falciparum* infection [28]. The authors characterized the intestinal microbiotas of 200 of the 695 cohort members (n = 106 female and n = 94 male; aged 3 mo to 25 y) and identified 2 distinctive intestinal bacteriotypes, one predominating in younger subjects (mean age, 1.4 y) and the other in older subjects (mean age, 9.1 y). The taxonomic profile of the first was characterized by a greater abundance of *Bifidobacterium* spp., *Streptococcus* spp., and the Enterobacteriaceae *Escherichia* spp. (unclassified) and *Shigella* spp. relative to the second profile, which featured a predominance of Ruminococcaceae and Lachnospiraceae. Time-to-infection analysis conditioned on age and other covariates showed that study subjects harboring the first enterotype were protected against *P. falciparum* infection relative to the other (hazard ratio, 0.42; 95% confidence interval .23–.75; $P = .004$). Interestingly, the authors found no significant association between enterotype and incident clinical (febrile) malaria ($P = .55$). One posited interpretation is that the protective enterotype conferred partial sterile and/or causal immunity against the sporozoite and/or liver stage, but not the erythrocytic stage, or, conversely, that the susceptible gut enterotype predisposes to the establishment of *Plasmodium* infection. The first supposition is suggested by Yilmaz and colleagues' findings discussed previously [27].

Intestinal Microbiota and Modulation of Malaria Disease Severity

In mice, gut bacterial communities influence malaria disease severity. Villarino and colleagues observed that isogenic

Table 1. Features of Included Studies

Study[a]	Mosquito Vector or *Plasmodium* Species[b]	Host Type	Use of Germ-free Control	Microbiota Community	Sample Type	Method of Microbiome Characterization
Braks and Takken 1999 [36]	*An. gambiae s.s.*	Human	Yes	Skin (forehead)	Droplet collection of sweat	Culture on nonselective media incubated for 24 h at 37°C
Verhulst et al 2009 [37]	*An. gambiae s.s.*	Human	Yes	Skin (foot)	Skin swab via sterile sampling ring with washing buffer	Culture on selective media incubated for 0, 6, 12, 24, 36, and 72 h at 34°C
Verhulst et al 2011 [38]	*An. gambiae s.s.*	Human	Yes	Skin (foot)	Skin swab, as above	Culture on selective media incubated at 34°C
Verhulst et al 2011 [24]	*An. gambiae s.s.*	Human	Yes	Skin (foot)	Skin swab, as above	16S rRNA amplicon (V2) sequencing; culture on selective media incubated for 12 and 24 h at 34°C
Yilmaz et al 2014 [27]	*P. berghei, P. yoelii*	C57BL/6 mice 8–10 wk old[c]	Yes	Intestinal	NA	Experimental inoculation of study animals with gut pathobionts *E. coli* O86:B7 and K12
Yooseph et al 2015 [28]	*P. falciparum*	Human adults and children in Mali	NA	Intestinal	Stool	16S rRNA amplicon (V1–V3) sequencing
Mooney et al 2015 [25]	*P. yoelii*	Female C57BL/6 and Swiss Webster mice 6–8 wk old	Yes	Intestinal	Fecal pellet	16S rRNA amplicon (V3–V4) sequencing
Taniguchi et al 2015 [26]	*P. berghei*	Male BALB/c and C57BL/6 mice 6–12 wk old	No	Intestinal	Fecal pellet	16S rRNA amplicon (V4) sequencing
Villarino et al 2016 [30]	*P. berghei, P. chabaudi, P. yoelii*	Female BALB/c and C57BL/6 mice 6–8 weeks old	Yes	Intestinal	Distal half small intestine, cecum, and colon	16S rRNA amplicon (V4) sequencing
Stough et al 2016 [23]	*P. yoelii*	Female C57BL/6 mice 6–8 wk old	No	Intestinal	Whole cecum	Metatranscriptomic analysis

Abbreviations: *An. gambiae s.s., Anopheles gambiae sensu stricto*; *E. coli, Escherichia coli*; NA, not applicable; *P. berghei, Plasmodium berghei*; *P. chabaudi, Plasmodium chabaudi*; *P. falciparum, Plasmodium falciparum*; *P. yoelii, Plasmodium yoelii*.

[a]Ordered chronologically by publication date.

[b]For studies of the skin microbiota association with mosquito attraction, the vector genus and species are given.

