Microbiota of Sand Flies

Subjects: Tropical Medicine

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Sand flies are a significant public health concern in many parts of the world where they are known to transmit agents of several zoonotic diseases to humans, such as leishmaniasis. Vector control remains a key component of many anti-leishmaniasis programs and probably will remain so until an effective vaccine becomes available. The sand fly gut microbiota has emerged as an encouraging field for the exploration of vector-based disease control. In particular, the gut microbiome was previously reported to either enhance or inhibit parasite activity depending on the species of bacteria and, thus, has the potential to alter vector competence.

Keywords: sand fly ; microbiome ; Leishmania ; vector competence

1. Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are tiny blood-sucking insects that feed on a wide range of hosts and potentially act as vectors of pathogens responsible for human and animal diseases worldwide. Of the more than 1000 sand fly species that have been described to date, approximately 10% are suspected or proven vectors of various pathogens, including arboviruses and bacteria, but are best known as the principal vectors of *Leishmania*, the etiological agent of leishmaniasis, a neglected tropical disease [1][2]. *Leishmania* have two main lifecycle stages: the motile flagellated promastigote, which is present in the sand fly vector, and the intracellular non-flagellated amastigote, which is present within mammalian host cells. Around 20 *Leishmania* species are known to be pathogenic to humans with clinical symptoms varying from localized, self-limiting cutaneous lesions and severe diffuse and destructive mucocutaneous lesions, to a disseminating visceral infection that is fatal in the absence of treatment [1]. The disease remains a public health problem worldwide, affecting approximately 12 million people in 98 countries, where 350 million people are at risk of infection [3]. An estimated 1.3 million new cases occur each year and cause 20,000 to 30,000 deaths per year [4]. At present, leishmaniasis is undergoing a type of synergy between natural phenomena and man-made conditions that facilitate the spread of the disease, with limited vaccines available and no effective therapeutic treatments against any form of human leishmaniasis [5][6]. Vector control is currently one of the most effective ways to prevent disease transmission. Advances that support evidence-based vector control can be leveraged to further optimize planning and implementation.

Over the last decade, microbial communities associated with sand flies gained relevance, as they have an important impact on the development of the parasite *Leishmania* in the host's digestive tract [7]. Since the application of molecular methods, novel culture-independent techniques have been developed to study microbial communities in sand flies [8][9], while several studies have used culture-dependent approaches [10][11]. The sand fly microbiota is a dynamic community mostly acquired from the environment, as in other Insecta or Diptera. Sand flies become infected with *Leishmania* when they feed on the blood of an infected host in order to develop eggs and reproduce. The *Leishmania* developmental life cycle within the sand fly vector occurs exclusively in the mid- and hindgut with the presence of symbiotic bacteria; therefore, possible bacteria-parasite interactions occur between the microbial community of the gut and parasite [12][13]. In addition to blood, they take sugar meals from a number of different sources, including the sap of plants or honeydew, through which they may acquire plant bacteria [14].

Although the role of the gut microbiome in the biological cycle of insects is widely recognized, factors modulating the composition of the gut microbiota of sand flies are still poorly defined. Lastly, many studies have aimed to clarify interactions among insect gut microbiota and diet, the local ecosystem and climatic conditions, phylogeny, and life stage. Previous studies reported that variation in the microbiota residing in the insect gut might be mainly explained by the host habitat, diet, developmental stage, and phylogeny all contributing to the structure of insect gut microbiota [15]. Sampling location has been reported to strongly affect the gut microbial community structure of mosquitoes [16], while the composition of the gut microbial community in turtle ants was better explained and influenced by host species [17].

A better understanding of vector-microbiota-pathogen interactions is vital, as it can lead to the discovery of new tools to block disease transmission and provide critical information for the development of intervention strategies for sand fly control. Scientists have discovered a new malaria transmission-blocking microbe in *Anopheles* mosquitoes [18] and *Wolbachia* endosymbionts have already been used successfully when introduced in *Aedes aegypti* to block the transmission of the dengue virus [19][20][21].

Paratransgenesis is an alternative approach to control disease transmission. In this strategy, bacterial flora native to disease-transmitting vectors are engineered to interfere with pathogen transmission [22][23]. The principal and essential step in paratransgenesis is the identification of suitable bacteria in the vector. These microorganisms should possess the main advantages of being non-pathogenic, easy to cultivate, and easy to genetically manipulate. For example, *Bacillus* (*B.*) *subtilis* and *Brevibacterium linens* isolated from the kala-azar vector *Phlebotomus* (*Ph.*) *argentipes* are currently being considered as possible candidates for paratransgenesis aimed at preventing *Leishmania* (*L.*) *donovani* transmission [24]. The same researchers illustrated the initial proof-of-concept of the paratransgenic approach to *Ph. argentipes* under laboratory conditions. They demonstrated the ability of Green Fluorescent Protein (GFP)- expressing *B. subtilis* to colonize the insect gut [25].

