FUNDAÇÃO OSWALDO CRUZ
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Programa de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa

TESE DE DOUTORADO

CARACTERIZAÇÃO DA MICROBIOTA DE ARTRÓPODES VETORES ASSOCIADA A COMPETÊNCIA VETORIAL

TIAGO FEITOSA MOTA

Salvador - Bahia
2023

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Tese apresentada ao curso de Biotecnologia em Saúde e Medicina Investigativa, Fundação Oswaldo Cruz, como requisito para obtenção do título de Doutor em Ciências.

Orientador: Dr. Artur Trancoso Lopo de Queiroz
Coorientadora: Dra. Deborah Bittencourt Mothé Fraga

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TIAGO FEITOSA MOTA

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MOTA, Tiago Feitosa. **Caracterização da microbiota de artrópodes vetores associada a competência vetorial.** 2023. 105 f. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 2023.

RESUMO

INTRODUÇÃO: Doenças transmitidas por vetores impactam a saúde em todo o mundo colocando em risco em torno de 80% da população mundial. Dentre os vetores artrópodes responsáveis pela transmissão dessas doenças destacam-se mosquitos e carrapatos, onde algumas espécies possuem competência vetorial (CV) de transmitirem um patógeno, vetores mono competentes (VMC), ou diversos patógenos, vetores pluri competentes (VPC). Além disso, em algumas infecções virais em artrópodes há diferenças na susceptibilidade em diferentes espécies de um determinado grupo de artrópodes. Tais diferenças na CV podem estar associadas à microbiota do artrópode que participa na modulação da resposta imune ou homeostase metabólica do hospedeiro. O estudo da microbiota permite detectar algumas espécies ou gêneros de bactéria que estão associadas a um perfil de susceptibilidade ou refratariedade. Essas bactérias são importantes para o estudo de novos métodos de controle biológico que visam modular a microbiota diminuindo ou bloqueando a infecção de um patógeno no artrópode. **OBJETIVO**: Assim, o objetivo do presente trabalho foi de identificar biomarcadores bacterianos em mosquitos e carrapatos associados à CV. **MÉTODO**: Para classificar as espécies de carrapato quanto a um perfil VMC ou VPC, foi realizada uma revisão de literatura sobre estudos de CV de espécies de carrapato com dados de sequenciamento de 16S rRNA bacteriano. Após a classificação, os dados de microbioma de carrapatos foram selecionados, processados e analisados em conjunto comparando a microbiota de carrapatos VMC e VPC. No caso de mosquitos, foram selecionados dados de 16S rRNA bacteriano de espécies refratárias ou suscetíveis ao vírus Zika (ZIKV). Assim, amostras de espécies de mosquito refratárias e suscetíveis foram selecionadas, processadas e analisadas em conjunto comparando os perfis de susceptibilidade ao ZIKV. Em ambos os trabalhos a composição bacteriana, diversidades alfa e beta, análise diferencial de abundância, análise funcional e redes de co-ocorrência foram avaliadas a fim de caracterizar a microbiota. **RESULTADOS**: Bactérias dos gêneros *Rickettsia*, *Staphylococcus* e *Corynebacterium* foram associadas à carrapatos VPC. A diversidade alfa foi maior em carrapatos VPC e houve diferenças estatisticamente significantes na diversidade beta. Vias metabólicas associadas ao controle do estresse oxidativo, dTDP-L-rhamnose e resistência a β-Lactam foram mais abundantes neste grupo. Além disso, a comunidade bacteriana se mostrou menos complexa e conectada em carrapatos VPC. Em relação aos mosquitos suscetíveis ou refratários ao ZIKV, *Pseudomonas*, *Serratia*, *Halomonas* e *Wolbachia* associadas a um perfil de susceptibilidade. Não houve diferenças estatisticamente significantes na diversidade microbiana entre os mosquitos suscetíveis ou refratários. Por outro lado, os mosquitos suscetíveis ao ZIKV se mostraram com um metabolismo mais enriquecido com redes de co-ocorrência menos densas e conectadas. **CONCLUSÃO**: O presente trabalho caracterizou a microbiota de vetores artrópodes associada à sua CV identificando alguns gêneros de bactéria que podem ser investigadas em futuros trabalhos a fim de reduzir a CV de artrópodes. Além disso, identificou algumas vias metabólicas que podem estar relacionadas a uma maior susceptibilidade para um ou mais patógenos.

Palavras-chave: Microbioma. Competência vetorial. Carrapato. Mosquito. Susceptibilidade.

MOTA, Tiago Feitosa. **Characterization of arthropod vectors' microbiota associated with vector competence**. 2023. 105 f. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 2023.

ABSTRACT

INTRODUCTION: Vector-borne diseases impact public health around the world, putting around 80% of the world's population at risk. Among the arthropod vectors responsible for the transmission of these diseases, mosquitoes and ticks stand out, where some species have vector competence (VC) to transmit one pathogen, mono-competent vectors (MCV), or several pathogens, pluri-competent vectors (PCV). Furthermore, in some viral infections in arthropods there are differences in susceptibility in different species of a given group of arthropods. Such differences in VC may be associated with the arthropod's microbiota that participates in modulating the host's immune response or metabolic homeostasis. The study of the microbiota makes it possible to detect some bacterial species or genera that are associated with a susceptibility or refractoriness profile. These bacteria are important for the study of new biological control methods that aim to modulate the microbiota by reducing or blocking the infection of a pathogen in the arthropod. **OBJECTIVE**: Thus, the objective of the present work was to identify bacterial biomarkers in mosquitoes and ticks associated with VC. **METHOD**: To classify tick species according to a MCV or PCV profile, a literature review on VC studies of tick species with bacterial 16S rRNA sequencing data was performed. After classification, tick microbiome data were selected, processed and analyzed together by comparing MCV and PCV tick microbiota. In the case of mosquitoes, bacterial 16S rRNA data from species refractory or susceptible to the Zika virus (ZIKV) were selected. Thus, samples of refractory and susceptible mosquito species were selected, processed and analyzed together comparing susceptibility profiles to ZIKV. In both studies, bacterial composition, alpha and beta diversities, differential abundance analysis, functional analysis and co-occurrence networks were evaluated in order to characterize the microbiota. **RESULTS**: Bacteria from the genera *Rickettsia*, *Staphylococcus* and *Corynebacterium* were associated with PCV ticks. Alpha diversity was higher in PCV ticks and there were statistically significant differences in beta diversity. Metabolic pathways associated with the control of oxidative stress, dTDP-L-rhamnose and resistance to β-Lactam were more abundant in this group. Furthermore, the bacterial community was less complex and connected in PCV ticks. In relation to ZIKV susceptible or refractory mosquitoes, *Pseudomonas*, *Serratia*, *Halomonas* and *Wolbachia* were associated with a susceptibility profile. There were no statistically significant differences in microbial diversity between susceptible or refractory mosquitoes. On the other hand, ZIKV susceptible mosquitoes showed a more enriched metabolism with less dense and connected co-occurrence networks. **CONCLUSION**: The present work characterized the microbiota of arthropod vectors associated with their VC, identifying some bacterial genera that can be investigated in future work in order to reduce the VC of arthropods. Furthermore, it identified some metabolic pathways that may be related to greater susceptibility to one or more pathogens.

Keywords: Microbiome. Vector competence. Tick. Mosquito. Susceptibility.

LISTA DE FIGURAS

Manuscrito 1

Manuscrito 2

LISTA DE ABREVIAÇÕES E SIGLAS

ANOSIM	ANalysis Of SIMilarities
ASV	Amplicon Sequence Variant
bp	base pairs
CHIKV	CHIKungunya Virus
CLR	Centered Log-Ratio
DENV	DENgue Virus
DNA	DeoxyriboNucleic Acid
FDR	False Discovery Rate
GEO	Gene Expression Omnibus
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genome
LDA	Linear Discriminant Analysis
LEfSe	Linear discriminant analysis Effect Size
NCBI	National Center for Biotechnology Information
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polimerase Chain Reaction
PERMANOVA	PERmutational Multivariate ANalysis Of VAriance
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
RNA	RiboNucleic Acid
rRNA	ribosomal RiboNucleic Acid
RNA-seq	RiboNucleic Acid sequencing
RVFV	Rift Valley Fever Virus
SRA	Sequence Read Archive
TBEV	Tick-Borne Encephalitis Virus
TOSV	TOScana virus
WGS	Whole Genome Shotgun
WNV	West Nile Virus
YFV	Yellow Fever Virus
ZIKV	ZIKa Virus

SUMÁRIO

1 **INTRODUÇÃO**

Doenças transmitidas por vetores artrópodes são responsáveis por mais de 700.000 mortes por ano e põem em risco em torno de 80% da população mundial (WHO, 2017a). Dentre os vetores artrópodes, os mosquitos e carrapatos são os que mais contribuem na transmissão de doenças transmitidas por vetores (Franklinos et al., 2019). Uma ampla variedade de doenças infecciosas, tanto de origem viral quanto bacteriana e causadas por protozoários, possuem vetores artrópodes como principal forma de transmissão impactando fortemente tanto na saúde humana como na saúde animal.

Algumas espécies como o *Aedes aegypti*, *Culex quinquefasciatus*, *Rhipicephalus microplus* e *Ixodes scapularis* se destacam por serem responsáveis pela transmissão de diversos patógenos. O *Ae. aegypti*, por exemplo, é uma espécie de mosquito que permite a transmissão e replicação viral de vírus como os da febre amarela (YFV), dengue (DENV), chikungunya (CHIKV) e zika (ZIKV), bem como o desenvolvimento de helmintos da espécie *Dirofilaria immitis* (Adegoke et al., 2020; Souza-Neto; Powell; Bonizzoni, 2019). Tal competência vetorial ampla, ou seja, de permitir o desenvolvimento, reprodução e transmissão de mais de um patógeno de domínios distintos, também está presente em carrapatos como a espécie *Ixodes scapularis*. Esta espécie é responsável pela transmissão de bactérias patogênicas como a *Borrelia burgdorferi* (doença de Lyme) e *Anaplasma phagocytophilum* (anaplasmose granulocítica humana), além de protozoários como a *Babesia microti* (babesiose humana) e vírus como o de Powassan (Eisen; Eisen, 2018).

Sob um outro de vista, observando a competência vetorial de diversos artrópodes para um mesmo patógeno, foi observado que somente espécies de mosquito do gênero *Aedes* demonstraram competência vetorial para ZIKV (Bisia et al., 2023). Dentre os mosquitos do gênero *Culex* somente o *Cx. quinquefasciatus* foi susceptível a infecção por ZIKV em alguns trabalhos (Guedes et al., 2017; Guo et al., 2016; Smartt et al., 2018). No entanto, tal resultado de susceptibilidade ao ZIKV por *Cx. quinquefasciatus* não foi replicado em diversos outros estudos sugerindo que seja improvável esta espécie de mosquito ser competente para transmitir ZIKV (Macleod; Dimopoulos, 2020). Tais diferenças na competência vetorial de diversos artrópodes podem estar associada à sua microbiota.

Estudos vêm demonstrando a interação entre a microbiota, vetor e patógeno destacando a influência de algumas espécies de bactéria presentes no intestino médio de mosquitos e carrapatos no desfecho da infecção nos hospedeiros invertebrados e na competência vetorial

(Saldaña; Hegde; Hughes, 2017). Dentre tais bactérias destacam-se cepas do gênero *Wolbachia* que participam na pré ativação da resposta imune por modulação da expressão gênica de genes associados a vias da resposta imune inata em *Ae. aegypti* e *Cx. quinquefasciatus* restringindo a replicação de DENV e vírus do oeste no Nilo (WNV) (Niang et al., 2018). Da mesma forma, em carrapatos da espécie *Ixodes scapularis* foi observado que alterações na microbiota estavam associadas à redução da expressão de peritrofina, causando perturbações na integridade da matriz peritŕofica do carrapato (De La Fuente et al., 2017). Esse tipo de descoberta possibilita o desenvolvimento de novos métodos de controle focados no vetor e na sua interação com os patógenos como o controle paratrangênico e/ou de origem genética.