[c]The study also included human subjects for whom anti-α-galactosyl antibody levels were correlated with malaria outcomes without direct assessment of the microbiome.

mice from 2 vendors experienced markedly less severe disease compared with counterparts from other vendors, a phenomenon that they convincingly showed to be explained by differences in bacterial enterotypes [30]. Following infection with *Plasmodium*-parasitized erythrocytes, protected mice exhibited lower parasite densities and longer survival [23, 30]. The effect was species- and strain-transcendent, observed in infections with *P. berghei*, *Plasmodium chabaudi*, and *P. yoelii* in both BALB/c and B6 mice. Under controlled conditions, fecal transplant from mice with mild disease phenotypes to germ-free mice conferred protection [30]. The protective enterotype was enriched for *Lactobacillus* and *Bifidobacteria* spp., and

Table 2. Risk of Bias

Type of Bias	Risk of Bias, No. of Studies			
	Low	High	Unclear	Does Not Apply
Laboratory animal experiments				
Selection bias (baseline characteristics, sequence generation, and allocation concealment)	6	1	2	1
Performance bias (random housing, masking of investigators)	5	0	4	1
Detection bias (random outcome assessment, masking of assessors)	1	0	8	1
Attrition bias (missing and/or incomplete outcome data)	8	1	0	1
Reporting bias (selective outcome reporting)	9	0	0	1
Other bias (contamination, outside influence, unit of analysis, replacements)	8	1	0	1
Human cohort studies[a]				
Selection (eg, representativeness, selection of controls, ascertainment of exposure)	2	0	0	8
Comparability (comparability of cohorts on basis of design or analysis)	2	0	0	8
Outcome (blind assessment and record linkage, adequacy of follow-up duration, and completeness)	2	0	0	8

[a]≥50% of the maximum number of stars for each category was considered low risk.

Table 3. Intestinal Bacterial Commensals With Observed Associations to Malaria Outcomes

Phylum	Family	Genus	Observed Associations
Actinobacteria	Bifidobacteriaeceae	*Bifidobacterium*	Associated with reduced incidence of *P. falciparum* infection in a Malian cohort [28]; associated with attenuated malaria severity in mice [30]
Bacteroidetes	Unclassified	Unclassified	Enriched in *P. yoelii* infection in mice [25]
Firmicutes	Lactobacillaceae	*Lactobacillus*	Depleted in *P. yoelii* and *P. berghei* infection in mice [25, 26]; associated with attenuated malaria severity in mice [30]; associated with reduced incidence of *P. falciparum* infection in a Malian cohort [28]
	Streptococcaceae	*Streptococcus*	Associated with reduced incidence of *P. falciparum* infection in a Malian cohort [28]
Proteobacteria	Enterobacteriaceae	*Escherichia*	Associated with attenuated malaria severity in mice [30]; colonization by *E. coli* O86:B7 associated with humoral immunity to *Plasmodium* sporozoites in mice [27]; associated with reduced incidence of *P. falciparum* infection in a Malian cohort [28]; unspecified Enterobacteriaceae associated with higher parasitemia and severe disease [26]; increased susceptibility to *E. coli* and nontyphoidal *Salmonella* gut colonization in mice infected with *P. yoelli* [25]
		Shigella	Associated with reduced incidence of *P. falciparum* infection in a Malian cohort [28]

Abbreviations: *E. coli, Escherichia coli*; *P. berghei, Plasmodium berghei*; *P. falciparum, Plasmodium falciparum*; *P. yoelii, Plasmodium yoelii*.

its effects were corroborated in additional experiments with diet alterations and probiotic administration [30]. In a separate series of studies of murine malaria, Stough and colleagues examined associations between malaria severity and transcriptional and metabolomic characteristics of enteric commensals. The study identified several differentially expressed metabolic pathways between *P. yoelii*–resistant and –susceptible mice (eg, protein metabolism, motility and chemotaxis, sulfur metabolism), as well as differences in microbe-associated metabolic profiles [23].