2. Diversity and Composition of the Microbiome in Sand Flies

2.1. Microbiota of Different Sand Fly Developmental Stages

Ingested food and environmental microbes generate larval and adult gut content microbiota. Previous studies on gut microbiota abundance showed that most of these larval-stage bacteria undergo biodegradation during the pupal stage and the bacterial load suddenly and significantly decreases after adult emergence [12][26]. The bacterial composition of larvae, pupae, and newly emerged adults of colony-reared *Ph. duboscqi* was initially investigated using standard bacteriological approaches [26]. *Ochrobactrum anthropi* was consistently the predominant bacterium at different growth stages, suggesting the presence of bacterial transtadial passage. A more recent study showed the occurrence of *Microbaterium* sp. in immature and adult stages of the same colony-reared sand fly species [12]. On the other hand, many bacterial species were identified in field and laboratory-reared sand flies from the same species [10]. This bacterium is known as a soil microorganism, indicating the influence of external microbial populations on the gut microbiota of immature sand fly stages [27]. This finding suggests that environments play important roles in the colonization of sand flies. The gut composition microbiota across larvae, pupae, and adults of *Lu. evansi* collected from the same locality was recently identified and included a number of soil microorganisms such as *Enterobacter*, *Pseudomonas*, *Bacillus*, and *Lysobacter* genera [28]. The presence of microbial strains in both larvae and adult sand flies will help to implement new efficient biological approaches for the control of sand fly populations in order to prevent *Leishmania* transmission. In a recent study, the genus *Lysinibacillus* was found in the immature (larvae) and adult stages, suggesting that species within this genus could remain transstadially associated with the sand fly [29]. It is important in this context to note that most reports focused on the gut microbial content of field and reared adult sand flies due to the inaccessibility of larvae and pupae in the field.

2.2. Microbiota of Wild and Laboratory Sand Flies

2.2.1. New World Sand Flies

Microbiota composition was analyzed in both laboratory strains and wild populations. The first publication on the gut microbiota composition of New World adult sand flies used a reared laboratory colony and different *Lu. longipalpis* populations collected from Brazil [30][31][32]. The composition of the adult midgut microbiota among different *Lu. longipalpis* populations and reared laboratory colonies showed shared opportunistic pathogenic, environmental, and gut-associated bacterial species including *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, *Enterobacter cloacae*, *Pseudomonas* sp., and *Serratia marcescens*. More recent studies on this sand fly species collected from field and laboratory-reared colonies showed that shared bacteria were *Pantoea*, *Serratia*, *Stenotrophomonas*, and *Erwinia* genera [13][14][30][31][32][33][34]. In the same way, *Staphylococcus*, *Clostridium*, and *Bacillus* genera belonging to the *Firmicutes* phylum were identified in *Lu. longipalpis* colony and field-captured insects [35][36][37]. These bacterial genera are known as pathogenic to several organisms, and the *Bacillus* genus is currently being considered as a possible candidate for paratransgenesis aimed at preventing *Leishmania* infection [38][39]. These findings indicate the capacity of some bacteria to persist in this sand fly species, despite the difference in field and laboratory conditions.

2.2.2. Old World Sand Flies

Microbiota composition was analyzed in both laboratory strains and wild *Ph. perniciosus* populations collected from Tunisia [10]. *Stenotrophomonas maltophilia*, *Bacillus* sp., and *Lysinibacillus* sp. were identified in both groups of sand flies. These findings indicate that vector control strategies based on modern biotechnological tools in the laboratory might be applicable in the field.

2.3. Microbiota and Sand Fly Species

The microbiota composition of sand flies was largely summarized in a previous meta-analysis study that included all data obtained until 2017 [10].

2.3.1. New World Sand Fly Species

The bacterial flora shared between different New World sand fly species collected from the field can be compared with the microbiota of field and laboratory-reared sand flies from the same species. Currently, bacterial communities have been investigated in seven New World sand fly species collected in the field: *Lu. evansi* [28], *Lu. longipalpis* [14][30][32][33], *Lu. cruzi* [14], *Lu. intermedia* [40], *Nyssomyia* (*Ny.*) *neivai* (synonymous *Lu. neivai*) [41], *Lu. ayacuchensis* [42], and *Pintomyia (Pi.) (Pifanomyia) evansi* [43]. Very few shared bacteria were identified in these sand fly species collected from the field, probably due to diverse habitats and blood host origins. *Staphylococcus agnetis*, potentially pathogenic to poultry [44] and associated with bovine mastitis [45], was shared among *Lu. cruzi*, *Lu. evansi*, and *Lu. longipalpis*. *Pelomonas* sp. was shared between *Ny. neivai* and *Lu. intermedia*, also found in other insects [46]. Three universal bacterial species were identified in three sand fly species: *Lu. evansi*, *Lu. intermedia*, and *Lu. longipalpis: Acinetobacter calcoaceticus*, known for triggering a detectable immune response in tsetse flies [47], *Enterobacter aerogenes* (found in other insects and potential pathogen to humans) [48], and *Pseudomonas putida* (associated with soil and water) [49][50]. On the other hand, *Ralstonia* sp. (a plant-associated species) [14] was shared among *Ny. neivai*, *Lu. intermedia*, and *Pi. evansi*; *Lawsonella* sp. and *Corynebactrium* sp. (found in other insects) [51][52] between *Lu. ayacuchensis* and *Lu. evansi*; *Escherichia* sp. between *Lu. longipalpis* and *Lu. evansi*.