O uso de abordagens ômicas também podem ser empregadas para entender a relação entre patógeno e hospedeiro invertebrado no estudo de doenças transmitidas por artrópodes, bem como o desenvolvimento de novos métodos de controle (Rinker; Pitts; Zwiebel, 2016). Análises transcriptômicas foram empregadas em *Cu. pipiens* experimentalmente infectados por RVFV (Rift Valley fever Virus) sendo observada uma maior expressão do gene Piwi4 que está associado a regulação da resposta imune do mosquito e a consequente replicação viral (Núñez et al., 2020). Em carrapatos da espécie *Ix. ricinus* foram detectados genes e proteínas associados a transmissão e sucesso da infecção no local da picada com potencial de uso para desenvolvimento de vacinas (Trentelman et al., 2020). Em vista do potencial das ferramentas ômicas para criação de novos métodos de combate de doenças transmitidas por vetores, abordagens multi-ômicas se tornam bastante promissoras. Estas permitem um melhor entendimento de forma mais holística para encontrar pontos importantes na interação patógeno-hospedeiro que podem ser explorados para o controle de doenças infecciosas bem como para o diagnóstico e tratamento (Yan et al., 2017).

2 **REVISÃO DE LITERATURA**

2.1 **Epidemiologia de doenças transmitidas por vetores**

Doenças transmitidas por vetores ocorrem em todos os continentes devido a grande capacidade adaptativa de artrópodes hematófagos aos ambientes onde seus hospedeiros são encontrados (Huntington; Allison; Nair, 2016). Além disso, tais doenças possuem íntima relação histórica com seres humanos que vai desde a redução populacional europeia durante a peste negra até o impacto econômico e social da malária na construção de rodovias na Amazônia (Athni *et al.*, 2021).

Vetores artrópodes são responsáveis pela transmissão de diversos domínios de patógenos como vírus, bactérias, protozoários e helmintos, colocando em risco bilhões de pessoas (WHO, 2017a). A distribuição dos artrópodes transmissores de doenças infecciosas varia de acordo com a biologia e adaptabilidade de cada tipo de vetor. Espécies de mosquitos dos gêneros *Aedes*, *Anopheles* e *Culex* estão presentes em todos os continentes do mundo excetuando-se a Antártica e são responsáveis pela transmissão de diversas arboviroses bem como filariose e malária (Wilke; Beier; Benelli, 2019). Triatomíneos dos gêneros *Triatoma*, *Rhodnius* e *Panstrongylus*, vetores de tripanossomatídeos, estão distribuídos principalmente nas Américas com algumas espécies sendo encontradas na África, Oriente Médio, sudoeste asiático e pacífico (Vieira *et al.*, 2018). Flebotomíneos estão distribuídos em regiões quentes nas Américas, África, sul europeu, Ásia e Austrália sendo responsáveis pela transmissão de vírus do gênero Phlebovírus como o vírus Toscana (TOSV) além de tripanossomatídeos (Maroli *et al.*, 2013); (Alkan *et al*., 2013). Carrapatos transmitem diversas doenças infecciosas de origem bacteriana, viral e protozoária por todos os continentes apesar de sua baixa motilidade sendo transportados por hospedeiros como aves migratorias e mamíferos (Piotrowski; Rymaszewska, 2020).

Dentre as principais doenças transmitidas por mosquitos, a dengue se destaca pela ampla distribuição em todo o mundo e sua alta morbidade (Ogunlade *et al.*, 2021). Esta é causada pelo DENV que pertence à família Flaviviridae e está presente em ambientes urbanos com milhões de casos anualmente em regiões tropicais e temperadas mais quentes (Gould *et al.*, 2017). Sua transmissão é causada pela picada de culicídeos das espécies *Ae. aegypti* e *Ae. albopictus* que depositam seus ovos em corpos de água parada sendo este um importante fator de risco ambiental (Khan *et al.*, 2018). Além da dengue, a malária também é uma importante doença transmitida por mosquitos que põe em risco cerca de metade da população mundial, ocorrendo

em 87 países da África, América latina e sul asiático (WHO, 2020). Protozoários do gênero *Plasmodium*, em especial as espécies mais patogênicas, *Pl. falciparum* e *Pl. vivax*, são os agentes causadores da malária transmitidos por mosquitos do gênero *Anopheles* (Minwuyelet et al., 2020). Crianças abaixo de 5 anos são mais susceptíveis e a ausência de mosquiteiros durante a noite está associada a um maior risco de infecção devido ao hábito alimentar noturno do vetor (Liu *et al.*, 2021). Helmintos como filárias da espécie *Wuchereria bancrofti* também são transmitidos por mosquitos causando a filariose linfática que é endêmica em 72 países do sudoeste asiático, África subsaariana, ilhas do Pacífico e alguns locais na América latina (Zulfiqar; Malik, 2021). A sua transmissão ocorre pela picada de mosquitos de diversos gêneros como *Aedes*, *Culex*, *Anopheles* e *Mansonia* e baixas condições sócio-econômicas estão associadas a uma maior prevalência dessa doença (Mutheneni *et al*., 2016).

A principal doença transmitida por triatomíneos é a doença de chagas que está presente principalmente na América latina com casos ocorrendo na Europa e Estados Unidos devido a imigração de pessoas infectadas que podem transmitir verticalmente ou por meio de transfusão sanguínea (Antinori *et al*., 2017). A transmissão do agente etiológico, *Trypanosoma cruzi*, se dá principalmente no repasto sanguíneo de triatomíneos de diversos gêneros como *Triatoma*, *Rhodnius* e *Panstrongylus* representados por aproximadamente 140 espécies (Añez *et al*., 2020). A doença de chagas possui diferentes apresentações clínicas geralmente crônicas que podem acometer o coração e o sistema gastrointestinal sendo registrado em torno de 28.000 novos casos e estimativa de 6 a 8 milhões de infecções anualmente (Norman; López-Vélez, 2019). Animais no peridomicílio aliado a construções com fissuras em madeira e concreto bem como a presença de entulho de madeira e tijolos foram associados como fatores de risco para a presença de triatomíneos infectados, dado que estas condições favorecem a manutenção de populações deste artrópode (Daflon-Teixeira *et al*., 2019).

Em relação a flebotomíneos, as leishmanioses são um grupo de doenças causadas por protozoários do gênero *Leishmania* com apresentações clínicas tegumentares ou viscerais e acometem cerca de mais de um milhão de pessoas no mundo (Mann *et al*., 2021). O Brasil pertence ao grupo de países que concentram mais de 90% dos casos tanto para leishmaniose tegumentar quanto visceral (WHO, 2017b). Casos de leishmaniose ocorrem nas Américas, África, sul europeu, Oriente Médio e sul asiático sendo associados a baixa infraestrutura sanitária, pobreza, migração populacional e imuno deficiência (Mann *et al*., 2021). Além de tripanossomatídeos, os flebotomíneos também são responsáveis pela transmissão de vírus do gênero Phlebovírus como o TOSV que causa uma doença febril podendo evoluir para uma infecção neuro invasiva (Anagnostou *et al*., 2011). Na Tunísia foram encontrados

flebotomíneos do gênero *Phlebotomus* infectados tanto por *Le. infantum* quanto por TOSV que pode contribuir na patogênese da leishmaniose comprometendo a imunidade do hospedeiro e favorecendo o estabelecimento da infecção de *Le. infantum* (Fares *et al*., 2020).

A borreliose de Lyme está entre uma das doenças causadas por bactérias transmitidas por carrapato mais importantes sendo a doença vetorial mais prevalente no hemisfério norte (Boulanger *et al*., 2019). Os principais vetores pertencem ao gênero *Ixodes* sendo o *Ix. pacificus* e *Ix. scapularis* responsáveis pela transmissão na América do norte e o *Ix. ricinus* pela transmissão na Europa (Rodino; Theel; Pritt, 2020). A doença é causada por bactérias do gênero *Borrelia* e cursa com inflamação cutânea próximo ao local da picada podendo haver visceralização para tecido cardíaco, articular e nervoso (Stanek *et al*., 2012). Carrapatos também transmite a babesiose humana causada por protozoários do gênero *Babesia* que parasitam células sanguíneas e podem causar hemólise, problemas respiratórios e lesões em diversos órgãos podendo levar à morte (Onyiche *et al*., 2021). Sua transmissão também ocorre pela picada de carrapatos das espécies *Ix. ricinus* e *Ix. scapularis* assim como a borreliose de Lyme. Nos Estados Unidos as áreas endêmicas para babesiose também são endêmicas para *Borrelia burgdorferi* e *Ix. scapularis* (Krause, 2019). Adicionalmente, alguns vírus também são transmitidos por carrapatos, destacando-se o vírus da encefalite transmitida por carrapatos (TBEV - tick-borne encephalitis). Este pertence à família *Flaviviridae* e pode causar sintomas neurológicos como paralisia muscular, distúrbios de consciência, dificuldades de deglutição e comunicação verbal, além de meningites e meningoencefalites (Pulkkinen; Butcher; Anastasina, 2018). O *Ix. ricinus* também é responsável pela transmissão do TBEV na Europa, enquanto outros carrapatos do mesmo gênero são vetores de outros genótipos de TBEV presentes na Sibéria e leste asiático (Shi et al., 2018).

2.2 **Dinâmica populacional de vetores e introdução de doenças**

A transmissão e ocorrência de todas as doenças infecciosas supracitadas está intimamente relacionada à dinâmica populacional e biologia de seus respectivos vetores artrópodes que são essenciais para a disseminação ou o controle destas doenças *(WILSON et al*., 2020). Tanto humanos quanto animais servem como fontes alimentares sanguíneas para a manutenção de populações destes artrópodes que podem albergar patógenos colocando em risco a saúde de seus hospedeiros. De forma geral, a dinâmica populacional de artrópodes vetores de doenças está associada com condições climáticas específicas que favorecem o desenvolvimento e sobrevivência das larvas e adultos (Semenza; Suk, 2018).

Em regiões tropicais as populações de vetores como flebotomíneos, mosquitos e carrapatos tendem a ser menos sazonais e terem picos populacionais associados à pluviosidade e outros fatores climáticos abióticos (López; Müller; Sione, 2018). Dessa forma, doenças transmitidas por vetores ocorrem mais frequentemente entre os trópicos de capricórnio e câncer. No entanto, carrapatos conseguem se manter em regiões mais temperadas e consequentemente preservam o ciclo de transmissão de doenças como a borreliose e encefalite viral (Randolph, 2004).

Com o aquecimento global, algumas espécies de flebotomíneos e mosquitos vêm expandindo sua distribuição para regiões temperadas colocando em risco populações de humanos e animais susceptíveis a doenças até então não endêmicas (Caminade; Mcintyre; Jones, 2019). Nas últimas décadas, flebotomíneos da espécie *Phlebotomus perniciosus* foram encontrados em localidades ao noroeste da Alemanha próximos à Bélgica e Luxemburgo (Naucke *et al*., 2008). Esta espécie é responsável pela transmissão de *Le. infantum* no sul da Europa e o aumento da temperatura média anual na Alemanha pode estar associado com a detecção desta espécie de flebotomíneo tão ao norte neste país, bem como a migração sazonal no verão de pessoas para áreas endêmicas no sul europeu (Naucke; Lorentz, 2012). De forma similar, mosquitos das espécies *Ae. albopictus* e *Ae. aegypti* foram encontrados ao sul do estado de Ontário no Canadá próximo à fronteira americana por pelo menos dois anos consecutivos em locais distintos (Giordano *et al*., 2020). Mesmo com maiores temperaturas no inverno canadense ainda não está claro se ovos e larvas de *Ae. aegypti* conseguiriam invernar até a chegada do verão.