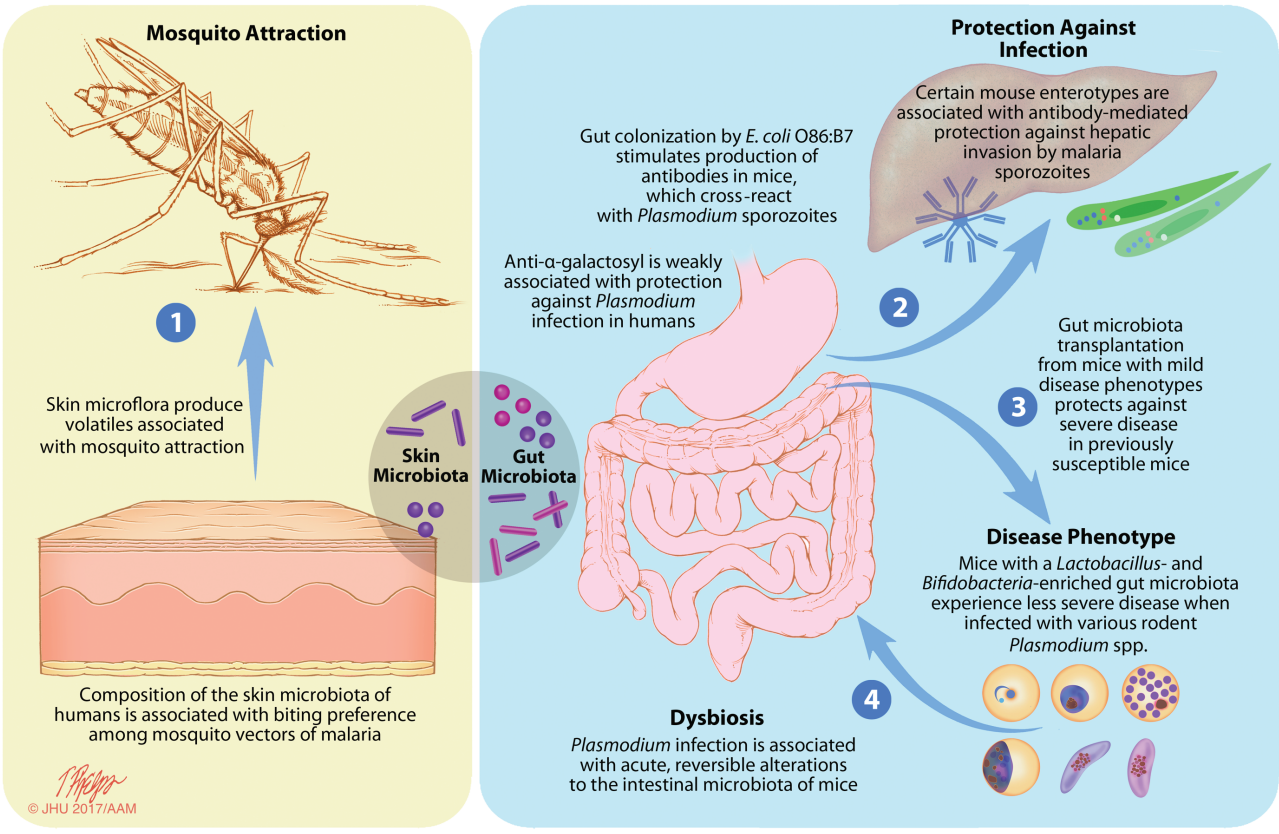

Figure 2. Observed associations between malaria and the mammalian microbiome. Abbreviation: *E. coli, Escherichia coli*.

In addition to their studies of *P. berghei*–induced dysbiosis, Taniguchi et al analyzed associations between the intestinal microbiota and malaria severity [26]. Distinct bacterial community compositions measured 4–9 days after infection were associated with degree of illness. Seventeen genera were found to have either a positive or negative association with parasite burden in BALB/c mice, and 22 genera were associated with parasitemia in B6 mice [26]. Specifically, the presence of *Lactobacillus* was associated with lower parasitemia and less severe cerebral malaria, and unspecified Enterobacteriaceae were associated with higher parasitemia and worse disease [26].