2.3.2. Old World Sand Fly Species

The microbial gut content of *Ph. papatasi* females has been largely explored. The first publication identified a species pathogenic to humans, *Enterobacter cloaceae* [53], from Egyptian sand flies [54]. More recently, *Microbacterium* sp., pathogenic to insects [55], was detected in Moroccan sand flies [12]. Several groups of researchers conducted the same kinds of experiments in Tunisia, Turkey, and India, and the *Bacillus* genus was the most dominant among genera [56]. Similar results were obtained in Iran and several bacteria genera and species were identified including *Acinetobacter*, *Enterobacter*, *Microbacterium*, *Staphylococcus*, *Terribacillus*, *B. cereus*, *B. flexus*, *B. licheniformis*, *B. pumilus*, *B. subtilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*. This last species was previously found associated with wild *Lu. longipalpis* [33] and is also lethal to *Leishmania* in vitro [57]. A recent study showed that *B. subtilis* and *Enterobacter cloacae* were shared among the *Ph. papatasi* habitat, rodent *Rhombomys opimus*, and sand fly gut [58]. Other studies on the gut microbial contents of different sand fly species in Iran and India including *Ph. sergenti*, *Ph. kandelakii*, *Ph. perfiliewi*, *Ph. halepensis*, and *Ph. argentipes* showed that they share the *Bacillus* genus with *Ph. papatasi* [11][24][59][60]. The *Pseudomonas* genus was identified recently in *Ph. chinensis* collected from China and shared with the sand flies cited above, except *Ph. argentipes* [8]. In a report, the researchers showed the influence of both sand fly species and habitats on the microbial gut content of *Ph. perniciosus* collected from Tunisia [10]. An extensive meta-analysis showed proportions of *Acinetobacter baumanii*, *Escherichia coli*, *Stenotrophamonas maltophila*, *B. subtilis*, *Staphylococcus epidermidis*, *Acinetobacter* sp., *Enterobacter* sp. *Klebsiella ozaenae*, and *Serratia* sp. among at least three phlebotomine insects from the Old and New World. A more recent paper showed that host species determine the composition of the prokaryotic microbiota in *Phlebotomus* sand flies collected from Greece [61]. *Ph. papatasi* microbiota was the most distinct from *Ph. Neglectus*, *Ph. tobbi*, and *Ph. similis*, dominated by *Spiroplasma*, *Wolbachia*, and *Paenibacillus.*

3. Gut Microbiota Alterations and Their Impact on Flies' Life Traits and *Leishmania* Infection

3.1. Gut Microbiota Alterations and Their Impact on Flies' Life Traits

A previous study reported that the sand fly gut microbiota influences different aspects of flies' life traits [62]. According to this report, the process of laying eggs was more efficient in *Lu. longipalpis* flies fed on rabbit feces than those fed on sterilized feces, which eliminates all rabbit intestinal track-supplied bacteria. The larvae from this last habitat showed delayed hatching and lower survival rates. When different bacteria were reintroduced into sterile feces, there were wide differences in hatching time and survival. On the other hand, it has been demonstrated that all L1-larvae were hatched

from homogeneous disinfected eggs and developed on sterilized material [63]. It is important to mention that although bacterial diversity decreases after a blood meal, bacterial numbers actually increase [13][26]. These findings provide new data on microbial dynamics in the sand fly gut which may be used for the development of novel control strategies. The larval nutrition associated with the putative breeding sites of the sand fly *Lu. longipalpis* might affect their oviposition, development, microbiome, and susceptibility to *Leishmania* which plays an important role in the epidemiology of leishmaniasis [63].

