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Review Article

Exploring the Gut–Skin Microbiome and Leishmaniasis: A Systematic Review of Current Evidence

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Abstract

Background: This review investigated the impact of skin and gut microbiomes on the development of leishmaniasis. Dysbiosis in these microbiomes is associated with various skin disorders through mechanisms related to immune modulation and inflammation. Leishmaniasis primarily presents as cutaneous wounds. The immune response of the host to *Leishmania* plays a crucial role in determining the disease's severity and treatment effectiveness. Gaining insight into these microbiome-immune dynamics is essential for improving the prevention and management of leishmaniasis.

Methods: A thorough analysis of the most recent research was done, emphasizing the functions of chemokines and cytokines in *Leishmania* infection. Synthesizing key findings from research indexed by PubMed, Scopus, Web of Science, Science Direct, EMBASE, and Magiran up to June 2024 provided an extensive understanding of immunological mechanisms. Eventually, 19 manuscripts were included in this review and thoroughly investigated and reported.

Results: The delicate balance between pro- and anti-inflammatory signals in the immune response to *Leishmania* infection largely depends on the commensal microbial populations.

Conclusion: Future research should concentrate on modifying these reactions to improve treatment outcomes for leishmaniasis.

Introduction

The human skin and gut microbiomes are vital for supporting our health, especially skin health. Numerous studies have explored the association between various skin conditions and infections related to dysbiosis (1-3).

The skin microbiome is made up of various microorganisms, such as bacteria, fungi, and viruses. These microorganisms interact with each other and with the host's immune system.

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Similar to the skin microbiome, the gut microbiome is composed of numerous microorganisms that inhabit the gastrointestinal tract and interact with our immune system and metabolic functions (2, 4, 5).

Numerous dermatological conditions have been linked to changes in the skin microbiome. Two of the most studied instances are acne vulgaris and its association with *Cutibacterium acnes* proliferation and atopic dermatitis and the connection between its clinical features and *Staphylococcus aureus*. Moreover, a varied proliferation of pathogenic microorganisms has been observed in chronic wounds (1, 2). The concept of the gut-skin axis considers the interaction between the gut and the skin via diverse mechanisms like the immune system, the nervous system, and the generation of metabolites. Disruptions in the gut microbiome can induce systemic inflammation, which may subsequently lead to skin manifestations, influencing the onset of conditions such as acne, rosacea, etc. (2, 3, 6).

Leishmaniasis is one of the skin infectious diseases that is still under investigation. Although multiple studies have been conducted on this matter, it is still considered one of the neglected tropical diseases, as mentioned by WHO. *Leishmania* is transmitted to the mammalian host through sandflies via the injection of infectious metacyclic promastigotes. The clinically important leishmaniasis includes three primary forms: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and cutaneous mucosal leishmaniasis, and among these, CL is the most common type. Leishmaniasis is mainly present in Central and South America, South Europe, Central Africa, and parts of South and Central Asia and the Middle East (7-11).

One of the most important components in leishmaniasis' pathogenesis and disease course is the effect of the immune system and the cutaneous inflammation caused after exposure to the parasite. The course of inflammation and the severity of the immune reaction determine not only the severity of the disease but also the response to treatment and the clinical outcome (12). Like other skin diseases, leishmaniasis can also be subject to change in course and response to treatment, considering the microbial changes in our gut or skin (13, 14).

Understanding the relationship between the skin and gut microbiomes and the host's immune system is crucial for developing effective strategies to prevent, diagnose, and treat skin diseases such as leishmaniasis. In this review, we aimed to highlight the effect of skin and gut microbiota on different aspects of leishmaniasis, such as immune response, clinical course, and treatment outcome. We hypothesize that a well-balanced microbiota can modulate host immunity in ways that improve disease control and healing, whereas dysbiosis may exacerbate inflammation and delay recovery.

Methods

Search strategy

We tried to retrieve English and Persian language manuscripts related to leishmaniasis and its relation to the host's immune system activity and microbiota. PubMed, Scopus, Web of Science, Science Direct, EMBASE, and Magiran were thoroughly searched up to June 2024.

The search query included the following keywords: “leishmaniasis”, “gut microbiota”, “intestinal microbiota”, “skin microbiota”, “normal microbial flora", and "microbiome", which were combined using the Boolean operators "OR" and "AND". We searched by hand and scanned previous manuscripts’ reference lists to identify other related studies.

Selection Criteria and Data Extraction

All the studies on the different interactions of leishmaniasis and skin or gut microbiota were included in this review, and studies with insufficient data, studies in languages other than English and Persian, and studies that did not pass quality assessments were excluded.

After removing the duplicates, the titles and abstracts were screened, and in the next step,

the full text of the eligible studies was retrieved and assessed. Two different reviewers evaluated the quality of the final manuscripts, and the data were eventually extracted (Fig. 1).