Além de questões climáticas, hábitos migratórios humanos e animais, especialmente das aves, também podem estar associados a expansão na distribuição de vetores artrópodes. Em 2018 novas espécies de carrapato do gênero *Hyalomma* foram encontrados parasitando animais e humanos na Alemanha possivelmente introduzidas por aves migratórias, associados ao fato de 2018 ter sido o ano mais quente até então registrado, possibilitando a manutenção destas novas espécies (Chitimia-Dobler *et al.*, 2019). Adicionalmente, a infraestrutura urbana e condição socioeconômica em determinados locais de centros urbanos também podem contribuir para a expansão de populações de mosquitos facilitando o estabelecimento de criadouros (Eder *et al*., 2018). Ademais, no Brasil, a expansão de centros urbanos em regiões de mata associada com o hábito de pessoas criarem animais no peridomicílio e a ineficiência no controle de flebotomíneos permitiram a urbanização das leishmanioses que atualmente ocorrem majoritariamente em centros urbanos (Reis *et al*., 2017).

Apesar da importância da expansão de populações de vetores artrópodes para o surgimento de casos autóctones para novas doenças em uma determinada região geográfica, a introdução do patógeno também é necessária. O fluxo de pessoas infectadas migrando de cidades dentro de um país pode contribuir na introdução de patógenos em locais onde há populações de vetores competentes estabelecidas (Zinszer *et al.*, [s.d.]). Além disso, o turismo ou curtas estadias de estrangeiros doentes também podem favorecer a ocorrência de doenças não autóctones, dado que há populações de vetores suscetíveis e competentes, como no caso do vírus Zika no Brasil (Massad *et al*., 2017).

2.3 **Competência vetorial**

Determinar a competência vetorial de artrópodes definindo-os como responsáveis pela transmissão de patógenos não é uma questão simples e trivial indo além da detecção do patógeno em populações do artrópode. Além de verificar a infecção natural em artrópodes coletados em área endêmica é necessário demonstrar que o patógeno consegue se reproduzir ou replicar sem causar morte do hospedeiro invertebrado (Azar; Weaver, 2020). Adicionalmente, é importante que sejam apresentadas evidências de transmissão para hospedeiros vertebrados susceptíveis. Alguns pontos da relação entre patógeno e hospedeiro invertebrado podem variar de acordo com a biologia da espécie. Em carrapatos é importante demonstrar transmissão transestadial, ou seja, larvas ou ninfas que adquirem infecção natural ou experimental se mantêm infectadas após ecdise para ninfas e adultos respectivamente (Eisen, 2020). Essa questão se torna importante para definir uma determinada espécie de carrapato como vetor de um patógeno devido a biologia deste artrópode que possui três estágios de vida durante a ecdise e entre os estágios é possível que a infecção seja eliminada (Breuner et al., 2020). Tal característica biológica também está presente em triatomíneos e da mesma forma é importante demonstrar que há transmissão transestadial nestes artrópodes (Blakely; Hanson; Romero, 2018).

Em alguns casos a confirmação da competência vetorial pode demorar mais de uma década. A espécie de flebotomíneos *Lu. cruzi* foi detectada com infecção natural por *Le. infantum* em 1998 no estado de Mato Grosso do Sul e por muitos anos ela foi considerada como provável vetor da leishmaniose na região de Corumbá (Dos Santos et al., 1998). No entanto, somente em 2017 a sua competência vetorial foi demonstrada usando *Lu. cruzi* coletados em Corumbá-MS. Após infecção, flebotomíneos desta espécie transmitiram *Le. infantum* para hamsters que após alguns meses desenvolveram a doença (De Oliveira *et al*., 2017).

Outra questão importante para avaliar a competência vetorial de alguma espécie de artrópode é a transmissão vertical de patógenos para a prole. Este tipo de transmissão foi demonstrado por *Ae. albopictus* experimentalmente infectados por ZIKV e representa um mecanismo que permite a sobrevivência do vírus na população de mosquitos quando há condições adversas para o repasto sanguíneo (Guo *et al*., 2020). A transmissão vertical também já foi demonstrada em mosquitos *Ae. aegypti* e *Ae. albopictus* sendo detectadas DENV-1 na prole de fêmeas experimentalmente infectadas (Buckner; Alto; Lounibos, 2013). No entanto, as taxas de transmissão vertical foram baixas, variando de 8,33% para o *Ae. aegypti* e 11,11% para o *Ae. albopictus*. Em carrapatos a transmissão transovarial ocorre com protozoários do gênero *Babesia* mas também foi experimentalmente comprovada para bactérias da espécie *Bo. miyamotoi* por *Ix. scapularis* (Breuner *et al.*, 2018). Além de indicar competência vetorial desde os primeiros estágios de vida do carrapato, a transmissão vertical juntamente com a transestadial demonstram que uma espécie de carrapato é tanto um vetor quanto um reservatório de um determinado patógeno (Orkun, 2019).

Dentro de uma mesma espécie de vetor artrópode pode haver diferenças genéticas em populações distintas que influenciam na competência vetorial. Mosquitos da espécie *Ae. aegypti* de diferentes linhagens variaram em susceptibilidade para infecções com o ZIKV em decorrência da diversidade genética entre colônias diferentes (Uraki *et al*., 2018). De forma similar, mosquitos da espécie *Cx. quinquefasciatus* variaram suas taxas de infecção e transmissão de WNV quando populações geograficamente distintas foram comparadas (Goddard *et al*., 2002). Tal observação também foi realizada em populações diferentes de carrapatos da espécie *Ornithodoros turicata* que demonstraram diferentes taxas de transmissão de *Bo. turicatae* de acordo com a população de carrapato avaliada (Krishnavajhala; Armstrong; Lopez, 2018).

A microbiota também tem relação com a competência vetorial de artrópodes vetores de patógenos. Em flebotomíneos, tanto em *Lu. longipalpis* infectados por *Le. infantum* quanto em *Ph. duboscqi* infectados por *Le. major* os parasitas não se desenvolveram na sua forma metacíclica infectante e se replicaram menos nos grupos de flebotomíneos tratados com antibiótico previamente à infecção (Kelly *et al*., 2017; Louradour *et al*., 2017). Bactérias simbiontes do gênero *Rickettsia* reduziram a taxa de infecção por *Bo. burgdorferi* em carrapatos da espécie *Ix. scapularis* quando comparados com carrapato livres deste simbionte evidenciando a interação da microbiota com patógenos que reduzem a competência vetorial de carrapatos (Steiner *et al*., 2008). Por outro lado, a interação entre patógenos e microbiota também podem favorecer a infecção por patógenos. Em experimentos entre carrapatos *Ix.*

scapularis infectados por *An. phagocytophilum* foi observado que a sua presença altera a composição do microbioma de forma a facilitar a sua passagem pela parede intestinal com consequente maior taxa de infecção (Abraham *et al.*, 2017). Em mosquitos foi demonstrado que a microbiota pode influenciar sua competência vetorial para patógenos virais e protozoários por meio de mecanismos diretos e indiretos como ativação da resposta imune do mosquito previamente a infecção ou pela produção de metabólitos nocivos ao patógeno (Dennison; Jupatanakul; Dimopoulos, 2014).

Tais interações entre microbiota e competência vetorial possibilitam o surgimento de métodos de controle paratrangênicos com bactérias simbiontes modificadas para reduzir ou bloquear a transmissão de patógenos (Saraiva *et al*., 2016). Neste sentido, estudos com *Wolbachia* observaram que cepas do gênero *Wolbachia* modulam a expressão de genes relacionados à resposta imune inata em *Ae. aegypti* e *Cu. quinquefasciatus* restringindo a replicação de (Denv; Wnv; Niang *et al*., 2018). Outros estudos avaliaram a estratégia de soltar mosquitos transfectados com *Wolbachia* para que ela fosse assimilada à população de *Ae. aegypti* demonstrando que além da bactéria se espalhar na população selvagem de mosquitos ela se mantia em altas taxas mesmo após dois anos de soltura (Hoffmann *et al.*, 2011; Hoffmann *et al*., 2014).

Apesar de promissores, os estudos até o presente momento avaliaram a interação entre um patógeno e um vetor específicos. No entanto, algumas espécies de vetores como *Ix. ricinus* e *Ae. aegypti* são responsáveis pela transmissão de diversos patógenos mesmo de domínios diferentes. Um estudo na Sérvia detectou oito patógenos diferentes em *Ix. ricinus* que estavam parasitando humanos mostrando sua alta capacidade como vetor pluri competente e o risco que eles representam (Banović *et al*., 2021). Em alguns casos há grandes perdas econômicas devido a carrapatos pluri competentes como o *Rh. microplus* que parasita os bovinos e transmite doenças como babesiose e anaplasmose (Karim *et al.*, 2017). Estima-se que por conta desta única espécie de carrapato mais de $3 bilhões deixaram de ser ganhos no Brasil em 2011 devido aos custos de medicamentos, carrapaticidas e descarte de leite durante tratamento farmacológico (Grisi *et al*., 2014). Outro vetor promíscuo, o *Ae. aegypti*, também causa grandes perdas econômicas e em 2016 estima-se que o Brasil teve um impacto econômico na ordem de R$2,3 bilhões no combate a esse vetor e custos diretos e indiretos das doenças por ele transmitidas (Teich; Arinelli; Fahham, 2017). Devido aos impactos à saúde e econômico que vetores pluri competentes causam, se torna cada vez mais importante estudá-los de forma mais holística a fim de buscar pontos em comum na competência vetorial de diversos patógenos para o desenvolvimento de métodos de controle que atinjam múltiplas doenças.

2.4 **Análise de microbioma**

A análise de microbioma consiste na identificação e estudo da composição de microrganismos (bactéria, eucariotos, vírus e archaea) presentes, seus genomas e condições do ambiente em uma determinada amostra (Marchesi; Ravel, 2015). Dessa forma os estudos que avaliam somente a composição de microrganismos estudam a microbiota que faz parte do microbioma. Frequentemente são empregadas duas técnicas, sequenciamento de genoma por shotgun (WGS - Whole Genome Shotgun) ou sequenciamento de amplicons de marcador genético específico para bactérias, fungos ou eucariotos unicelulares (Kim; Kim; Jung, 2020). A primeira técnica permite o sequenciamento do genoma de diversos microrganismos que serão mapeados com genomas de interesse sendo interessante para estudar o microbioma. No entanto, ele possui um custo relativamente alto, requer maior capacidade computacional e possui acurácia limitada na identificação de genes individuais sendo necessária uma alta cobertura no sequenciamento (Ranjan *et al*., 2016). Assim, menos amostras são sequenciadas em uma mesma reação aumentando o custo total no processamento de diversas amostras. Por outro lado, o sequenciamento de amplicons de marcador genético específico está cada vez mais barato, permitindo o sequenciamento de diversas amostras ao mesmo tempo e sua análise é computacionalmente mais leve (Schriefer *et al.*, 2018).

Para a análise da composição de bactérias em uma determinada amostra o gene rRNA 16S de bactérias é utilizado. Nesta abordagem é realizada uma PCR de uma ou mais regiões variáveis do gene que posteriormente tem os amplicons filtrados e sequenciados. Previamente ao sequenciamento é realizada uma outra PCR para adicionar adaptadores às sequências de cada amostra, importantes para o sequenciamento, bem como pequenas sequências marcadoras que servem como código de barras para identificar as sequências de cada amostra (Liu et al., 2020). Estas duas etapas de PCR e a extração de DNA prévias ao sequenciamento são consideradas gargalos no fluxo de trabalho dado que apresentam riscos de contaminação durante a pipetagem de reagentes (Muhamad rizal *et al*., 2020).