3.2. Gut Microbiota Alterations and Their Impact on Leishmania Infection

The influence of the microbial contents on *Leishmania* development in sand flies was investigated in several previous studies. *Serratia marcescens*, which are considered to be pathogenic bacteria for many insects [64], negatively affect *L. infantum chagasi* and *L. braziliensis* by inducing lysis in vitro of the parasite cell membrane [57][65]. Furthermore, it has been demonstrated in vivo that the infection rate of *L. mexicana* in *Lu. longipalpis* sand flies was reduced when fed on *Pseudozyma* sp., *Asaia* sp., or *Ochrobactrum intermedium* [66]. The same experiments, in which *L. mexicana* colonized the sand fly gut prior to being fed *Serratia marcescens*, showed that the survival of flies with a *Leishmania* infection was significantly higher compared with those without *Leishmania* infection. This might be due to the protection offered by *Leishmania* to the sand fly from the bacterial infection or to modulation of the host immunity response by this parasite, as reported in other models such as *Anopheles gambiae* infected with *Plasmodium* [67]. In the same context, *Ph. papatasi* was treated with *an* antibiotic cocktail to deplete gut bacteria and was experimentally infected by *Leishmania*. The bacterial composition of the gut was previously reported to either enhance or inhibit *Leishmania* activity. Previous studies showed that treatment with antibiotics reduces the richness and diversity of microbiota, but *Leishmania* infection increases, indicating that the microbiota can be a barrier to the establishment and development of promastigotes in *Ph. papatasi* and *Pi. evansi* [43][68]. These findings strengthened the theory that any manipulation that reduces the size and/or diversity of the natural microbiota should enhance the ability of *Leishmania* to establish infections in sand flies or other pathogens in mosquitoes [69]. It has been demonstrated that endosymbionts, such as Microsporidia infections, were more frequently associated with guts without *Leishmania* infection, whereas *Arsenophonus* was only found in guts with a high load of *Leishmania* infection and treated with antibiotics [43]. It has been shown that Microsporodia impairs *Plasmodium falciparum* transmission in *Anopheles arabiensis* mosquitoes [70]. This finding is in agreement with the previous study of the potential influence of this endosymbiont on *Leishmania*. On the other hand, in *Ph. dubosqui* and *Lu. longipalpis*, treatment with antibiotics results in females being highly refractory to the development of transmissible infections [7][13]. It has been demonstrated, for example, that sucrose utilization by the microbiota is essential to promote the appropriate osmotic conditions required for the survival of infective stage promastigotes in vivo [7]. Together, these diverse data suggest that the sand fly midgut microbiome is a critical factor for *Leishmania* growth and differentiation to its infective state prior to disease transmission. As part of a paratransgenic approach, further studies are needed to identify candidate bacteria that can be used, or other biological approaches, to control sand fly populations and *Leishmania* transmission [10]. A more recent study showed that *Lysinibacillus*, *Pseudocitrobacter*, and *Serratia*, which are potential candidates for paratransgenic or biological control, strongly inhibited *Leishmania* growth and survival in vitro and co-infected *Lu. longipalpis* [29].

4. Fungi Associated with the Midgut of Sand Flies

Although the bacterial component of sand fly microbiota has been investigated in several studies, few papers reported on the fungal diversity in sand flies [33][59][71]. A comparative analysis of fungal communities revealed the absence of fungi in *Lu. longipalpis* guts collected from an endemic area, whereas fungi were found in a non-endemic area, including *Cunninghamella bertholletiae*, *Peronospora conglomerata*, *Mortierella verticillata*, and *Toxicocladosporium irritans* [33], suggesting that fungi are excluded in the presence of *Leishmania*. However, contradictory findings identified fungal genera in sand flies collected from endemic areas of northern Iran and southern Peru, including *Ph. papatasi*, *Ph. sergenti*, *Ph. kandelakii*, *Ph. perfiliewi*, *Ph. halepensis*, and *Lu. ayachensis* [42][59]. In these areas, species belonging to *Penicillium*, *Aspergillus*, *Acremonium*, *Fusarium*, *Geotrichum*, *Candida*, and *Malassezia* genera were identified [42][59][71]. However, it was not possible to elucidate their role or conclude any outcomes regarding potential pathogenic effects or interactions with *Leishmania*.

References

1. Maroli, M.; Feliciangeli, M.D.; Bichaud, L.; Charrel, R.N.; Gradoni, L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. Med. Vet. Entomol. 2013, 27, 123–147.