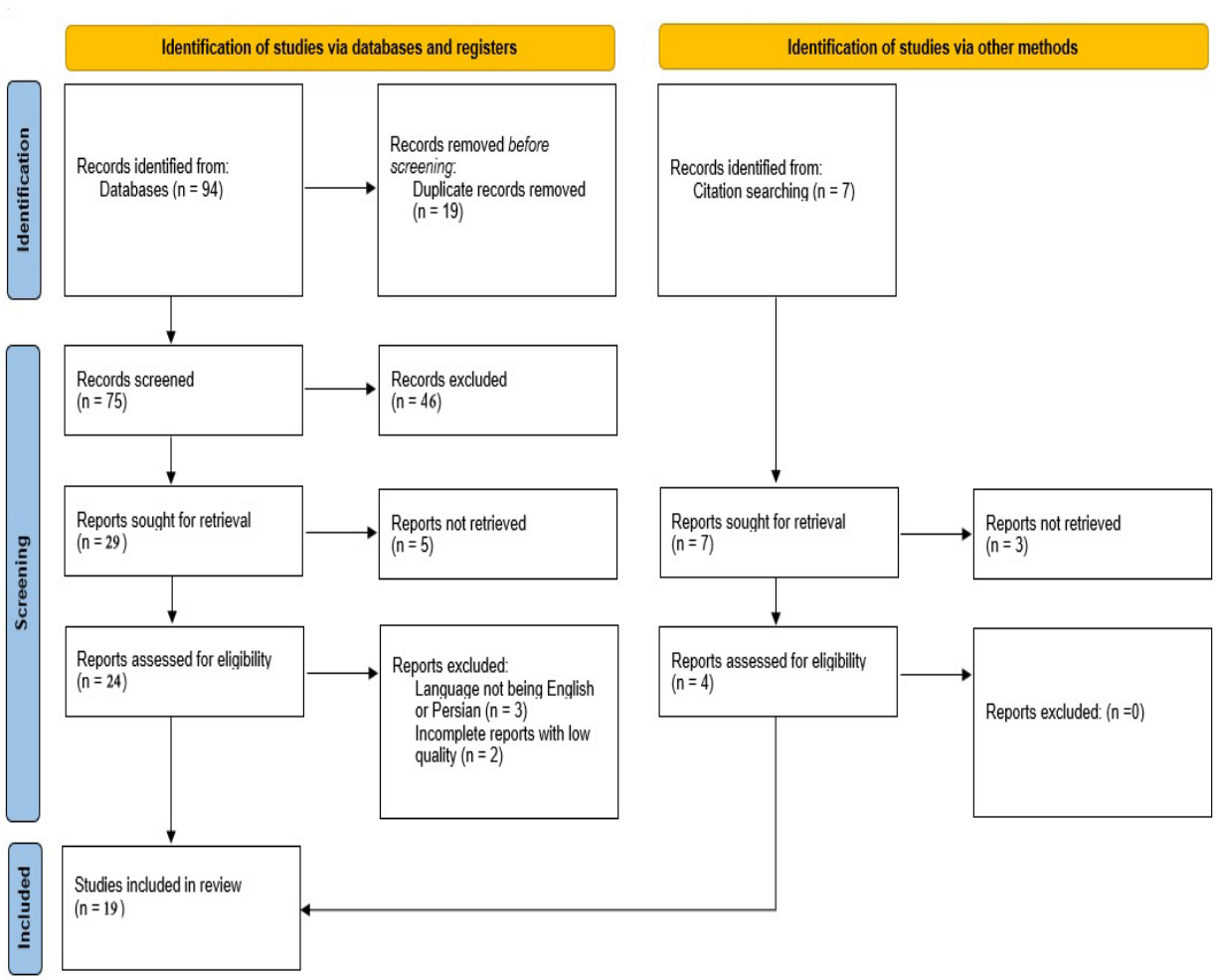

Fig. 1: Flow diagram for included studies

Quality Assessment of Included Articles

National Institutes of Health **(**NIH) quality assessment forms were used to evaluate the scientific quality of the selected manuscripts. According to these protocols, the following domains were scored: 1) correct selection of the study group, 2) control of confounding factors, and 3) outcome investigation. The papers were then categorized as having qualities of good, fair, or poor. Studies with a quality assessed as "poor" were excluded from this review. The process of selecting and matching the criteria was conducted by three independent reviewers. The quality assessment of the included studies is presented in Table 1.