A fim de minimizar o viés causado pela presença de contaminantes introduzidos no processamento das amostras, controles positivos e negativos podem ser sequenciados juntamente com as amostras de interesse. Atualmente existem controles positivos comerciais onde é sabido tanto a espécie de bactéria quanto a sua quantidade (Li *et al*., 2022). Tais controles podem ser utilizados na análise dos dados para identificar os contaminantes e assim removê-los ou ajustar a sua abundância nas amostras do estudo (Davis *et al.*, 2018). Dessa forma, o risco da presença de contaminantes nas amostras pode ser reduzido e controlado evitando que tais

bactérias sejam associadas às características de interesse das amostras. Outro ponto importante da análise da composição bacteriana por meio do sequenciamento do gene 16S ribossomal são as regiões variáveis do gene.

A classificação bacteriana varia quando diferentes regiões variáveis são comparadas e alguns grupos de bactérias são mais facilmente detectados quando certas regiões variáveis são usadas (Bukin et al., 2019). Dessa forma, uma meta-análise envolvendo dados de sequenciamento do 16S ribosomal bacteriano deve levar essas questões em consideração a fim de obter um grupo de dados comparável. Um estudo avaliou in silico diferentes protocolos de sequenciamento e as regiões variáveis V3 e V4 se mostraram mais adequadas para avaliação da composição bacteriana (Mizrahi-Man; Davenport; Gilad, 2013). Adicionalmente, a região V3 demonstrou possuir tamanhos diferentes entre espécies distintas de bactérias com maior número de variações em comparação com outras regiões variáveis (Vargas-Albores et al., 2017). Dessa forma, esta região variável se mostra promissora para classificação taxonômica dado que apesar de variar bastante, os extremos da sequência são bastante conservados entre as bactérias (Vargas-Albores et al., 2017).

Em alguns casos específicos como a identificação de bactérias associadas a doenças respiratórias, a região V1-V2 se mostrou com maior acurácia em comparação às outras regiões variáveis do gene 16S (López-Aladid et al., 2023). Por outro lado, a região V6 pode ser mais interessante em casos onde bactérias de interesse pertencem à Classe *Alphaproteobacteria* dado que esta região é bem representada na região V6 (Kerrigan; Kirkpatrick; D'hondt, 2019). Alguns autores indicam o sequenciamento de todo o gene 16S para obter maior precisão na caracterização de uma comunidade bacteriana (Klemetsen; Willassen; Karlsen, 2019). Assim, ao sequenciar o gene 16S para analisar a composição microbiana, é importante selecionar a região variável que mais se adequa à pergunta e objetivo do trabalho a fim de melhor caracterizar a microbiota bem como obter inferências a seu respeito.

2.4.1 Do processamento dos dados à classificação taxonômica

Após o sequenciamento do gene 16S rRNA contido das amostras as sequências são processadas para posterior classificação taxonômica e análises (Galloway-Peña; Hanson, 2020). O processamento inicial das sequências se dá com a remoção dos adaptadores, primers e identificadores para garantir que as sequências estarão livres de fragmentos sintéticos que não fazem parte da microbiota de estudo. Adicionalmente são removidas sequências que possuam bases ambíguas ou homopolímeros longos uma vez que podem representar artefatos da

plataforma de sequenciamento. Também são removidas sequências com tamanhos que não condizem com a região delimitada pelos primers por serem artefatos do sequenciamento ou contaminações. Por fim, sequências quiméricas e com escore de qualidade Phred inferiores a um determinado ponto de corte também são removidas. Tais processos de controle de qualidade das sequências são importantes para garantir que as sequências analisadas não comprometam as futuras etapas de análise (De La Cuesta-Zuluaga; Escobar, 2016).

Depois deste processo de filtragem e controle de qualidade as sequências são agrupadas para formar unidades taxonômicas operacionais (OTU - Operational Taxonomic Unit) que são sequências representativas das sequências de todas as amostras. Geralmente o OTU é formado utilizando uma identidade entre 97% e 99% entre as sequências sendo aceita a faixa de 1% a 3% de divergência entre as sequências que cada OTU representa. Entretanto, este foi um ponto criticado na literatura por ser definido de forma arbitrária e os protocolos de agrupamento influenciavam nas futuras análises, superestimando a diversidade da comunidade bacteriana (Xue; Kable; Marco, 2018). Assim, foi indicada a substituição do uso de OTU por variantes de sequências de amplicon (ASV - Amplicon Sequence Variant) que levam em consideração uma identidade de 100% (Callahan; Mcmurdie; Holmes, 2017). Dessa forma, cada ASV representa sequências únicas presentes nas amostras (Kim et al., 2017). Utilizando a identificação do OTU/ASV e o número de sequências que deram origem ao OTU é montada uma tabela com frequências absolutas de cada OTU por amostra (tabela de OTU/ASV). Esta abordagem com sequências representativas traz vantagens computacionais uma vez que futuras etapas de análise utilizam um menor número de sequências (De La Cuesta-Zuluaga; Escobar, 2016).

A classificação taxonômica é realizada com bancos de dados de 16S rRNA sendo o Silva e o Greengenes mais comumente empregados. O banco de Silva possui um calendário de atualizações mais frequente, enquanto o Greengenes não é atualizado desde 2013 (Balvočiūtė; Huson, 2017). Como resultado da classificação temos uma tabela com os mesmos identificadores das sequências representativas e suas respectivas classificações taxonômicas. Dessa forma, é possível cruzar as informações contidas na tabela de OTU/ASV com a classificação taxonômica e saber quantas cópias de cada taxa estava presente em cada amostra. Em seguida essas informações são utilizadas para as análises de diversidade alfa e beta a fim de avaliar se há diferenças na comunidade bacteriana associadas com variáveis de interesse das amostras como infectados e não infectados.

2.4.2 Composicionalidade do dado

O sequenciamento de DNA na plataforma Illumina é amplamente empregado em estudos de microbioma. Essa plataforma trabalha contabilizando as sequências até um limite de contagens de acordo com o chip de sequenciamento e equipamento utilizados (Slatko; Gardner; Ausubel, 2018). Assim, devido à forma como o sequenciamento do gene 16S rRNA ocorre, a quantidade de sequências contabilizadas em uma corrida de sequenciamento é limitada ao número de leituras determinados pelo sequenciamento. Durante o sequenciamento, o número de leituras por amostra varia e o limite de leituras do chip de sequenciamento não permite o sequenciamento de todas as bactérias presentes na amostra (Gloor; Reid, 2016). Ademais, o limite de contagem total de uma corrida de sequenciamento cria uma dependência na contagem de diferentes sequências dado que uma sequência com muitas contagens limita o quanto outras sequências podem ser lidas (Gloor et al., 2017). Uma outra característica do dado oriundo do sequenciamento na plataforma Illumina é a esparsidade do dado onde muitas sequências estão ausentes em muitas amostras (Silverman et al., 2020). Assim, a matriz de contagem de bactérias se torna majoritariamente composta por zeros. Tais características em conjunto com diferenças do número de leituras das amostras impossibilitam a comparação direta entre OTUs/ASVs de amostras distintas. Isto ocorre uma vez que contagens similares de duas amostras podem representar diferentes proporções de um dado OTU/ASV dentre diferentes totais de sequências lidas por amostra (Greenacre, 2021). Todos esses pontos que caracterizam o dado obtido do sequenciamento Illumina indicam que esse conjunto de dados representa um dado composicional (Gloor et al., 2017).

Em decorrência da composicionalidade do dado de sequenciamento do gene 16S, a análise deve levar em consideração tal característica em seus métodos. Assim, previamente às análises, a matriz de contagem de OTUs/ASVs passa por uma transformação ou normalização que torna a comparação entre amostras mais coerente dado o tipo de dado que está sendo analisado. Inicialmente foram aplicados métodos de normalização comumente utilizados em dados de RNA-seq como os aplicados nos pacotes edgeR e DESeq. Contudo, eles podem aumentar erros do tipo 1 na análise quando empregados para analisar microbioma (Nookaew et al., 2012). Dessa forma, a transformação de razão de log centralizada (CLR) foi sugerida para lidar com a característica composicional do dado de microbioma. Ela centraliza o log das contagens de cada OTU/ASV por amostra pela média geométrica de toda a matriz reduzindo a influência das diferenças de contagens entre amostras (Gloor et al., 2016). Outras transformações de razão de log como a razão de log aditiva (ALR - *Additive Log Ratio*) ou

isométrica (ILR - *Isometric Log Ratio*) também podem ser utilizadas em dados de microbioma. Assim como no CLR um denominador comum é utilizado para o cálculo das razões de log. No caso do ALR um componente é usado como referência, enquanto o ILR representa uma composição em função de uma base ortonormal (Egozcue et al., 2003; Greenacre; Martínez-Álvaro; Blasco, 2021). Utilizando tais transformações, métodos que levam em consideração a composicionalidade do dado foram desenvolvidos, CoDA - *Compositional Data Analysis*, e vêm sendo cada vez mais empregados em análises de dados ômicos (López-García et al., 2023). Estes serão abordados nas sessões respectivas de cada etapa de análise do microbioma.

2.4.3 Diversidade alfa

A diversidade alfa representa a diversidade intra amostra comparando tanto a quantidade (riqueza) quanto a proporcionalidade (uniformidade) de bactérias presentes em cada amostra (Mcart; Cook-Patton; Thaler, 2012). Ela é representada por um número que sumariza tanto a riqueza quanto a uniformidade das bactérias que compõem a microbiota (Willis, 2019). Sua mensuração é importante uma vez que indica um dos tipos de disbiose da microbiota associado à uma redução da diversidade de microrganismos de forma geral. Assim, é possível inferir que, baseado em diferenças na diversidade alfa, o tratamento ou condição testada no experimento promovem um situação de disbiose na microbiota dos indivíduos testados. A disbiose por sua vez é caracterizada por uma alteração na composição da microbiota onde a proporção de bactérias patogênicas se encontra aumentada ou a de não patogênicas está reduzida bem como pode haver uma redução geral de toda a microbiota (Petersen; Round, 2014).

A mensuração da diversidade alfa é realizada por métricas comumente empregadas em estudos de ecologia como o índice de Shannon ou Chao1. O primeiro é calculado levando em consideração tanto a riqueza de espécies quanto a uniformidade de cada bactéria presente na amostra, enquanto que o segundo somente considera a riqueza (Kim et al., 2017). Além destes índices há o índice de Simpson e suas variantes que se assemelha ao de Shannon avaliando tanto a riqueza quanto a uniformidade (Galloway-Peña; Hanson, 2020). No entanto, o índice de Simpson dá mais peso para a uniformidade no seu cálculo enquanto o de Shannon valoriza mais a riqueza (Kim et al., 2017). Adicionalmente, o índice de Shannon é mais influenciado por OTUs/ASVs raros ao passo que o índice de Simpson é mais influenciado por taxa abundantes e dominantes na amostra (Bent; Forney, 2008). Os resultados dos cálculos dos índices de Chao1 e Shannon variam de zero ao infinito e representam maior diversidade quanto maior for o índice. Por outro lado, o índice de Simpson varia entre zero e um, indicando maior diversidade quanto

mais próximo de zero ele for. Para facilitar a interpretação o índice inverso de Simpson é frequentemente apresentado dado que ele é medido dividindo o número um pelo índice de Simpson. Dessa forma, quanto maior for o índice inverso de Simpson maior é a diversidade alfa da amostra.