2. Akhoundi, M.; Kuhls, K.; Cannet, A.; Votypka, J.; Marty, P.; Delaunay, P.; Sereno, D. A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. PLoS Negl. Trop. Dis. 2016, 10, e0004349.
3. Alidosti, M.; Heidari, Z.; Shahnazi, H.; Zamani-Alavijeh, F. Behaviors and perceptions related to cutaneous leishmaniasis in endemic areas of the world: A review. Acta Trop. 2021, 223, 106090.
4. World Health Organization (WHO). Leishmania Fact Sheet Number 375. 2016. Available online: http://www.who.int/mediacentre/factsheets/fs375/en/ (accessed on 3 February 2022).
5. Sereno, D.; Maia, C.; Aït-Oudhia, K. Antimony resistance and environment: Elusive links to explore during Leishmania life cycle. Int. J. Parasitol. Drugs Drug Resist. 2012, 2, 200–203.
6. Seblova, V.; Oury, B.; Eddaikra, N.; Ait-Oudhia, K.; Pratlong, F.; Gazanion, E.; Maia, C.; Volf, P.; Sereno, D. Transmission potential of antimony-resistant Leishmania field isolates. Antimicrob. Agents Chemother. 2014, 58, 6273–6276.
7. Louradour, I.; Monteiro, C.C.; Inbar, E.; Ghosh, K.; Merkhofer, R.; Lawyer, P.; Paun, A.; Smelkinson, M.; Secundino, N.; Lewis, M.; et al. The midgut microbiota plays an essential role in sand fly vector competence for Leishmania major. Cell. Microbiol. 2017, 19, e12755.
8. Li, K.; Chen, H.; Jiang, J.; Li, X.; Xu, J.; Ma, Y. Diversity of bacteriome associated with Phlebotomus chinensis (Diptera: Psychodidae) sand flies in two wild populations from China. Sci. Rep. 2016, 6, 36406.
9. Vivero, R.J.; Villegas-Plazas, M.; Cadavid-Restrepo, G.E.; Herrera, C.X.M.; Uribe, S.I.; Junca, H. Wild specimens of sand fly phlebotomine Lutzomyia evansi, vector of leishmaniasis, show high abundance of Methylobacterium and natural carriage of Wolbachia and Cardinium types in the midgut microbiome. Sci. Rep. 2019, 9, 17746.
10. Fraihi, W.; Fares, W.; Perrin, P.; Dorkeld, F.; Sereno, D.; Barhoumi, W.; Sbissi, I.; Cherni, S.; Chelbi, I.; Durvasula, R.; et al. An integrated overview of the midgut bacterial flora composition of Phlebotomus perniciosus, a vector of zoonotic visceral leishmaniasis in the Western Mediterranean Basin. PLOS Negl. Trop. Dis. 2017, 11, e0005484.
11. Karimian, F.; Vatandoost, H.; Rassi, Y.; Maleki-Ravasan, N.; Mohebali, M.; Shirazi, M.H.; Koosha, M.; Choubdar, N.; Oshaghi, M.A. Aerobic midgut microbiota of sand fly vectors of zoonotic visceral leishmaniasis from northern Iran, a step toward finding potential paratransgenic candidates. Paras. Vector 2019, 12, 10.
12. Guernaoui, S.; Garcia, D.; Gazanion, E.; Ouhdouch, Y.; Boumezzough, A.; Pesson, B.; Fontenille, D.; Sereno, D. Bacterial flora as indicated by PCR-temperature gradient gel electrophoresis (TGGE) of 16S rDNA gene fragments from isolated guts of phlebotomine sand flies (Diptera: Psychodidae). J. Vector Ecol. 2011, 36, S144–S147.
13. Kelly, P.H.; Bahr, S.M.; Serafim, T.D.; Ajami, N.J.; Petrosino, J.F.; Meneses, C.; Kirby, J.R.; Valenzuela, J.G.; Kamhawi, S.; Wilson, M.E. The Gut Microbiome of the Vector Lutzomyia longipalpis Is Essential for Survival of Leishmania infantum. Mbio 2017, 8, e01121-16.
14. Sant'Anna, M.R.V.; Darby, A.C.; Brazil, R.P.; Montoya-Lerma, J.; Dillon, V.M.; Bates, P.A.; Dillon, R.J. Investigation of the Bacterial Communities Associated with Females of Lutzomyia Sand Fly Species from South America. PLoS ONE 2012, 7, e42531.
15. Yun, J.-H.; Roh, S.W.; Whon, T.W.; Jung, M.-J.; Kim, M.-S.; Park, D.-S.; Yoon, C.; Nam, Y.-D.; Kim, Y.-J.; Choi, J.-H.; et al. Insect Gut Bacterial Diversity Determined by Environmental Habitat, Diet, Developmental Stage, and Phylogeny of Host. Appl. Environ. Microbiol. 2014, 80, 5254–5264.
16. Muturi, E.J.; Lagos-Kutz, D.; Dunlap, C.; Ramirez, J.L.; Rooney, A.P.; Hartman, G.L.; Fields, C.J.; Rendon, G.; Kim, C.-H. Mosquito microbiota cluster by host sampling location. Paras. Vector 2018, 11, 468.
17. Sanders, J.G.; Powell, S.; Kronauer, D.J.; Vasconcelos, H.L.; Frederickson, M.E.; Pierce, N.E. Stability and phylogenetic correlation in gut microbiota: Lessons from ants and apes. Molec. Ecol. 2014, 23, 1268–1283.
18. Wang, S.; Jacobs-Lorena, M. Genetic approaches to interfere with malaria transmission by vector mosquitoes. Trends. Biotechnol. 2013, 31, 185–193.
19. Moreira, L.A.; Iturbe-Ormaetxe, I.; Jeffery, J.A.; Lu, G.; Pyke, A.T.; Hedges, L.M.; Rocha, B.C.; Hall-Mendelin, S.; Day, A.; Riegler, M.; et al. Wolbachia symbiont in Aedes aegypti limits infection with dengue, Chikungunya, and Plasmodium. Cell 2009, 139, 1268–1278.
20. Ye, Y.H.; Carrasco, A.M.; Frentiu, F.D.; Chenoweth, S.F.; Beebe, N.W.; van den Hurk, A.F.; Simmons, C.P.; O'Neill, S.L.; McGraw, E.A. Wolbachia reduces the transmission potential of dengue-infected Aedes aegypti. PLOS Negl. Trop. Dis. 2015, 9, e0003894.
21. Joshi, D.; Pan, X.; McFadden, M.J.; Bevins, D.; Liang, X.; Lu, P.; Thiem, S.; Xi, Z. The maternally inheritable Wolbachia wAlbB induces refractoriness to Plasmodium berghei in Anopheles stephensi. Front. Microbiol. 2017, 8, 366.

22. Coutinho-Abreu, I.V.; Zhu, K.Y.; Ramalho-Ortigao, M. Transgenesis and paratransgenesis to control insect-borne diseases: Current status and future challenges. Parasitol. Int. 2010, 59, 1–8.