Table 1: Details of included studies and their quality

Author	Country	*Leishmania* spp.	Human/ Animal	Microbial Community	Characteristics of immune response	NIH quality	Ref
Oliveira et al.	Brazil	*L. major*	Mice	-	Worse prognosis in the germ-free mice compared to conventional mice	fair	(16)
Julia et al.	USA	*L. major*	Mice	Germ-free mice showed lower levels of lymphocytic activity and infiltration. Extracts from *E.coli* and *E. faecalis,* but not those from *P.mirabilis* and *C.perfringens*, induced IL-2 secretion, and the memory cells in this matter might help the animal fight Leishmania.	Mice which had contact with stimulant bacteria showed a better response to leishmaniasis	fair	(17)
Fontes et al.	Brazil	*L. braziliensis*	Human	Secondary infections, mainly by *S.aureus* and *S.pyogenes* in leishmanial lesions	*Staphylococcus*-related toxins might exacerbate the status of inflammation	Good	(33)
Oliveira et al.	Brazil	*L. major*	Mice	Worse prognosis in the germ-free mice compared to conventional mice	Lower interferon and cytokine levels in germ-free mice	Good	(23)
Naik et al.	USA	*L. major*	Mice	*S.epidermidis* was introduced as skin commensal / Poor prognosis in the absence of *S.epidermidis*	Germ-free mice had a lower level of dermal CD4+ T cells, IL-1, IL-17, and IFN-ɣ.	Good	(22)
Lamour et al.	UK	*L. major*	Mice	Higher level in self-healing mice's gut microbiota: Gammaproteobacteria, Erysipelotrichia, Decreased abundance in self-healing mice after infection: Actinobacteria	No significant changes in fecal bacterial composition were evident over the study period in non-healing mice	Good	(18)

Table 1: Continued…

				Increased abundance in self-healing mice after infection: Alphaproteobacteria, *Bacteroides*			
Gimblet et al.	USA	*L. braziliensis* / *L. major*	Human / mice	A higher number of *S.aureus* and *Streptococcus* in leishmanial lesions and nearby skin	Microbiota changes increase the inflammation severity	Good	(30)
Lappan et al.	India	*L. donovani*	Human	lower in the gut microbiota of cases than controls: Ruminococcaceae, *Gastranaerophilales*, *Entamoeba* Higher in cases than controls: *Pentatrichomonas*	No different communities and diversity of gut microbiota	Good	(27)
Passos et al.	Brazil	*L. infantum*	hamsters	No significant changes related to the infection, but the association of the abundance of *Bifidobacterium* and lactobacilli with the parasite load in infected mice	Possible protective role of gut microbiota	Good	(29)
Lewis et al.	UK	*L. donovani*	Mice and Hamster	Better prognosis in antibiotic-treated and dysbiotic hamsters after *Rodentibacter* removal from gut microbiota.	No difference in disease prognosis in dysbiotic mice.	Good	(19)
Kaluarachchi et al.	Sri Lanka	*L. donovani*	Human	A higher number of Enterobacteriaceae and *Pseudomonas* in leishmanial lesions / lower diversity in bacterial compositions of lesions	Secondary infections/ biofilm formation / higher Bacilli in wound swabs compared to contralateral skin	Good	(45)
Singh et al.	USA	*L. major*	Human	*Staphylococcus* used to show the changes in inflammation.	More severe inflammation in the presence of *Staphylococcus*	Good	(24)

Table 1: Continued…

Lopes et al.	Brazil	*L. major*	mice	*Lactobacillus* and *Streptococcus* and gram-negative Enterobacteriaceae were depleted in mice with antibiotic therapy, which resulted in a worse prognosis in *Leishmania* lesions.	Germ-free mice's innate immune system is less effective against resistant species of leishmaniasis	fair	(20)
Olias-morelo et al.	Spain	L. infantum	hamsters	No significant changes in gut microbiota	No significant changes in gut microbiota	Good	(28)
Meazzi et al.	Italy	-	dogs	Firmicutes was seen as significantly more abundant in healthy dogs compared with the other two groups. Conversely, Proteobacteria were more abundant in symptomatic dogs.	decreased Th1 response in symptomatic dogs	fair	(32)
Amorim et al.	USA	*L. braziliensis*	Human	Delayed healing in the presence of the *S.aureus*	Increased IL-1B in the presence of higher lesional *S.aureus*	Good	(13)
Singh et al.	USA	*L. braziliensis*	Mice	*S.aureus* abundance in infected mice	Delayed healing and worse prognosis in the presence of *S.aureus*	Good	(31)
Jaimes et al.	Colombia	*L. panamensis / L. braziliensis*	Human	Rise in Firmicutes, Proteobacteria, *Staphylococcus, Malassezia*, and *Trichomonas* in lesional skin.	Different communities and diversity of skin and lesion microbiota	Good	(25)
Mrazek et al.	Czech Republic	*L. major*	Mice	Higher diversity in colon and ileum microbiota in susceptible mice / a rise in Muribaculaceae and Lactobacillaceae in colon microbiota of infected mice / a rise in Proteobacteria in the ileum microbiota of infected mice	Changes in ileum microbiota were imminent, but the colon microbiota was more stable.	Good	(21)

The included studies were also investigated regarding risks of bias using the Risk of Bias in Non-randomized Studies of Exposures (ROBINS-E) tool (15). Details of the risk of bias assessment are presented in Fig. 2.