Skin Microbiota and Attraction of Malaria Mosquito Vectors

Anopheline mosquitoes, dozens of species that are responsible for malaria transmission, are anthropophilic and have long been known to exhibit differential attraction among different individual humans [31–33]. They home to their targets in response to several cues, including chemotaxis to human body odor attractants (kairomones), which are constituted in large part by volatile compounds produced by skin flora [34, 35]. The composition of skin microbial communities appears to explain, at least in part, host preference of malaria-transmitting mosquitoes. A preliminary study of the potential influence of skin microflora on the differential attractiveness of human sweat to female *Anopheles gambiae sensu stricto* showed a positive correlation between attractiveness and the duration of incubation of sweat samples in aerobic conditions, characterized by bacterial colony counts on agar plates [36]. In subsequent experiments, female *Anopheles gambiae s.s.* mosquitoes were preferentially attracted to baits containing skin microorganisms cultured from the feet of healthy volunteers compared with control traps with sterile agar and clean air with or without carbon dioxide [37]. Using selective culture media, the authors identified *Staphylococcus*, aerobic *Corynebacterium*, *Micrococcus*, *Propionibacterium*, *Pseudomonas*, and *Brevibacterium* spp., and the yeast *Pityrosporum* spp. as the potential chemoattractant-producing microbes. Additional genera were identified by 16S rRNA amplicon sequencing, and modeled data showed a relative abundance of *Leptotrichia*, *Delftia*, and *Acidobacteria* spp. in skin swabs of study participants who were highly attractive to anopheline mosquitoes (n = 9 from a group of 48 healthy volunteers) compared with those who were least attractive (n = 7). Attraction was demonstrated to be mediated by microorganism-generated volatiles [24]. Although experiments inconsistently identified specific taxa associated with greater attraction potential, they consistently showed that richer microbial diversity attenuated mosquito attraction and greater abundance of *Staphylococcus* spp. amplified attraction [24]. Results of similar experiments performed in a rural Kenyan village were suggestive but inconclusive. Skin microbiota-inoculated traps captured 6.0 ± 1.54 anopheline mosquitoes (mean and standard deviation of 16 collection time periods) compared with 3.6 ± 1.0 caught in control traps, but the difference was not significant once adjustments for house and collection time were made (P = .08) [38].

DISCUSSION

This systematic review summarizes the nascent literature describing malaria and the microbiome of mammalian hosts. Whereas the influence of the mosquito midgut microbiota on infectious dynamics of *Plasmodium* spp. is fairly well established [14–18], the role of mammalian host microbial communities in malaria infection is less delineated. Preliminary evidence shows infection with *Plasmodium* spp. is associated with transient alterations to the intestinal microbiota of mice, and distinct microbial communities of the intestine and skin have correlates with susceptibility to *Plasmodium* infection, disease phenotype, and vector mosquito affinity [23–28, 30, 36–38].

Intestinal microbiotas of healthy mice and humans share common enterotypes shaped mainly by host genetics and dietary balances of proteins, lipids, and carbohydrates [39–41]. The dysbiosis that occurs in murine *Plasmodium* infection can be due to perturbation of the normal gut architecture from tissue injury caused by parasite sequestration in intestinal vascular beds, dietary changes that accompany acute illness, and host immune and inflammatory responses. Results of 2 independent studies of *Plasmodium* infection in mice showed shifts in bacterial assemblages toward a relative depletion of Firmicutes [25, 26], which was correlated with more severe disease outcomes [26]. The altered gut environment promoted colonization by bacterial pathobionts, which may relate to the clinical observation of frequent bacterial coinfection among patients with malaria, including with nontyphoidal *Salmonella* [25].

Extending studies of malaria and microbiome dysbiosis to humans could be accomplished in clinical and epidemiological studies of chronic (asymptomatic) malaria, uncomplicated malaria, and severe malaria, in which investigators must be careful to address the potential confounding effect of diet (eg, milk-fed infants differ in microbiome composition from older children and adults, and some antimalarial drugs require coadministration with fatty food). Pharmacologic studies in healthy human subjects or patients with malaria that examine the potential bidirectional influences of antimalarial drugs and the intestinal microbiota upon each other might also be valuable. Composition of the intestinal microbiota influences the metabolism of many key pharmaceuticals, and, conversely, antimalarial agents may impact gut microbial communities through activity against commensal bacteria and fungi [42–44].

Reduced susceptibility to infection by *Plasmodium* sporozoites in certain mouse enterotypes was mediated by anti-α-galactosyl IgM, production of which was stimulated by gut inoculation with an α-galactosyl–expressing serovar of *E. coli* [27]. This and other gut microbes, via antigen-presenting

cells of the intestine, facilitate immunoglobulin production by B cells, which can be distributed widely [45, 46]. Thirty years ago, Ravindran et al detailed the role of a similar humoral response with anti-α-galactosyl against the erythrocytic stage of *P. falciparum* [47]. More recently, malaria vaccinologists showed that long-lived IgM antibodies to *P. falciparum* sporozoite proteins inhibit invasion of hepatocytes and fix complement to sporozoites [48]. Microbiome studies of patients with systemic lupus erythematosus similarly identified an immunopathological role of IgM, the expression of which corresponded to the presence of bacterial commensals of the anaerobic Synergistetes phylum that, in turn, were associated with an increased Firmicutes/Bacteroidetes ratio [49]. Further animal and human studies that investigate the role of enteric bacteria in the production of anti-α-galactosyl or other posited *Plasmodium*-reactive antibodies and their associations with incident malaria would be informative.