Dentre outras métricas de mensuração da diversidade alfa o estimador de cobertura baseado em abundância (ACE - Abundance-based Coverage Estimator) representa uma abordagem não paramétrica onde as probabilidades das bactérias observadas são sumarizadas em um número (Chao; Lee, 1992). Nesta métrica, bactérias raras e abundantes são avaliadas separadamente e a informação de ausência e presença delas é levada em consideração no cálculo da probabilidade de ocorrência em cada amostra (Kim et al., 2017). Além da riqueza e uniformidade das espécies de bactérias presente na microbiota, a distância filogenética entre estas também pode ser empregada na mensuração da diversidade alfa. A diversidade filogenética de Faith traz uma abordagem diferente ao levar em consideração a soma dos comprimentos dos ramos da árvore filogenética construída com todos os taxa presente na microbiota (Faith, 1992). Assim, uma microbiota mais diversa seria a que tivesse uma distância filogenética global maior entre os indivíduos que a compõe. No entanto, está métrica leva em consideração somente a presença e ausência das espécies de bactéria e não considera as diferenças na abundância delas (Chao; Chiu; Jost, 2016). Assim, modificações na composição microbiana não são identificadas dificultando a caracterização de uma disbiose com a diversidade filogenética de Faith.

A diferença do número de leituras por amostra supracitada pode influenciar nos resultados de diversidade alfa uma vez que a abundância de um OTU/ASV reflete as limitações do sequenciamento (Weiss et al., 2017). Uma forma de lidar com essa questão é utilizar a rarefação para ajustar as diferenças no números de leitura totais de cada amostra. Ela se baseia em uma reamostragem dos OTUs/ASVs de cada amostra igualando o número de contagem de todas as amostras tornando-as comparáveis (Willis, 2019). A rarefação é realizada reamostrando aleatoriamente sem reposição todas os OTUs/ASVs do estudo por amostra biológica para ficarem com o número de leituras da amostra com menor contagem. Assim, todas terão números iguais de leituras para a análise de diversidade. Entretanto, tal abordagem também sofre críticas na literatura dado que este tipo de normalização promove a remoção de informação válida presente nas amostras (Mcmurdie; Holmes, 2014).

Transformações para adequar o dado composicional como CLR tornam as contagens em números que não pertencem ao conjunto de números naturais impossibilitando o cálculo das métricas de diversidade alfa supracitados. Dessa forma, é indicado que para analisar a

diversidade alfa, seja realizada ao menos a rarefação das contagens das amostras para evitar um viés decorrente das diferenças de profundidade do sequenciamento entre amostras.

2.4.4 Diversidade beta

A diversidade beta mede a diversidade entre amostras por meio da comparação da dissimilaridade produzindo matrizes de distância entre as amostras. Amostras com valores baixos de dissimilaridade indicam comunidades bacterianas semelhantes, enquanto altos valores representaram distinções entre a microbiota de duas amostras (Wu; Chen; Shen, 2020). Assim, ao comparar amostras em conjunto por grupos de interesse é possível observar se as comunidades bacterianas são similares ou não, sendo um indicativo de que algo na microbiota pode estar diferenciando-as.

As matrizes de dissimilaridade, por sua vez, são calculadas por medidas de distância como Bray-Curtis, Jaccard e Unifrac. As métricas de Bray-Curtis e Jaccard são análogas, porém a primeira leva em consideração as diferenças de abundância de taxa entre comunidades bacterianas enquanto a segunda não considera a abundância dando mais peso para presença e ausência de taxa (Staley; Kaiser; Khoruts, 2018). A métrica Unifrac é um método filogenético que utiliza a distância filogenética entre os taxa de comunidades bacterianas distintas (Galloway-Peña; Hanson, 2020). Ela pode ser dividida em duas versões, a *Weighted* e *Unweighted* Unifrac, sendo que a primeira também leva em consideração a abundância dos taxa assim como Bray-Curtis e a segunda considera a presença e ausência assim como Jaccard (Lozupone et al., 2007). Assim, as métricas de dissimilaridade utilizadas na análise de diversidade beta podem ser classificadas em dois grupos, as quantitativas que consideram a abundância de bactérias como Bray-Curtis e *Weighted* Unifrac e as qualitativas que avaliam a presença e ausência como Jaccard e *Unweighted* Unifrac (Knight et al., 2018). No entanto, tais medidas de dissimilaridade não levam em consideração a característica composicional do dado. Dentro da abordagem CoDa, uma vez em que a contagem de bactérias é transformada com uma razão de log, não há necessidade da aplicação de uma medida de dissimilaridade. Assim, uma análise de componentes principais (PCA) seria suficiente para analisar a diversidade beta (Gloor et al., 2017). Tal abordagem configura a medida de distância de Aitchison que aplica a distância Euclidiana no dado transformado, razão de log, uma vez que tal transformação traz o dado do espaço simplex para o espaço Euclidiano (Aitchison, 1982). Recentemente foi desenvolvido um método que leva em consideração a composicionalidade do dado bem como a distância filogenética como nas métricas Unifrac. Este método se baseia na transformação ILR seguida

de um balanceamento utilizando a distância filogenética entre taxa (Silverman et al., 2017). Dessa forma, é possível lidar com a característica composicional do dado de microbioma bem como incorporar a distância filogenética na análise da diversidade beta.

Independente da matriz de distância utilizada, a visualização e interpretação da diversidade beta é realizada por meio de análises multivariadas como a análise de coordenadas principais (PCoA), PCA ou escalonamento multidimensional não métrico (NMDS) (Sudarikov; Tyakht; Alexeev, 2017). Tais métodos de análise permitem reduzir dimensionalmente das variáveis para duas ou três dimensões que mais explicam a distância entre amostras de acordo com a métrica empregada facilitando a interpretação e visualização (Vázquez-Baeza et al., 2013). Entretanto as análises multidimensionais não permitem avaliar estatisticamente se há diferenças entre as comunidades bacterianas nos grupos estudados. Uma alternativa para analisar estatisticamente a diversidade beta entre comunidades bacterianas é a análise multivariada permutacional de variância (PERMANOVA) ou análise de similaridades (ANOSIM) (Anderson; Walsh, 2013). Ambos os métodos são similares aplicando permutação às matrizes de dissimilaridades da diversidade beta e aplicam a análise multivariada testando suas hipóteses nulas.

A PERMANOVA testa a hipótese nula de que os centroides dos grupos são equivalentes para todos os grupos (Galloway-Peña; Hanson, 2020). A ANOSIM avalia a similaridade intra e intergrupos testando a hipótese nula de que a média do rank de similaridade das amostras dentro de um grupo e entre grupos é igual (Xia; Sun, 2017). Por ter uma hipótese mais robusta, a PERMANOVA acaba sendo empregada com mais frequência (Anderson; Walsh, 2013). No entanto, é importante avaliar o uso dos métodos estatísticos supracitados dado que o valor de p é influenciado pelo número de permutações (Paliy; Shankar, 2016). Outro fator importante é a homocedasticidade, homogeneidade da dispersão dos pontos em relação ao centróide de seus respectivos grupos. Tanto o ANOSIM quanto PERMANOVA e o teste de Mantel são sensíveis a diferenças na dispersão de cada grupo (Anderson, 2017). Embora o PERMANOVA tenha se mostrado mais robusto na questão da homocedasticidade, quando a dispersão é diferente entre os grupos, o valor de p pode estar inflado dificultando a interpretação dos resultados dos testes supracitados (Bruneel et al., 2021). Assim, é importante observar a homocedasticidade para melhor interpretar os resultados dos testes estatísticos ao avaliar as diferenças entre as comunidades bacterianas nos grupos estudados.

2.4.5 Análise diferencial de abundância

Além das análises de diversidade alfa e beta, foram desenvolvidos métodos para determinar espécies ou grupos de bactérias diferentemente abundantes entre grupos. O método mais empregado é a análise de discriminante linear e tamanho de efeito (LEfSe - *Linear discriminant analysis effect size*). Ele utiliza o Kruskal-Wallis para determinar os taxa diferentemente abundantes entre os grupos e depois realiza uma análise de discriminante linear (LDA) para medir o tamanho do efeito destes taxa em cada grupo medindo a força dessa associação entre taxa diferentemente abundante e grupo (Segata et al., 2011). Uma análise filogenética também é realizada em conjunto com o LEfSe dando visibilidade às bactérias diferentemente abundantes nos grupos e suas proximidades filogenéticas (Palmer et al., 2019).

Métodos comumente empregados em análises de transcriptoma como o DESeq2 e EdgeR também foram empregados em análises de microbioma para determinar bactérias diferentemente abundantes (Ma; Luo; Jiang, 2020). Ambos são amplamente empregados em dados de sequenciamento de RNA e utilizam uma regressão binomial negativa para determinar as bactérias diferentemente abundantes no comparativo caso-controle (Lutz et al., 2022). No entanto, o dado é normalizado com métodos diferentes, sendo a expressão log relativa (RLE - *Relative Log Expression*) utilizada no DESeq2 e a média aparada dos valores M (TMM - *Trimmed Mean of M-values*) empregada no EdgeR (Abbas-Aghababazadeh; Li; Fridley, 2018). Apesar das similaridades entre o dado de sequenciamento de RNA e do gene 16S rRNA de bactéria, estes dois métodos de análise diferencial apresentaram ineficiências no controle de falsos positivos quando aplicados em dados de microbioma (Yang; Chen, 2022). Além disso tais métodos não levam em consideração a composicionalidade do dado de microbioma. Recentemente, foi desenvolvido um método chamado LinDA (*Linear models for Differential Abundance analysis*) que aplica regressão linear logarítmica nas contagens transformadas por CLR para lidar com a composicionalidade do dado (Zhou et al., 2022). Em seguida é aplicada uma correção de viés e são calculados os valores de p corrigidos para taxa de falsas descobertas. O LinDA também permite a utilização de uma regressão de efeito misto para acomodar desenhos experimentais longitudinais que não são contemplados nos outros métodos de análise diferencial supracitados (Spies et al., 2017).

Este tipo de análise é bastante importante para detectar bactérias mais abundantes em grupos de interesse com potencial de uso para futuras estratégias paratrangênicas de controle. Recentemente, algoritmos de aprendizagem de máquina vêm sendo aplicados em estudos de microbioma para predizer os taxa de bactérias associados com características de interesse

(Zhou; Gallins, 2019). Tal abordagem se mostra bastante interessante por permitir lidar com grande quantidade de dados principalmente em situações de meta análises de diversos estudos (Cammarota et al., 2020). Na comparação entre os diferentes métodos de análise diferencial da microbiota, resultados muito distintos foram encontrados (Nearing et al., 2022; YANG; Chen, 2022). Tais divergências na determinação de bactérias diferentemente abundantes entre grupos podem ser decorrente das diferentes formas de normalização e detecção destas bactérias empregadas em cada método. Assim, é importante levar em consideração como cada método conduz a análise bem como as premissas que consideram em relação ao dado como esparsidade e composicionalidade na hora de escolher o método de análise.

2.4.6 Análise funcional

Adicionalmente às análises de microbioma supracitadas é possível realizar a análise funcional do microbioma identificando diferenças de funções metabólicas entre populações de bactérias de grupos de interesse. Dependendo da abordagem de sequenciamento empregada o fluxo de trabalho da análise funcional varia. Quando WGS é empregado, são realizadas predições genéticas dos metagenomas montados seguida de identificação dos genes e anotação funcional por meio de buscas baseadas em homologia em bancos de dados de enzimas (KEGG - *Kyoto Encyclopedia of Genes and Genomes*) ou proteínas (Liu, G. et al., 2020). Em seguida é realizada análise de enriquecimento funcional, LDA ou redes metabólicas de acordo com a comparação de interesse do estudo (Niu et al., 2018). Esta abordagem por meio de WGS é mais generalista e permite detectar tanto funções metabólicas das bactérias quanto de células do hospedeiro presentes nas amostras facilitando uma melhor compreensão da interação entre microbioma e hospedeiro.