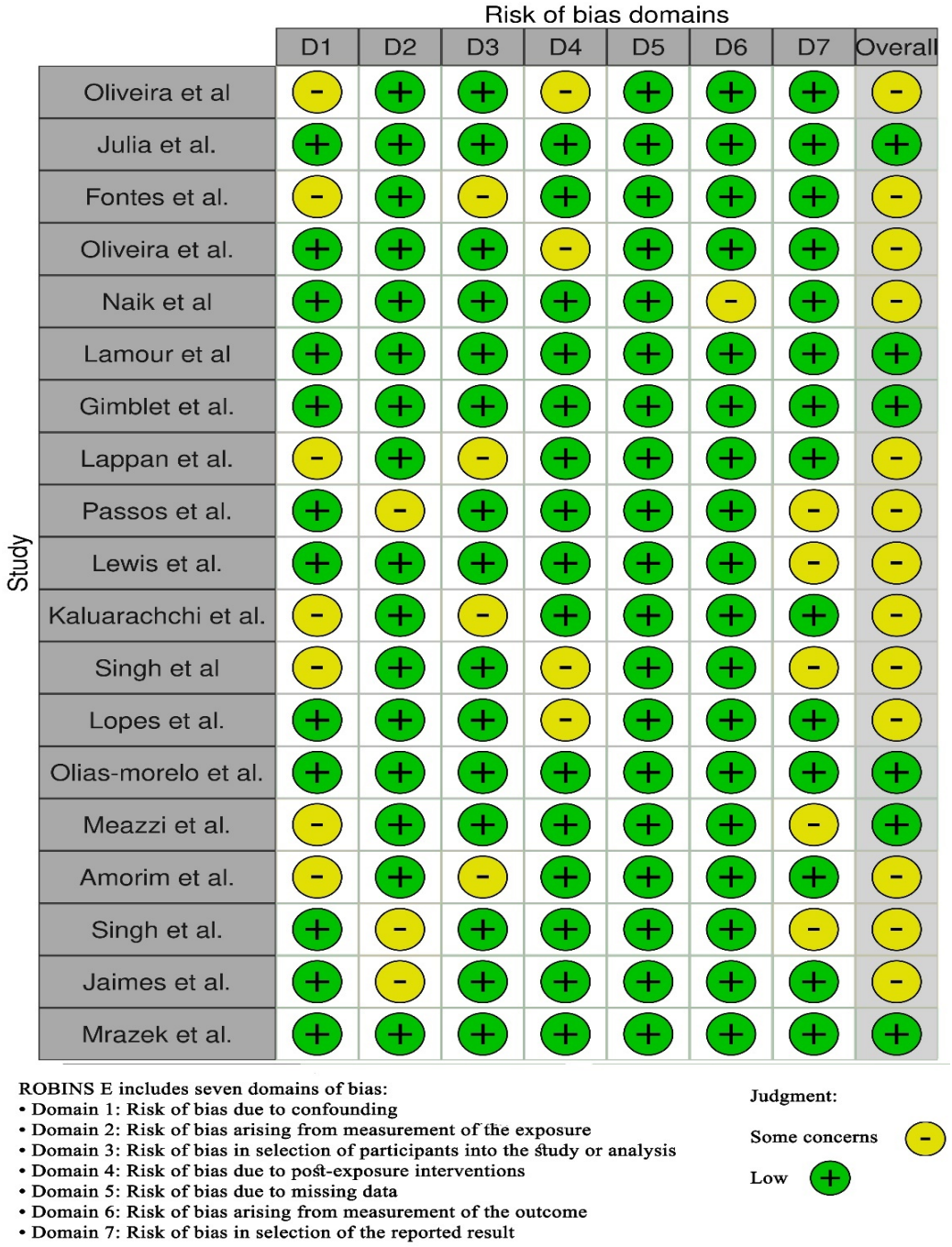

Fig. 2: Risk of bias assessment of the included studies

Results

After searching the databases using the keywords mentioned, we identified 94 studies, and 75 remained after removing duplicates. After scanning topics and abstracts, 30 studies were retrieved. The studies were thoroughly examined, and those in other languages or without an accessible full text were excluded. Out of the total, 19 studies met our inclusion criteria.

Fig. 1 depicts the flowchart of the included studies, and Table 1 provides details of the reviewed studies.

All in all, there were 13 animal studies on this matter, and 7 studies have investigated human microbial changes in leishmanial infection (one manuscript considered both humans and mice). Multiple approaches were taken in the process of investigating the interaction between microbiota and Leishmaniasis infection.

Nine of the included studies investigated *L. major* (16-24), four articles reported on *eishmania braziliensis*, and *eishmania infantum* and *Leishmania donovani* were each mentioned in three articles (19, 25-29), and only one article investigated *L. panamensis* (25).

The bacterial strain most commonly mentioned was *S. aureus*. This bacterium was investigated in two different ways. The growth rate of leishmanial lesions and the nearby skin has been researched, and it was reported to be higher in leishmanial lesions (25- 30). Moreover, two studies depicted that the presence and abundance of Staphylococci in leishmanial lesions may lead to delayed healing (13, 31). A more severe inflammatory response was shown in the cases of *Staphylococcus* infection (24).

Four studies mentioned Proteobacteria in their investigations. The number of Proteobacteria was increased in leishmanial lesions in mice, dogs, and even humans (18, 25, 32). A rise in Proteobacteria count was also observed in the ileal microbiota of infected mice (21).