23. Hurwitz, I.; Fieck, A.; Read, A.; Hillesland, H.; Klein, N.; Kang, A.; Durvasula, R. Paratransgenic control of vector borne diseases. Int. J. Biol. Sci. 2011, 7, 1334–1344.

24. Hillesland, H.; Read, A.; Subhadra, B.; Hurwitz, I.; McKelvey, R.; Ghosh, K.; Das, P.; Durvasula, R. Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: A platform for potential paratransgenic manipulation of sand flies. Am. J. Trop. Med. Hyg. 2008, 79, 881–886.

25. Hurwitz, I.; Hillesland, H.; Fieck, A.; Das, P.; Durvasula, R. The paratransgenic sand fly: A platform for control of Leishmania transmission. Parasit. Vectors 2011, 4, 82.

26. Volf, P.; Kiewegova, A.; Nemec, A. Bacterial colonisation in the gut of Phlebotomus duboseqi (Diptera: Psychodidae): Transtadial passage and the role of female diet. Folia. Parasitol. 2002, 49, 73–77.

27. Zhang, H.B.; Shi, W.; Yang, M.X.; Sha, T.; Zhao, Z.W. Bacterial diversity at different depths in lead-zinc mine tailings as revealed by 16S rRNA gene libraries. J. Microbiol. 2007, 45, 479–484.

28. Vivero, R.J.; Jaramillo, N.G.; Cadavid-Restrepo, G.; Soto, S.I.; Herrera, C.X. Structural differences in gut bacteria communities in developmental stages of natural populations of Lutzomyia evansi from Colombia's Caribbean coast. Parasit. Vector 2016, 9, 496.

29. Campolina, T.B.; Villegas, L.E.M.; Monteiro, C.C.; Pimenta, P.F.P.; Secundino, N.F.C. Tripartite interactions: Leishmania, microbiota and Lutzomyia longipalpis. PLoS Negl. Trop. Dis. 2020, 14, e0008666.

30. Oliveira, S.M.; Moraes, B.A.; Goncalves, C.A.; Giordano-Dias, C.M.; D'Almeida, J.M.; Asensi, M.D.; Mello, R.P.; Brazil, R.P. Prevalence of microbiota in the digestive tract of wild females of Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae). Rev. Soc. Bras. Med. Trop. 2000, 33, 319–322.

31. Perira de Oliveira, S.M.; de Morais, B.A.; Goncalves, C.A.; Giordano-Dias, C.M.; Vilela, M.L.; Brazil, R.P.; D'Almeida, J.M.; Asensi, M.D.; Mello, R.P. Digestive tract microbiota in female Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae) from colonies feeding on blood meal and sucrose plus blood meal. Cad. Saude Publica. 2001, 17, 229–232.

32. Gouveia, C.; Asensi, M.D.; Zahner, V.; Rangel, E.F.; Oliveira, S.M. Study on the bacterial midgut microbiota associated to different Brazilian populations of Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae). Neotrop. Entomol. 2008, 37, 597–601.

33. McCarthy, C.B.; Diambra, L.A.; Rivera Pomar, R.V. Metagenomic analysis of taxa associated with Lutzomyia longipalpis, vector of visceral leishmaniasis, using an unbiased high-throughput approach. PLoS Negl. Trop. Dis. 2011, 5, e1304.

34. Pires, A.; Villegas, L.E.M.; Campolina, T.B.; Orfano, A.S.; Pimenta, P.F.P.; Secundino, N.F.C. Bacterial diversity of wild-caught Lutzomyia longipalpis (a vector of zoonotic visceral leishmaniasis in Brazil) under distinct physiological conditions by metagenomics analysis. Parasit. Vector 2017, 10, 627.

35. Gupta, A.K.; Rastogi, G.; Nayduch, D.; Sawant, S.S.; Bhonde, R.R.; Shouche, Y.S. Molecular phylogenetic profiling of gut-associated bacteria in larvae and adults of flesh flies. Med. Vet. Entomol. 2014, 28, 345–354.

36. Ngo, C.T.; Romano-Bertrand, S.; Manguin, S.; Jumas-Bilak, E. Diversity of the bacterial microbiota of anopheles' mosquitoes from Binh Phuoc Province, Vietnam. Front. Microbiol. 2016, 7, 2095.

37. Garofalo, C.; Osimani, A.; Milanovic, V.; Taccari, M.; Cardinali, F.; Aquilanti, L.; Riolo, P.; Ruschioni, S.; Isidoro, N.; Clementi, F. The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. Food Microbiol. 2017, 62, 15–22.

38. Robert, L.L.; Perich, M.J.; Schlein, Y.; Jacobson, J.L. Bacillus sphaericus inhibits hatching of phlebotomine sand fly eggs. J. Am. Mosq. Control Assoc. 1998, 14, 351–352.

39. Wahba, M.M.; Labib, I.M.; el Hamshary, E.M. Bacillus thuringiensis var. israelensis as a microbial control agent against adult and immature stages of the sandfly, Phlebotomus papatasi under laboratory conditions. J. Egypt. Soc. Parasitol. 1999, 29, 587–597.