Em estudos que aplicam o sequenciamento do gene 16S rRNA bacteriano outras abordagens foram desenvolvidas utilizando um banco de dados do KEGG que associa vias metabólicas a grupos de bactérias. Foram desenvolvidos alguns métodos que usam a abundância relativa da tabela de OTU/ASV e o banco de dados do KEGG para predizer as vias metabólicas mais ativas de acordo com as bactérias mais abundantes e suas respectivas vias metabólicas (Iwai et al., 2016). Um desses métodos é o PICRUSt que utilizava o banco de dados de 16S rRNA bacteriano Greengenes (Langille et al., 2013) enquanto que um outro método, Tax4Fun, utiliza o banco do Silva (Asshaue et al., 2015). Recentemente atualizado, o PICRUSt2, também utiliza o banco do Silva e trabalha com as ASVs do fluxo de análise do QIIME2 (Douglas et al., 2020). Com base nos resultados obtidos por tais métodos de predição

funcional da microbiota é possível aplicar ferramentas que determinam abundância diferencial, como as citadas no tópico 2.4.5. De forma análoga à análise de enriquecimento empregada na análise de dados de sequenciamento de RNA, é possível usar os identificadores do KEGG Orthology diferentemente abundantes para avaliar quais vias metabólicas estariam enriquecidas em cada grupo (Wu et al., 2021).

Apesar de promissora, é importante ressaltar que essa abordagem é uma aproximação e não leva em consideração a expressão de proteínas das vias metabólicas. Adicionalmente, o banco de dados com a associação entre bactéria e via metabólica do KEGG é limitado (Galloway-Peña; Hanson, 2020). Ainda assim, a análise funcional do microbioma permite apontar possíveis mecanismos metabólicos que possam estar influenciando na relação patógeno e artrópode, bem como na sua competência vetorial.

2.4.7 Redes de co-ocorrência

A co-ocorrência se dá pelo presença simultânea de duas unidades como espécies animais ou bactérias em mesmo ambiente ou microbioma (Goberna; Verdú, 2022). Assim, matrizes de co-ocorrência foram empregadas em estudos de ecologia para descrever a distribuição de espécies, taxa, em diferentes biomas ou ambientes (Goberna; Verdú, 2022). Mais recentemente, com o intuito de avaliar a estrutura da comunidade microbiana, redes de co-ocorrência vêm sendo cada vez mais aplicadas em estudos de microbioma na última década ao comparar a co-ocorrência em diferentes microbiomas com diferentes fenótipos, por exemplo caso-controle (Gao et al., 2022). Dentre as formas de avaliação da co-ocorrência de bactérias, métodos que envolvem correlações e regressões são os mais comumente empregados (Matchado et al., 2021). Tal abordagem permite compreender como se correlacionam as bactérias de uma microbiota bem como observar quais são importantes para a estrutura de toda a comunidade microbiana ou de módulos específicos. Inicialmente foram utilizadas as correlações de pearson ou spearman para avaliar as correlações entre bactérias de uma determinada microbiota (Layeghifard; Hwang; Guttman, 2017). No entanto, o uso desses métodos de correlação vem sendo criticado devido ao fato deles não levarem em consideração características importantes do dado de microbioma como a composicionalidade ocasionando correlações espúrias (Aitchison, 1982; Lovell et al., 2015).

Ao comparar diferentes métodos de correlação, foi observado que estas características do dado representam um gargalo na avaliação das correlações entre bactérias uma vez que estariam associadas a uma maior detecção de falsos positivos (Weiss et al., 2016). Assim, foram

desenvolvidos métodos que ajustam o dado para adequar essas questões permitindo a utilização das correlações de pearson ou spearman como o SparCC, *Sparse Correlations for Compositional data* (Friedman; Alm, 2012). Este método de correlação transforma o dado usando CLR para ajustar a questão da composicionalidade e faz uma aproximação iterativa da correlação entre bactérias (Matchado et al., 2021). Devido às múltiplas interações para inferir tanto a correlação bem como um pseudo valor de p, o SparCC é computacionalmente mais pesado em comparação com outras formas de correlação (Hirano; Takemoto, 2019). Um outro método que também ajusta para a composicionalidade porém não ajusta para a esparsidade é o CCLasso, *Correlation inference for Compositional data through Lasso* (Fang et al., 2015). Este também utiliza o dado transformado por CLR e aplica um modelo de variável latente com a abundância de cada bactéria, variável dependente, em relação às outras bactérias, variáveis independentes do modelo. Devido à alta dimensionalidade do dado é aplicada uma penalização como na regressão de Lasso para controlar e reduzir associações espúrias bem como o sobreajuste. Em comparação ao SparCC, o CCLasso tem uma estimativa mais acurada da correlação além de lidar melhor com correlações espúrias enquanto o SparCC é mais robusto em relação à esparsidade (Lutz et al., 2022).

Uma das vantagens de analisar uma rede de co-ocorrência é a possibilidade de observar bactérias ou grupos de bactérias importantes para a comunidade bacteriana, bem como capturar a dinâmica da microbiota em casos de estudos longitudinais (Layeghifard; Hwang; Guttman, 2017). Ela também contribui para a descrição de uma microbiota disbiótica dado que esta não está associada somente a uma menor diversidade bacteriana, mas também com uma desorganização das correlações entre bactérias (Chen et al., 2020). Ao comparar as redes de co-ocorrência microbiana de indivíduos com doença de Chron com indivíduos do grupo controle, foi observada uma rede menos conectada e mais esparsa nos indivíduos doentes (Mondot et al., 2016). Tal observação destaca a importância de avaliar outros aspectos da microbiota para descrever o estado de disbiose de uma determinada comunidade bacteriana.

Devido a forma como as redes de co-ocorrência são construídas, é importante destacar que correlações não informam a respeito de causalidade e assim tais redes não indicam uma interação ecológica entre as bactérias (Goberna; Verdú, 2022). No entanto, ainda que não informe diretamente a influência de uma determinada bactéria na microbiota, a rede de co-ocorrência possibilita identificar pontos de partida para estudos direcionados em bactérias possivelmente importantes na estruturação da microbiota (Kishore et al., 2023).

3 **OBJETIVOS**

3.1 **Objetivo geral**

Identificar biomarcadores bacterianos em vetores artrópodes associados à competência vetorial.

3.2 **Objetivos específicos**

- Comparar a comunidade bacteriana da microbiota de espécies de carrapato com ampla competência vetorial na transmissão de múltiplos patógenos;
- Determinar funções metabólicas associadas à competência vetorial de carrapatos pluri competentes;
- Comparar a composição microbiana de mosquitos com perfil de susceptibilidade ao vírus Zika com os refratários;
- Apontar possíveis marcadores microbianos de mosquitos associados à susceptibilidade ou refratariedade ao vírus Zika.

4 **RESULTADOS**

Os resultados da tese serão apresentados nos manuscritos a seguir:

MANUSCRITO 1: Another tick bites the dust: Exploring the association of microbial composition with a broad transmission competence of tick vector species.

MANUSCRITO 2: The Evil that bacteria do in ZIKV susceptibility in mosquitoes.

4.1 **Manuscrito 1 - Another tick bites the dust: Exploring the association of microbial composition with a broad transmission competence of tick vector species**

O manuscrito foi aceito para publicação em 21/08/2023 na revista ***Microbiology Spectrum*** (Fator de impacto = 9.043) como artigo científico e corresponde aos objetivos específicos da tese:

Caracterizar a comunidade bacteriana da microbiota de espécies de carrapato com ampla competência vetorial na transmissão de múltiplos patógenos;
Determinar funções metabólicas associadas à competência vetorial de carrapatos pluri competentes.

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Another tick bites the dust: Exploring the association of microbial composition with a broad transmission competence of tick vector species

Running Head: Microbiome pluri vectorial competence

Tiago F. Mota[a]#, Eduardo R. Fukutani[a], Kelsilandia A. Martins[b], Vanessa R. Salgado[c], Bruno B. Andrade[a], Deborah B. M. Fraga[a], Artur T. L. Queiroz[a]

[a]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Bahia, Brazil
[b]Division of Biomedical and Life Sciences, Lancaster University, Lancaster, United Kingdom
[c]Faculdade de Medicina Veterinária da União Metropolitana de Educação e Cultura (UNIME), Lauro de Freitas, BA 42700-000, Brazil

#Address correspondence to Tiago F. Mota, tiagofmotavet@gmail.com

Abstract

Ticks harbor and transmit many different pathogens. While some species are competent vectors of a single pathogen, Mono Competent Vectors (MCV), other species are proven to be competent vectors of several pathogens, Pluri Competent Vectors (PCV). Such a difference in vector competence might be related to the microbiome. To better comprehend its influence on the vector competence of ticks for one or several pathogens, a data driven approach using publicly available databases was applied on bacterial 16S rRNA from MCV and PCV tick species. Alpha and beta diversity, co-occurrence networks and functional profiles were analyzed. A differential analysis was performed to identify bacterial genera associated with PCV ticks. These tick species presented higher richness and the bacterial composition showed a significant difference between MCV and PCV ticks. The bacteria genera of PCV ticks demonstrated fewer correlations within each other in comparison with MCV ticks. The differential analysis revealed 14 bacteria genera related to PCV tick species, such as *Rickettsia*, *Staphylococcus* and *Corynebacterium*. Using 24 differently abundant genera, tick samples from another dataset could be classified into either PCV or MCV with high accuracy and concordance. Moreover, pathway regulation related to ROS detoxification, β-Lactam resistance and dTDP-L-rhamnose biosynthesis could be participating in competence of PCV ticks for several tick-borne pathogens. These findings enlighten our understanding of the bacteria community's role on some tick species' broad vector competence.

Importance

Some tick species are competent to transmit more than one pathogen while other species are, until now, known to be competent to transmit only one single pathogen. Such a difference

in vector competence for one or more pathogens might be related to the microbiome and understanding what differentiates these two groups of ticks could help us control several diseases aiming at the bacteria groups that contribute to such a broad vector competence. Using 16S rRNA from tick species that could be classified into these groups, genera such as *Rickettsia* and *Staphylococcus* seemed to be associated with such a broad vector competence. Our results highlight differences in tick species when they are divided based on the number of pathogens they are competent to transmit. These findings are the first step into understanding the relationship of one single tick species and the pathogens it transmits.

Keywords: Vector competence, Tick-Borne Diseases, Microbiome, Pathogen transmission.

Introduction

Vector-borne diseases are responsible for more than 700,000 deaths per year and around 80% of the world's population are at risk of infection (1). Among the arthropod vectors, ticks and mosquitoes are responsible for the majority of the vector-borne diseases transmission (2). Ticks are obligatory hematophagous arthropods that have a broad host spectrum and are spread worldwide, being an important vector linking pathogens to vertebrates. These characteristics assign them as an important public health problem. Several tick species such as *Ixodes scapularis*, *Ix. ricinus*, *Haemaphysalis longicornis* and *Rhipicephalus microplus* are known to be vectors to many pathogens from distinct domains, i.e. virus, bacteria and protozoa (3). Furthermore, they also impair the society's economy and public health (4, 5).