Three studies investigated the Lactobacilli family, all pointing out the possible protective effect of these bacteria. The Lactobacilli count in gut microbiota was higher in strains of mice resistant to *Leishmania*, and the depletion of bacteria using antibiotics may lead to a worse prognosis (20, 21, 29).

Streptococcus was investigated in 4 studies. There is a difference in the results caused by the abundance of Streptococci in the skin and gut. *Streptococcus* has been introduced as a possible cause of secondary infection in leishmanial lesions and was linked to a worse prognosis in the lesion's healing process (13, 30, 33-35). On the other hand, the depletion of Streptococci alongside other genera of gut microbiota after antibiotic therapy has been reported to be associated with a worse prognosis and a limited immune response (20). Enterobacteriaceae, were also shown to be at a lower count in the patient's gut microbiota than in controls, and the depletion of these bacteria with the use of antibiotics led to a worse prognosis (20, 27).

Firmicutes was also among the studied strains in 3 of the included studies. The number of *Firmicutes* was reported to rise in the gut and colon microbiota of canine leishmanial cases and mice (21, 32). *Firmicutes* have also been more abundant in the leishmanial lesions of human cases (25).

Discussion

Leishmaniasis is one of the skin infectious diseases that is still under investigation. Although multiple studies have been conducted on this matter, it is still considered a neglected tropical disease, as the WHO mentioned, and obviously can't be overlooked (8).

Germ-free animal models, specifically mice, have been widely employed to investigate the influence of microbiota on the advancement of diseases and immune reactions. Different mouse models that were studied in *Leishmania* research were mostly BALB/C, C57BL/6, and Swiss/NIH mice. BALB/C mice were known as a susceptible genus to leishmaniasis and were shown to be unable to heal lesions (23, 36). C57BL/6 has been used as a resistant model to

leishmaniasis, and conventional or non-conventional Swiss/NIH were used as a comparison (16, 23).

Transmission and disease progression in experimental leishmaniasis depend on parasite species/strain, host genetic background (e.g., BALB/c vs C57BL/6), route of inoculation (needle vs sand fly), and very early innate events—particularly neutrophil influx and their crosstalk with dermal macrophages and monocyte-derived cells that establish the inflammatory milieu for parasite survival or control. Skin commensals further tune these responses (37, 38).

Comparative work shows that susceptible and resistant strains develop distinct ileal (more than colonic) microbiome signatures after *L. major* infection, with strain-specific biomarkers emerging on LEfSe analysis; earlier systems studies similarly noted differential trajectories in taxa across self-healing vs. non-healing mice. These patterns likely shape local metabolites and downstream immunity (20, 21).

Germ-free mice mount high interferon-gamma (IFN-γ) yet fail to heal, with larger parasite burdens; dysbiotic or germ-free settings also show expanded infected dermal myeloid pools and altered reactive oxygen species (ROS), linking commensal education of innate and T-cell responses to lesion size and parasite control (23).

This article brings together outcomes from diverse research projects that compare germ-free and conventionalized mice, as well as clinical and immunological research, focusing on crucial similarities and differences in lesion progression, cytokine patterns, and immune cell functions concerning *Leishmania* infections.

While investigating the lesion progression and size in different strains and in the presence of other bacteria, it was depicted that, contrary to BALB/C mice, which had a continuous growth in lesion size and footpad swelling without resolution, the lesion size and footpad swelling in resistant C57B1/6 mice peaked at 4-5 wk and finally resolved to pre-infection size. This matter was further investigated by comparing germ-free mice to conventionalized Swiss/NIH mice. Although conventionalized mice showed a better response than germ-free mice, their lesion size was still larger than that of the conventional mice, which were in contact with microbiota from the beginning (16, 20, 23). The parasitic load was also reported to be higher in germ-free Swiss/NIH mice and failed to heal lesions (20, 23). On the other hand, Lewis et al. reported no difference in disease presentation in dysbiotic mice compared to conventional ones (19).

The clinical course of the disease was also investigated in other animals. The same research group of Lewis and associates also investigated hamsters. Dysbiotic hamsters also showed better prognosis and slower progression in the disease course. While searching for the cause and the difference in microbial and immunological circumstances in antibiotic-treated and conventional hamsters, *Rodentibacter* was absent in antibiotic-treated hamsters. This might be related to the gut-liver axis, such as intestinal parasitism, bacterial translocation to the liver, and even the amount of iron sequestration. Lewis et al. suggested that prophylactic antibiotic treatment may be positively effective (19).

The apparent discrepancy between Lewis et al. and other studies likely reflects differences in host species and microbiome composition. While dysbiotic mice showed no significant difference in disease progression, dysbiotic hamsters exhibited delayed disease progression when *Rodentibacter* was removed from the gut microbiota. This suggests species-specific microbiome–immune interactions and underlines the complexity of translating findings across models.