40. Monteiro, C.C.; Villegas, L.E.; Campolina, T.B.; Pires, A.C.; Miranda, J.C.; Pimenta, P.F.; Secundino, N.F. Bacterial diversity of the American sand fly Lutzomyia intermedia using high-throughput metagenomics sequencing. Parasit. Vector 2016, 9, 480.

41. Machado, V.E.; Martins, P.M.; Ferreira, H.; Ferro, M.; Bacci, M.; Pinto, M.C. Bacterial groups associated with Nyssomyia neivai (Diptera: Psychodidae) sand flies. J. Vector Borne Dis. 2014, 51, 137–139.

42. Tabbabi, A.; Watanabe, S.; Mizushima, D.; Caceres, A.G.; Gomez, E.A.; Yamamoto, D.S.; Cui, L.; Hashiguchi, Y.; Kato, H. Comparative Analysis of Bacterial Communities in Lutzomyia ayacuchensis Populations with Different Vector Competence to Leishmania Parasites in Ecuador and Peru. Microorganisms 2020, 9, 68.

43. Vivero, R.J.; Castañeda-Monsalve, V.A.; Romero, L.R.D.; Hurst, G.; Cadavid-Restrepo, G.; Moreno-Herrera, C.X. Gut Microbiota Dynamics in Natural Populations of Pintomyia evansi under Experimental Infection with Leishmania infantum. Microorganisms 2021, 9, 1214.

44. Poulsen, L.L.; Thofner, I.; Bisgaard, M.; Olsen, R.H.; Christensen, J.P.; Christensen, H. Staphylococcus agnetis, a potential pathogen in broiler breeders. Vet. Microbiol. 2017, 212, 1–6.

45. Lange, C.C.; Brito, M.A.; Reis, D.R.; Machado, M.A.; Guimaraes, A.S.; Azevedo, A.L.; Salles, E.B.; Alvim, M.C.; Silva, F.S.; Meurer, I.R. Species-level identification of staphylococci isolated from Bovine mastitis in Brazil using partial 16S rRNA sequencing. Vet. Microbiol. 2015, 176, 382–388.

46. Montoya-Porras, L.M.; Omar, T.C.; Alzate, J.F.; Moreno-Herrera, C.X.; Cadavid-Restrepo, G.E. 16S rRNA gene amplicon sequencing reveals dominance of Actinobacteria in Rhodnius pallescens compared to Triatoma maculata midgut microbiota in natural populations of vector insects from Colombia. Acta Trop. 2018, 178, 327–332.

47. Kaaya, G.P.; Otieno, L.H.; Darji, N.; Alemu, P. Defence reactions of Glossina morsitans morsitans against different species of bacteria and Trypanosoma brucei brucei. Acta Trop. 1986, 43, 31–42.

48. Memona, H.; Manzoor, F.; Anjum, A.A. Cockroaches (Blattodea: Blattidae): A reservoir of pathogenic microbes in human-dwelling localities in Lahore. J. Med. Entomol. 2017, 54, 435–440.

49. Nicoletti, G.; Corbella, M.; Jaber, O.; Marone, P.; Scevola, D.; Faga, A. Non-pathogenic microflora of a spring water with regenerative properties. Biomed. Rep. 2015, 3, 758–762.

50. Colauto, N.B.; Fermor, T.R.; Eira, A.F.; Linde, G.A. Pseudomonas putida Stimulates Primordia on Agaricus bitorquis. Curr. Microbiol. 2016, 72, 482–488.

51. McMullen, A.R.; Anderson, N.; Wallace, M.A.; Shupe, A.; Burnham, C.A. When Good Bugs Go Bad: Epidemiology and Antimicrobial Resistance Profiles of Corynebacterium striatum, an Emerging Multidrug-Resistant, Opportunistic Pathogen. Antimicrob. Agents Chemother. 2017, 61, e01111-17.

52. Panteli, N.; Mastoraki, M.; Lazarina, M.; Chatzifotis, S.; Mente, E.; Kormas, K.A.; Antonopoulou, E. Configuration of Gut Microbiota Structure and Potential Functionality in Two Teleosts under the Influence of Dietary Insect Meals. Microorganisms 2021, 9, 699.

53. Nagy, E.; Pragai, Z.; Koczian, Z.; Hajdu, E.; Fodor, E. Investigation of the presence of different broad-spectrum beta-lactamases among clinical isolates of Enterobacteriacae. Acta Microbiol. Immunol. Hung. 1998, 45, 433–446.

54. Dillon, R.J.; Kordy, E.E.; Shehata, M.; Lane, R.P. The prevalence of a microbiota in the digestive tract of Phlebotomus papatasi. Ann. Trop. Med. Parasitol. 1996, 90, 669–673.

55. Thakur, A.; Dhammi, P.; Saini, H.S.; Kaur, S. Pathogenicity of bacteria isolated from gut of Spodoptera litura (Lepidoptera: Noctuidae) and fitness costs of insect associated with consumption of bacteria. J. Invertebr. Pathol. 2015, 127, 38–46.

56. Mukhopadhyay, J.; Braig, H.R.; Rowton, E.D.; Ghosh, K. Naturally occurring culturable aerobic gut flora of adult Phlebotomus papatasi, vector of Leishmania major in the Old World. PLoS ONE 2012, 7, e35748.