Usually vector-pathogen studies on ticks focus on single-pathogen infections, despite co-infections cases being a commonly noted occurrence on field ticks (6). Although co-infected with many pathogens, these ticks should not be always considered responsible for their transmission. Vector competence represents the arthropod's ability to acquire a pathogen infection, maintain it with replication and further transmission to a susceptible host leading to disease development (7). Thus, the incrimination as a vector should be empirically determined to avoid misclassification regarding pathogen transmission (8). Some species, such as *Ix. scapularis* and *Ix. ricinus* were empirically described transmitting bacterial, protozoan and viral pathogens (9–12). On the other hand, other tick species like *Ix. holocyclus* and *Hyalomma dromedarii* were incriminated as vectors of many pathogens while having little empirical evidence for most of these pathogens. This classification regarding the number of transmitted pathogens was assumed in studies based on molecular detection, which does not prove vector

competence (8). However, until now, empirical experiment-based studies suggested that they are competent in transmitting only one single pathogen (13–16).

The vector competence of a given tick species to transmit multiple pathogens appears to be related to its microbiome. It was demonstrated that pathogen-microbiome interaction could influence the infection establishment and transmission. The study of tick microbiome enlightened the tick-pathogen relashionship's complexity, in which symbiont bacteria plays an important role in tick fitness and vector competence (17). This type of discovery with aid of high-throughput approaches enables the development of new control methods focused on the pathogen-microbiome-vector relationship (18). Moreover, a point of view from tick species that are competent to transmit several pathogens in comparison with those that have a narrower vector competence could bring us insights into control mechanisms to decrease such a broad competence. For instance, the introduction of modified symbiont *Sphingomonas* in tick microbiota has been demonstrated to reduce *Anaplasma phagocytophilum* infection in *Ix. scapularis* ticks (19). Along these lines, the first group of ticks could be assigned as being pluri-competent vectors (PVC) while the second group of ticks as being mono-competent vectors (MCV).

Thus, the aim of the present work was to explore the microbiome data from tick species identified as PCV in comparison with those that are, up to date, considered as MCV. This exploratory approach can help us investigate what tick microbiome can tell us about such a broad vector competence.

Results

For the literature review on vector competence of ticks that have 16S data deposited in Sequence Read Archive (SRA), 2651 papers have been screened and 226 empirical vector competence related studies were fully read, figure 1. The majority of these tick species with 16S data in SRA, 32 out of 58, could not be classified into MCV (competent to transmit one or any pathogen) or PCV (competent to transmit more than one pathogen) as only one or no papers have been found that have evaluated its vector competence for any pathogen. Among the 26 species that could be classified based on 226 studies, 17 species had samples with the inclusion criteria for the microbiome analysis herein proposed, i.e. 16S V3-V4 or V4 region, paired-end sequences, 250 to 300 bases, field collected, washed with ethanol, whole body tissue and free of pathogens. Among the 1480 samples meeting the inclusion criteria, 352 samples were divided into two datasets. This sample number was selected in order to balance the number of samples of each tick species within each group as some species were over represented.

The training dataset was used to characterize the microbiota and identify differentially abundant bacteria while the validation dataset provided samples to assess the accuracy of the bacteria associated with any of the groups to distinguish them. For the training dataset 225 samples of 7 species have been chosen, being the MCV group composed by *Dermacentor silvarum*, *De. occidentalis* and *Ix. holocyclus* while the PCV group by *Amblyomma cajennense*, *Ha. longicornis*, *Ix. ricinus* and *Rh. sanguineus*. The remaining samples, 127, were used for the validation dataset being the MCV group composed by *D. occidentalis*, *D. silvarum*, *Ha. leporispalustris*, *Ix. holocyclus* and *Ix. persulcatus*. The validation dataset's PCV group was composed of *De. marginatus*, *De. reticulatus*, *De. variabilis*, *Ha. longicornis*, *Ha. punctata*, *Ix. pacificus*, *Ix. ricinus* and *Rh. sanguineus*. A second validation dataset, 16S V1-V2 region, was composed of 100 samples from four tick species, being *Ha. humerosa* and *Ix. holocyclus* assigned to the MCV group while *Ix. ricinus* and *Rh. sanguineus* to the PCV group. For each dataset, samples were randomly selected in order to balance the number of samples per tick species within each group. Differences of sample number among groups was not statistically significant when compared with the other datasets by chi-square test. The whole classification results and list of tick species and pathogens pairs that have been evaluated can be seen in the supplementary material.

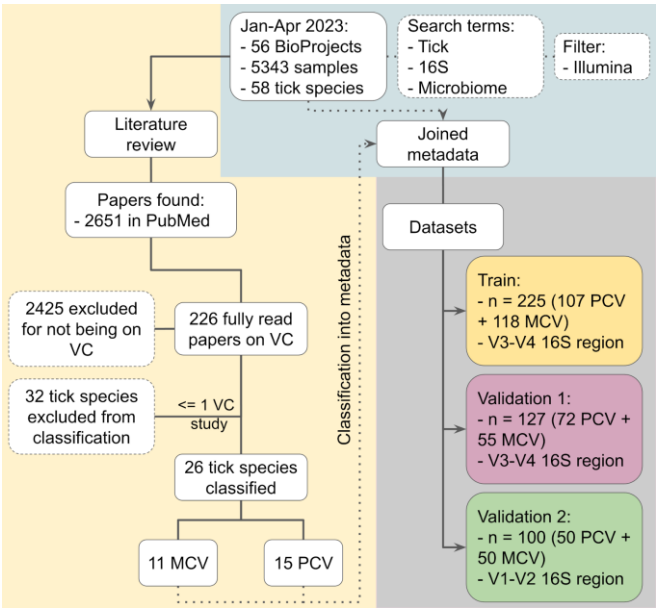

Figure 1: Flowchart of microbiome data search in NCBI's Sequence Read Archive followed by literature in PubMed review on tick species containing 16S rRNA data. The light blue area

represents the search in the SRA database. The light yellow area represents the literature review and classification into MCV and PCV tick species. The purple area represents the datasets prepared for the microbiome analysis. VC - Vector Competence; MCV - Mono Competent Vector; PCV - Pluri Competent Vector.

The bacterial community of ticks from each studied group (MCV and PCV) were composed by similar families and genera, however, with different centered log-ratio, CLR, abundances among the groups (Figure 2A). For instance, *Rickettsiaceae*, *Moraxellaceae*, *Staphylococcaceae* and *Corynebacteriaceae* families were more abundant in the PCV group. On the other hand, *Enterobacteriaceae*, *Streptococcaceae*, *Rhizobiaceae*, *Flavobacteriaceae*, *Sphingomonadaceae* and *Comamonadaceae* were more present in the MCV group when comparing both groups in the heatmap, figure 2A. In the beta diversity analysis using Phylogenetic Isometric Log-Ratio transformation (PhILR) followed by an Euclidean distance matrix, MCV and PCV groups presented different bacterial communities as seen in the Principal Coordinate Analysis (PCoA), figure 2B. This separation between groups was statistically significant (PERMANOVA, $F = 93.04$, $R^2 = 0.294$, $p < 0.001$). According to the PERMANOVA test, 29.4% (R^2) of the distance between groups could be explained by their vector competence grouping (MCV and PCV). There was no statistically significant difference in multivariate dispersion in the comparison between groups, $F = 0.18$ and $p > 0.674$. Thus, *p*-value inflation in the PERMANOVA test should not be expected.

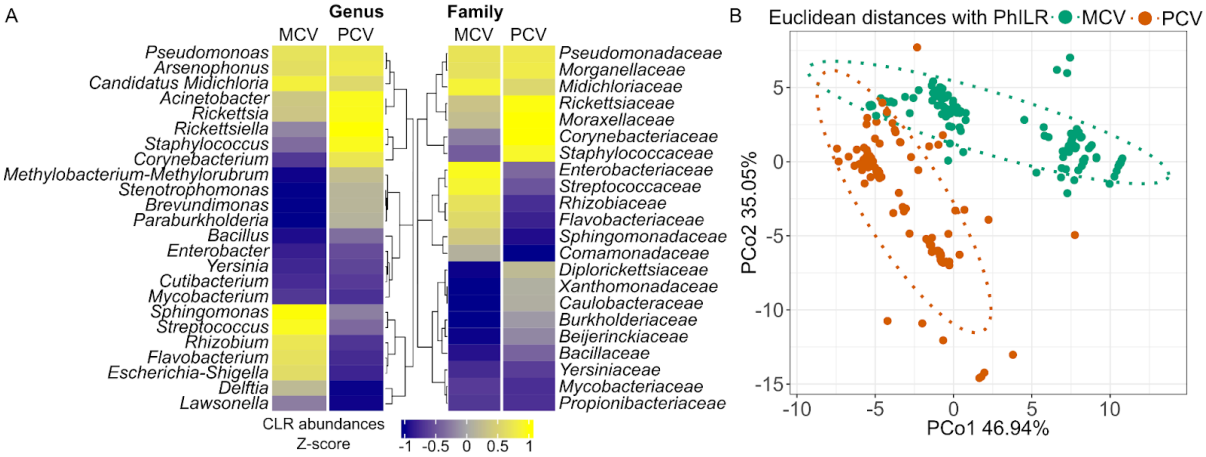

Figure 2: Microbial composition and beta diversity highlighting differences in the bacterial community of mono competent vectors, MCV, and pluri competent vectors, PCV. In A, a heatmap using mean CLR transformed abundances by group is represented. Z-score has been applied in order to reduce color scale range. In B, a PCoA from an Euclidean matrix of PhILR transformed counts between groups is represented.

In order to evaluate how bacteria genera correlated with each other within groups, networks using Sparse Correlations for Compositional data, SparCC, have been built for each group separately. The co-occurrence network of PCV tick species was less modular, with lower overall centrality and density as shown in figure 3. Due to this result, the alpha diversity has been evaluated using rarefied counts to see if the smaller and less dense network seen in PCV tick species was related to a lower diversity of bacteria. Contrasting this previous idea, alpha diversity was higher in PCV ticks as measured by both Shannon and inverse Simpson metrics with Mann-Whitney $p < 0.0001$, figure 3D.

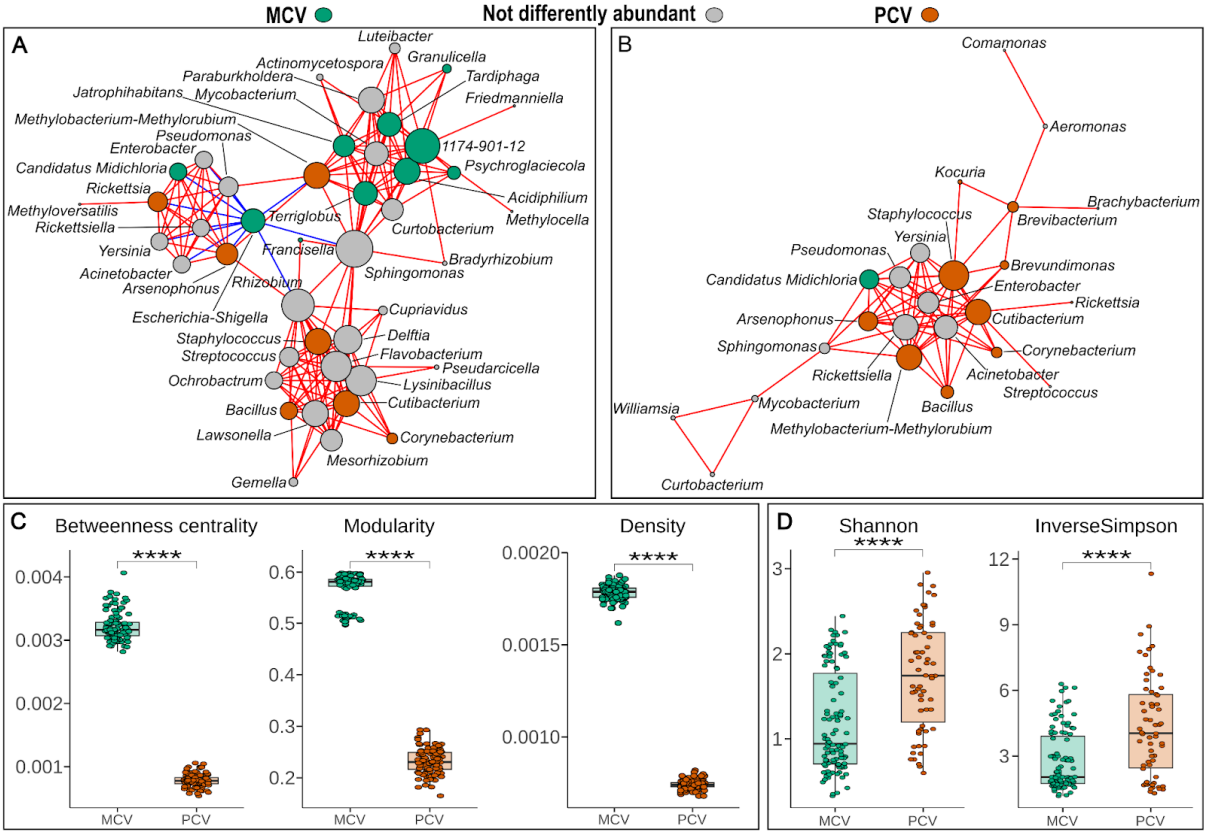

Figure 3: Co-occurrence networks of mono competent vectors, MCV (A), and pluri competent vectors, PCV (B) tick species. Node diameter was measured by each node degree. Color of nodes are related to the genera that were differently associated with each of the groups in the Linear models for Differential Abundance analysis. Color of edges represent (red) positive and (blue) negative correlations > 0.4 between genera. In C and D, boxplots of network performance metrics and alpha diversity metrics are shown. Comparisons between groups were done using Mann-Whitney test with $p < 0.0001$.