Conversely, some bacteria might play a protective role in hamsters. Two groups of infected and control hamsters were compared in research conducted by Passos et al., and although there were no significant differences found in the relative abundance of *Bifidobacterium* and *Lactobacillus* in the animals' gut microbiota, *Bifidobacteria* were shown to be negatively

correlated with parasite load. A significant increase was seen in the amount of *Bifidobacterium* in the gut microbiota of infected hamsters through the course of the disease (29). The changes throughout the course of infection in hamsters were once again reported as insignificant by Olias-Moreno et al., and only a minor insignificant rise in the ratio of *Firmicutes*/*Bacteriodes* was depicted (28).

Moreover, different bacterial compositions were studied in mice, dogs, and humans to determine their effect on the immune system.

The loads of *clostridia* were higher in BALB/C mice before getting infected with *L. major*, compared to C57BL/6 mice. On the other hand, C57BL/6 mice had a higher number of *Gammaproteobacteria*. Both strains showed a decrease in these bacterial dominations throughout the course of the disease (18). An increase in the number of *Alphaproteobacteria* and *Bacteroides* and a reduction in the levels of Actinobacteria and bacilli were seen in healing mice. At the same time, there were no significant changes in fecal bacterial compositions in non-healing mice (18). There was also a difference in the bacterial communities in the colon and ileum microbiota between susceptible and resistant mice. Susceptible mice had a higher diversity in the colon and ileum microbiota. Also, there was a rise in the numbers of *Muribaculaceae* and *Lactobacillaceae* in the colon microbiota and a rise in levels of Firmicutes and Proteobacteria in the ileum of infected mice (21).

In the only study conducted on dogs and this matter, 39 dogs were investigated by Meazzi et al. They were divided into three groups: healthy dogs, asymptomatic exposed dogs, and infected ones. Healthy dogs showed a more abundant Firmicutes level, such as Clostridia and *Erysipelotrichia*, while symptomatic dogs had a higher level of Proteobacteria and a dysbiotic gut microbiota dominated by *Escherichia. coli*, *Enterococci*, and *Proteus*. Spirochetes were also less abundant in symptomatic dogs than in healthy and resistant ones (32).

In the only study on dogs with visceral leishmaniasis, which are the natural reservoir for *L. infantum*, clear changes in the gut microbiome were seen. Symptomatic dogs had fewer *Firmicutes* and more *Proteobacteria*, together with weaker immune responses (32). This is different from laboratory models such as hamsters, where gut microbiota changes were much smaller and only seen at the genus level. These differences show why it is important to separate natural field infections from controlled lab infections. Field studies may better reflect real disease and transmission conditions, while lab studies are useful for understanding mechanisms under controlled settings. Both are important, but their results need to be interpreted with care when thinking about human disease.

The stool samples of human visceral leishmaniasis cases were also investigated and researched. The levels of *Ruminococcaceae*, *Gastranaerophilales*, and *Entamoeba* were higher in controls, besides a higher level of *Pentatrichomonas* was seen in cases (27). While gut microbiota appears to modulate systemic immunity in visceral leishmaniasis, its role in cutaneous disease remains poorly defined and largely speculative.

Multiple studies have shown before that the skin microbiota can also have a notable effect on skin disease progression and inflammatory responses, and the same also goes for leishmaniasis. As it was depicted, one of the main factors associated with the leishmaniasis clinical course and prognosis is inflammatory responses in the skin, and this can be even more influential than factors such as parasite burden or species (24, 33). One of the elements affecting the skin's inflammatory processes is skin commensal microbiota, and it was said to be related to levels of IL-17A and IL-1β (33).

Thecolonization with certain bacteria can exacerbate inflammatory responses. For instance, colonizing mice with *Staphylococcus epidermidis* or *Staphylococcus xylosus* followed by infection with *L. major* resulted in significantly higher early interleukin levels and a more robust inflammatory response compared to

controls. However, *S. epidermidis* alone did not elicit a response without *Leishmania* inoculation. Although interleukin levels and lymphocyte counts rose initially, they did not remain elevated at the disease's peak. Lesion size and pathology severity were more pronounced in *Staphylococcus*-treated mice, independent of parasite load (24, 31).

Bacterial contaminations in leishmanial lesions have long been investigated. *S. aureus* and *Staphylococcus Pyogenes* were seen as the most common pathogens in this matter. *S. aureus* is especially important because it can produce toxins such as SEA (staphylococcal enterotoxin A) and SEB (staphylococcal enterotoxin B), which lead to more severe inflammation and poor prognosis. The biofilms were seen in 64% of cutaneous leishmanial lesions. These biofilms were dominated by Pseudomonas, bacilli, and Enterobacteriaceae (33).

On the other hand, *Leishmania* infection itself can significantly alter the skin's microbial communities. In mice infected with *L. braziliensis*, a marked decrease in skin microbial diversity was observed, with *Staphylococcus* species becoming dominant, leading to increased IL-17 and a heightened inflammatory response. These changes were not confined to the lesion area but occurred globally on the skin and were transferable to uninfected mice, which then developed higher inflammatory responses upon *Leishmania* infection. Similar patterns were seen in human patients with *L. braziliensis*, where lesions were dominated by *S. aureus* and *Streptococcus*, and adjacent skin showed similar but less pronounced change (30).