57. Moraes, C.S.; Seabra, S.H.; Castro, D.P.; Brazil, R.P.; de Souza, W.; Garcia, E.S.; Azambuja, P. Leishmania (Leishmania) chagasi interactions with Serratia marcescens: Ultrastructural studies, lysis and carbohydrate effects. Exp. Parasitol. 2008, 118, 561–568.

58. Maleki-Ravasan, N.; Oshaghi, M.A.; Afshar, D.; Arandian, M.H.; Hajikhani, S.; Akhavan, A.A.; Yakhchali, B.; Shirazi, M.H.; Rassi, Y.; Jafari, R.; et al. Aerobic bacterial flora of biotic and abiotic compartments of a hyperendemic Zoonotic Cutaneous Leishmaniasis (ZCL) focus. Parasit. Vectors 2015, 8, 63.

59. Akhoundi, M.; Bakhtiari, R.; Guillard, T.; Baghaei, A.; Tolouei, R.; Sereno, D.; Toubas, D.; Depaquit, J.; Abyaneh, M.R. Diversity of the bacterial and fungal microflora from the midgut and cuticle of phlebotomine sand flies collected in North-Western Iran. PLoS ONE 2012, 7, e50259.

60. Gunathilaka, N.; Perera, H.; Wijerathna, T.; Rodrigo, W.; Wijegunawardana, N.D.A.D. The Diversity of Midgut Bacteria among Wild-Caught Phlebotomus argentipes (Psychodidae: Phlebotominae), the Vector of Leishmaniasis in Sri Lanka. Biomed. Res. Int. 2020, 2020, 5458063.

61. Papadopoulos, C.; Karas, P.A.; Vasileiadis, S.; Ligda, P.; Saratsis, A.; Sotiraki, S.; Karpouzas, D.G. Host Species Determines the Composition of the Prokaryotic Microbiota in Phlebotomus Sandflies. Pathogens 2020, 9, 428.

62. Peterkova-Koci, K.; Robles-Murguia, M.; Ramalho-Ortigao, M.; Zurek, L. Significance of bacteria in oviposition and larval development of the sand fly Lutzomyia longipalpis. Parasites and Vectors. Parasit Vectors 2012, 5, 145.
63. Aguiar Martins, K.; Meirelles, M.H.A.; Mota, T.F.; Abbasi, I.; de Queiroz, A.T.L.; Brodskyn, C.I.; Veras, P.S.T.; Mothé Fraga, D.B.; Warburg, A. Effects of larval rearing substrates on some life-table parameters of Lutzomyia longipalpis sand flies. PLoS Negl. Trop. Dis. 2021, 15, e0009034.
64. Grimont, P.A.; Grimont, F.; Le Minor, S.; Davis, B.; Pigache, F. Compatible results obtained from biotyping and serotyping in Serratia marcescens. J. Clin. Microbiol. 1979, 10, 425–432.
65. Moraes, C.S.; Seabra, S.H.; Albuquerque-Cunha, J.M.; Castro, D.P.; Genta, F.A.; de Souza, W.; Brazil, R.P.; Garcia, E.S.; Azambuja, P. Prodigiosin is not a determinant factor in lysis of Leishmania (Viannia) braziliensis after interaction with Serratia marcescens D-mannose sensitive fimbriae. Exp. Parasitol. 2009, 122, 84–90.
66. Sant'anna, M.R.; Diaz-Albiter, H.; Aguiar-Martins, K.; Salem, A.S.W.; Cavalcante, R.R.; Dillon, M.V.; Bates, P.A.; Genta, F.A.; Dillon, R.J. Colonisation resistance in the sandfly gut: Leishmania protects Lutzomyia longipalpis from bacterial infection. Parasit. Vectors 2014, 1, 329–339.
67. Simões, M.L.; Dimopoulos, G. A mosquito mediator of parasite-induced immune priming. Trends Parasitol. 2015, 31, 402–404.
68. Hassan, M.I.; Al-Sawaf, B.M.; Fouda, M.A.; Al-Hosry, S.; Hammad, K.M. A Recent Evaluation of the Sandfly, Phlepotomus Papatasi Midgut Symbiotic Bacteria Effect on the Survivorship of Leishmania Major. J. Anc. Dis. Prev. Rem. 2014, 2, 110.
69. Dong, Y.; Manfredini, F.; Dimopoulos, G. Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLoS Pathog. 2009, 5, e1000423.
70. Herren, J.K.; Mbaisi, L.; Mararo, E.; Makhulu, E.E.; Mobegi, V.A.; Butungi, H.; Mancini, M.; Oundo, J.W.; Teal, E.T.; Pinaud, S.; et al. A microsporidian impairs Plasmodium falciparum transmission in Anopheles arabiensis mosquitoes. Nat. Commun. 2020, 11, 2187.
71. Schlein, Y.; Polacheck, I.; Yuval, B. Mycoses, bacterial infections and antibacterial activity in sand flies (Psychodidae) and their possible role in the transmission of leishmaniasis. Parasitology 1985, 90, 57–66.