The attenuation of malaria severity in mice with characteristic microbiome enterotypes may similarly be mediated by specific immune responses. As alluded to above, certain commensal microbe-related antibodies recognize erythrocytic stages of *Plasmodium* [47]. Opsonization of parasitized erythrocytes by gut microbe–stimulated antibodies could accelerate their destruction and clearance, although this or another immune mechanism remains to be shown. Nonspecific immune and inflammatory responses may also play a mediating role. Interleukins linked to malaria pathogenesis have been shown to correlate with microbiome enterotypes [50, 51]. Bifidobacteriaeceae and other taxonomic groups are involved in priming phagocytes against other diseases [52, 53], raising the possibility of a similar effect in malaria. Systemic nitric oxide mitigates malaria disease severity [54–58], production of which relies in part on oral and intestinal commensal bacteria. Nitric oxide is a byproduct of denitrification, a biochemical pathway of anaerobic symbionts such as those that predominate in malaria-protective enterotypes (eg, *Lactobacillus*, *Bifidobacteria*) [59–61]. These and other potential explanations of the gut microbiota's association with malaria disease phenotypes remain unexplored. Additional laboratory investigations that elucidate specific and nonspecific immune and inflammatory mechanisms of microbiota protection against malaria disease severity are needed. Clinical studies of severe malaria in humans could incorporate measures of intestinal microbial composition and metabolomics for correlation with disease outcomes.

Villarino et al noted that the gut microbial communities associated with milder malaria phenotypes were previously seen to be differentially abundant in African children residing in a malarious region compared with Europeans in a nonendemic area [30, 62]. Whereas the drivers of coevolution between hosts and their microbiomes are controversial [63], it is reasonable to speculate that *Plasmodium* may have exerted a selective pressure on the human microbiome analogous to its natural selection in human genetic evolution [1–5].

Identifying the microbiome determinants of mosquito attraction to human hosts has implications for malaria control and vector biology. Recognition of the influence of skin microbial communities on mosquito attraction has led entomologists to manipulate microbe-associated volatile mixtures to develop new attractants for baited mosquito traps in efforts to augment malaria control and vector biology research [64]. Next steps may include application to personal-protection methods of mosquito bite avoidance.

The reviewed studies share several limitations. The potential exclusion of low-abundant but pathologically relevant commensals due to detection limits of 16S rRNA amplicon sequencing and differences in sequencing protocols (Table 1) and environmental controls may have biased some results. For example, 1 study reported associations with microbial taxa that are possible contaminants of commercial sequencing reagents (*Leptotrichia*, *Delftia*, and *Acidobacteria* spp.) [24]. Viruses, fungi, archaea, and intestinal eukaryotes were not characterized in the studies, a limitation shared by most microbiome studies published to date. Use of unbiased next-generation sequencing approaches may help illuminate the role, if any, of these other microorganisms in *Plasmodium* infection, malaria severity, and related outcomes.

There are limitations to the review itself. Although our systematic approach attempted to capture all relevant studies regardless of date, publication status, or language, there may be additional studies that were not identified. We found only 10 eligible studies; compared with microbiome-related studies of other parasitic and tropical diseases, the number is relatively large. Our risk of bias assessment was limited due to absence of details regarding blinding or lack thereof of outcome assessors and other study design features such as cage assignment and handling of laboratory animals. Because this is a qualitative review and we did not identify any unpublished studies, it is not possible to comment regarding the likelihood of publication bias.

CONCLUSIONS

Emerging evidence has begun to characterize the relationship between malaria and the mammalian microbiome. Laboratory experimentation that continues to unravel the complex interactions between the microbiome and malaria immunopathology are needed, and microbiome-based studies of malaria can be readily integrated into clinical and epidemiological research. Well-designed human translational studies are needed to investigate putative bidirectional interactions of malaria and the host microbiome. Field samples can be collected, stored, and transported with relative ease, and sequencing laboratories and biocomputational services are increasingly available at decreasing costs. A young but compelling body of literature, reviewed here, provides guidance.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. I. and C. L. conceived of the work, and M. I. developed the study protocol. M. I. did the literature search with assistance from Johns Hopkins William H. Welch Medical Library information specialist Maria Truskey. M. I. and J. D. reviewed the abstracts and conducted full-text review and data extraction. M. I., J. D., and N. S. contributed to the data analysis. All authors contributed to the first draft of the manuscript, critically revised all subsequent drafts, and approved the final version.

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