L. braziliensis infection led to a predominance of *Staphylococcus* spp., *Corynebacterium*, and *Streptococcus* in the skin microbiome. The dominance of *Staphylococcus* spp. Correlated with prolonged healing and increased inflammatory reactions, particularly involving IL-1 family members (13).

Jaimes et al. examined microbial changes in *Leishmania* lesions in male patients without superinfections or antibiotics. They noted an increase in Firmicutes and Proteobacteria in skin lesions, while Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, and Fusobacteria decreased. There were no significant differences between lesioned and healthy skin in prokaryotic communities, but eukaryotic communities showed increased Malassezia restricts in lesions (25).

All this research on microbiota eventually leads to its effect on the immune system. Commensal bacteria and immune cells interact constantly to provide a stable environment that is necessary for normal and balanced immune function.

Microbial substances, including bacterial lipopolysaccharides (LPS), can cause immune cells to release cytokines (39). This mechanism helps the immune system acquire tolerance to harmless antigens and initiate immune responses against infections (40). There has also been other studies considering the effects of other microbial products such as fungal metabolites on leishmaniasis (41).

A study comparing germ-free and conventionalized mice—such as Swiss-NIH and C57BL/6—inoculated with *L. major* was carried out by Oliveira et al. in 2005. They discovered that IFN-γ was hardly detected in BALB/C mice and secreted at nearly the same amount in Swiss-NIH and C57BL/6 mice, suggesting a robust Th1 response in resistant mice and a weak response in susceptible mice. In contrast, Swiss-NIH and C57BL/6 mice had lower IL-4 levels than BALB/C mice, suggesting a Th2-skewed reaction in susceptible mice (23). Julia et al. looked into the activity of several lymphocytes (CD4+, CD8+, and Tregs) as well as cytokine levels, such as IL-4 and IL-2. The interleukins were shown to be significantly less prevalent in germ-free mice. This decrease in cytokine levels was associated with the absence of commensal bacterial exposure, which typically aids in immune system priming. Bacterial extracts from *E. coli* and *Escherichia. faecalis* were found to be particularly effective in stimulating IL-2 secretion. In contrast, extracts from *Proteus mirabilis* and *Clostridium perfringens* did not exhibit the same effect. A *Leishmania-*

related antigen (LACK) induced a burst secretion of interleukins, facilitating an appropriate immune response to the parasite. Conventional mice exposed to various antigens from commensal bacteria had memory cells responsive to LACK, resulting in a more effective immune response to *Leishmania* infection. Further exploration involved introducing bacterial extracts of gut microbiota to germ-free mice to assess their effect on LACK-specific T cells. Extracts from *E. coli* and *Enterococcus faecalis* stimulated IL-2 secretion, while those from *Proteus mirabilis* and *Clostridium perfringens* did not. These memory cells potentially assist animals in combating *Leishmania* (17).

Singh et al. investigated mice infected with *L. major* after being colonized by *S. epidermidis* or *S. xylosus*. Even though *S. epidermidis* did not cause an inflammatory response on its own in the absence of *Leishmania* infection, they discovered that the presence of *S. epidermidis* considerably raised early IL-17A and IL-1β levels, aggravating the inflammatory response (24, 31).

Commensal bacteria can improve the performance of APCs or antigen-presenting cells, including dendritic cells. Without a doubt, this contact is essential for triggering immunological responses (40, 42).

The changes in gut microbiome also affect Treg differentiation and activity of different T cells, which is crucial for preserving immunological tolerance and averting autoimmune disorders. The production of Tregs is stimulated by certain gut bacteria, which improves the body's tolerance of non-pathogenic antigens while maintaining reactivity to infections (43). The germ-free mice could not heal *Leishmania* lesions, possibly due to a defect in Th2 function (17). Moreover, in 2005, conventionalized mice showed better responses than germ-free mice, though not as effective as fully conventional mice (23). Another interesting thing to mention is that conventional mice, exposed to commensal bacteria from the beginning, had memory cells responsive to *leishmania*-related antigens (LACK), facilitating a stronger immune response. This was in stark contrast to germ-free mice, which lacked such memory cells and thus had a diminished immune response (17).

On the other hand, Lopes et al. examined the innate immune system. In contrast to the findings focused on adaptive immunity, this study reported no significant differences in Th1 activity or pro-inflammatory cytokines (IFN-γ, TNF, IL) between germ-free and conventional mice. The numbers of CD4+, CD8+, and Tregs were not notably different between the two groups. The higher parasitic load and larger lesions in germ-free mice were attributed to innate immune system deficiencies. This study observed a higher number of CD11b+ myeloid cells, neutrophils, dermal macrophages, and monocyte-driven macrophages in germ-free mice. Additionally, the number of infected macrophages was higher and did not decrease over time compared to conventional mice. These differences were linked to impaired ROS activation of macrophages and their ability to polarize after antigen exposure (20).

In addition, compared to typical mice, these mice possessed more infected macrophages that did not diminish with time. Decreased ROS activation and macrophage polarization, essential for efficient pathogen clearance, have been connected to the compromised response in germ-free mice (20).

The importance of CD11b and neutrophils was also highlighted in the canine study. And all the exposed groups in this study had a higher level of these cells. In addition, a mild positive correlation was seen between the levels of fecal Actinobacteria and CD11+ cells (32).

Additionally, intentional microbial dysbiosis has been studied in the past. *Leishmania* infection dramatically reduced skin microbial diversity, with Staphylococcus species taking the lead, according to Gimblet et al. This change was associated with elevated levels of IL-17 and inflammatory reactions, suggesting a dysbiosis that may exacerbate the course of the illness. Interestingly, this microbial shift was not limited to the lesion area; it was also transferable

to other mice that were not affected, indicating that the *Leishmania* infection had a systemic influence on the skin microbiota (30) and Singh et al. found that, although unrelated to parasite load, the lesion size and level of pathology were greater in *Staphylococcus*-treated mice than in controls. This implies that by adjusting the immune response, the skin microbiota can affect how severe a disease is (31). To reduce the amount of *Lactobacillus* and *Streptococcus* in their usual microbiota, Lopes et al. also administered antibiotics to ordinary mice. Lower levels of ROS and macrophage activity were the outcome. On the other hand, germ-free mice's microbiota was restored, and the susceptibility phenotype was reversed when they were exposed to regular feces, indicating the important role that the microbiota plays in immunological function (20).

Similar findings were reported in human patients infected with *L. braziliensis*, where lesions showed decreased microbial diversity, dominated by *S. aureus* and Streptococcus (24, 26, 27, 30, 33).

All in all, preliminary evidence nominates *Bifidobacterium* and *Lactobacillus* as potentially beneficial taxa in VL models, while *Staphylococcus* control may be a priority in CL lesions; integrating host genetics, geography-shaped microbiomes, and vector ecology will be essential for any personalized microbiome-informed therapy.

Considering these findings on the effect of microbiota on the immune system and the great role of immune system responses on *Leishmania* pathogenicity and disease course, microbial modulation, such as the use of antibiotics and probiotics, can be more important than it is now. Further studies on this matter, especially on the use of probiotics in *Leishmania* patients, might be needed to illuminate this subject further. On the other hand, antibiotic-induced dysbiosis can impair macrophage ROS responses and skew myeloid populations, worsening lesion control in *L. major* models—an important caution for indiscriminate microbiota manipulation.

Geography, diet, sanitation, and exposure to other microbes/parasites shape baseline gut communities and may influence leishmaniasis outcomes and response to microbiota interventions; in CL, secondary bacterial infection and biofilms are common and can worsen healing, confounding lesion-level microbiome signals. Vector ecology (e.g., *Nyssomyia umbratilis* gut proteome/microbiota differences between endemic vs. non-endemic regions) adds another layer impacting transmission (44).

However, our study faced several limitations. A meta-analysis could have further enhanced the statistical robustness of our findings; yet, due to the considerable heterogeneity among the included studies—in methodology, outcome measures, and microbial analysis techniques—performing such an analysis was not feasible without risking misleading results. In addition, the current body of research on microbiota and leishmaniasis is still in its early stages, with a limited number of published studies available. Much of the evidence is derived from animal models, which, although informative, do not always fully replicate human disease conditions. Translation will require standardized sampling (skin vs. gut compartments), harmonized sequencing/analysis pipelines, and prospective human cohorts to test whether modulating microbiota changes outcomes. Innate cell programs differ between germ-free and conventional mice (e.g., $CD11b^{+}$ myeloid cells, neutrophils, dermal macrophages), which helps explain model-specific results and underscores the need to validate mechanisms in patients.

Furthermore, there is a lack of mechanistic and causal data, particularly concerning the role of the gut microbiota in leishmaniasis, making it difficult to establish direct functional relationships. Many findings also have uncertain clinical relevance, as they have not yet been validated in large, well-characterized human cohorts. These limitations highlight the need for future studies that incorporate standardized

methodologies, mechanistic investigations, and robust clinical data to advance our understanding of microbiota–parasite interactions.

Conclusion

All in all, in this review, we highlighted that leishmaniasis development and treatment are significantly influenced by the interplay between the immune system and the gut and skin microbiota. Most evidence derives from animal models, which limits clinical generalizability and highlights the need for more clinical human studies. However, it seems that maintaining a diversified and well-balanced microbiota is essential for a successful immune response and improved disease outcomes. Future treatment strategies must take the microbiota into account as a crucial element in the control of leishmaniasis.

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Ethics Statement

This manuscript has been approved by the Ethical Committee of Isfahan University of Medical Sciences. (Ethical Code: IR.ARI.MUI.REC.1403.066)

Conflict of interest

The authors declare no conflicts of interest.

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