Among the 408 pathways predicted with PICRUSt2's functional analysis, 197 were associated to any of the groups, MCV or PCV, with $\log_2$ fold change > 1 and BH adjusted *p*-value < 0.05, according to Linear models for Differential Abundance, LinDA. The pathways predicted to be more active in PCV tick species were related to the biosynthesis of dTDP-L-rhamnose, mycothiol, peptidoglycan as well as methyl ketone. On the other hand, the pathways which were predicted to be up-regulated in MCV tick species were L-tryptophan biosynthesis, sulfoglycolysis and degradation of D-galactarate and D-glucarate. A complete table of all differentially regulated pathways can be seen in the supplementary table 1.

Several bacteria genera were associated with each of the studied tick groups, presenting LinDA's $\log_2$ fold change > 0.5 and BH adjusted $p < 0.05$ (figure 4A). Genera such as

Candidatus Midichloria, Escherichia-Shigella, Francisella and Acidiphilum were related to the MCV group. To the same degree, *Rickettsia*, *Staphylococcus*, *Methylobacterium-Methylorubium* and *Corynebacterium* were associated with the PCV group. In order to validate LinDA analysis results, a PCoA with PhILR and Euclidean distance matrix was used to assess if separation was better using only the genera associated with each group. As seen in figure 4B, the clustering separation between the groups was greater than that observed before the filter. In both situations there was a statistically significant difference in centroid positions from both groups (PERMANOVA, $p < 0.001$), while there was an increased R^2 value in the above-mentioned validation dataset from 0.294 to 0.445. In both cases, the homogeneity dispersion of samples within each group showed no statistically significant differences, $F = 0.18$ and $p > 0.674$; $F = 3.60$ and $p = 0.057$, respectively. Using the train dataset, i.e. the same dataset used for the differential analysis, ticks were classified using a principal component regression followed by a Receiver operating curve (ROC) which showed an area under the curve (AUC) of 0.859. When another dataset with new samples that sequenced the same 16S variable region, V3-V4, was used a similar AUC of 0.878 was obtained. Such comparison could not be performed for the second validation dataset using samples that have sequenced the 16S V1-V2 region as the 24 differently associated bacteria genera by LinDA analysis were not present in this dataset. The model using the 24 genera most associated with MCV or PCV ticks demonstrated a high accuracy of 0.95 (95%CI 0.90-0.98) with a non-informative rate of 0.57 and high concordance, kappa coefficient of 0.90.

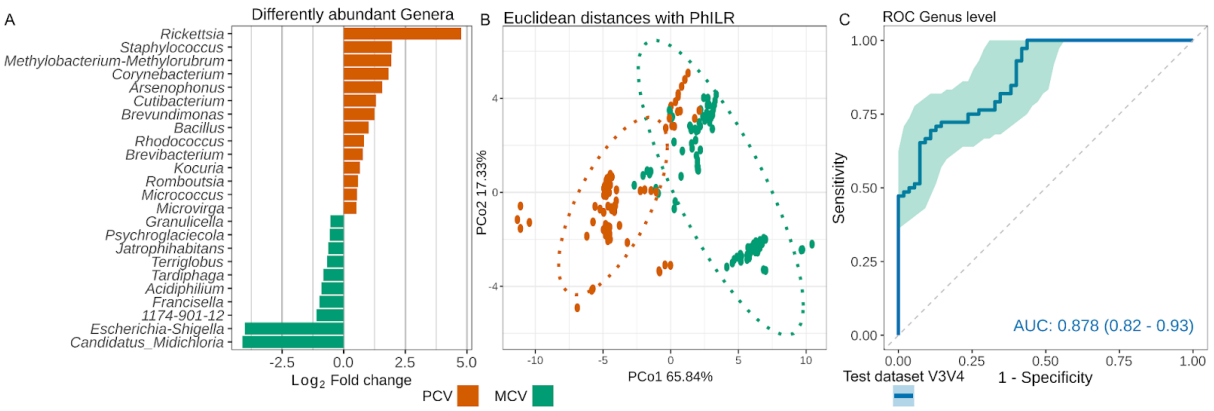

Figure 4: Differential analysis to identify to which group bacteria genera were associated with and validation of using these genera to measure the accuracy of using them to separate samples from another dataset into MCV or PCV ticks. In A, LinDA analysis of the microbiota from both mono competent vectors (MCV) and pluri competent vectors (PCV) groups. Only taxa with $\log_2$ fold change > 0.5 and Benjamini-Hochberg adjusted $p < 0.05$ are shown. In B, Principal

Coordinate Analysis of the beta diversity, with PhILR and Euclidean distance, when only this group of bacteria was kept. In C, Receiver operating curves (ROC) the area under the curve (AUC) in the train and validation (V3-V4) datasets based on principal component regression.

Discussion

The tick species herein evaluated were selected based on the presence of laboratory confirmation, and its vector competence being classified as MCV and PCV. Only species assessed under experimental conditions were used. This evaluation was performed by a comprehensive literature review of vector competence studies which highlighted a publication bias in such kind of studies. For instance, some tick species such as *Ix. ricinus*, *Ix. scapularis* and *Ha. longicornis*, have more studies on their vector competence for any pathogen. These species are most likely to be part of the PCV group as more information regarding their competence to transmit a broader range of pathogens is available in the literature. Regardless of such limitations, several tick species with microbiome data deposited in NCBI's SRA could be classified as being MCV or PCV allowing us to further evaluate differences in their microbiota.

The alpha diversity results showed a higher richness in PCV tick species. Such observation contrasts what has been seen under empirical condition where *Ix. scapularis* co-infected with three pathogens, *Bo. burgdorferi*, *An. phagocytophilum* and *Ba. microti*, did not show differences in alpha diversity's shannon index when compared with pathogen free ticks (20). Considering our network analysis, the richer PCV group had a network with less correlations among the genera. Such contrast demonstrates that although the microbiota composition of PCV ticks being richer, less correlated microbiota might be important before co-infection for multiple pathogen vector competence, since the samples herein evaluated were free of infection. The microbiota's beta diversity of MCV and PCV ticks differed in terms of phylogenetic distance as shown in the beta diversity evaluation, PERMANOVA $p < 0.001$, and as multivariate dispersion has not been observed, *p*-value tend not be over-inflated. Such a difference in beta diversity suggests unique microbial composition related to competence in transmitting several pathogens which was further evaluated with LinDA.

Considering the bacteria genera with LinDA's $\log_2 > 0.5$ and BH adjusted *p*-value < 0.05, *Francisella* genus was associated with the MCV group, suggesting that bacteria belonging to this genus should direct tick species towards transmitting less pathogens. This genus is composed of pathogenic species as well as endosymbionts that have been identified in many tick species (21). In particular, the latter which are detected in all life stages both in colony

reared and field collected ticks (21). High prevalence of *Francisella* was associated with decreased *Rickettsia* genus, which impairs tick borne pathogens transmission by outcompeting other bacterial genera (22, 23). In our analysis no direct correlation was observed between these two genera.

Candidatus Midichloria was also associated with the MCV group. This bacterial genus is closely related to symbionts such as *Midichloria mitochondrii.* This species has been recently demonstrated to be positively associated with *Bo. burgdorferi* and *Neoehrlichia mikurensis* while being negatively associated with *An. phagocytophilum (24)*. However, a method has been used which modeled co-occurrence of bacteria based on the chance of these bacteria be present together with *Mi. mitochondrii*. As it is a symbiont bacteria present in many ixodid tick species (25), co-occurrence with pathogens does not mean that it plays a role in infection or transmission. In our analysis the genus *Ca. Midichloria* was positively correlated with environmental genera such as *Staphylococcus*, *Enterobacter* and *Acinetobacter* (26) as well as some symbiont bacteria such as *Arsenophonus sp.* and *Rickettsia sp. (27)*. Such correlation with environmental and symbiont bacteria might be indicating a bridge role of *Ca. Midichloria* as environmental bacteria are acquired from habitat and blood meal shaping ticks microbiota (26). Some of these genera were also associated in the present work with PCV tick species indicating that *Ca. Midichloria* should be important for both evaluated tick groups as it showed positive correlations in both networks.

Recently, *Mi. mitochondrii*'s pathways and tissue tropism has been studied showing that it may play a role helping the tick by supplying nutrients, increasing its survival as well as maintaining homeostasis and antioxidant defenses (28). Thus, this symbiont might be beneficial for the survival of many bacteria that favor correlations of genera. Altogether, it should be promoting an environment associated with both mono and pluri competence as suggested by the results of the present work. The other bacteria genera associated with MCV group have not been related to vectorial competence or any kind of influence on microbial composition yet. Nonetheless, as demonstrated by the results of the present work, they might be somehow affecting bacterial composition leaning vectorial competence for several pathogens.

The *Staphylococcus* and *Corynebacterium* genera are known environmental bacteria that can be acquired by ticks and might interact with tick's microbiota (26, 29). These genera were associated with uninfected PCV ticks and might, directly or indirectly, favor the colonization of several pathogens in one single tick. The genus *Bacillus* was also associated with PCV ticks and correlated positively with other bacteria related to this same group such as *Staphylococcus* and *Cutibacterium*. In recent work, *Bacillus* has been negatively associated

with ticks infected by the pathogen *R. helvetica* while being positively associated with the genus *Rickettsia (30)*. In the present work such a positive correlation with *Rickettsia* was not observed, at least not directly in the PCV network. However, its positive correlation with other genera also related to PCV ticks indicates that it somehow should be helping to promote a favorable environment for the competence to transmit some pathogens.

Although the genus *Rickettsia* has been associated with PCV ticks, it showed more correlations with other genera in the MCV network demonstrating positive correlations with other bacteria related to both groups. Since pathogen infected samples were removed from the analysis and most of the species belonging to *Rickettsia* are non-pathogenic (31), we believe that this genus detected in our analysis refers to non-pathogenic symbionts. Rickettsial endosymbionts like *Ri. peacockii* and *Ri. buchneri* have been strongly related to rickettsial pathogens such as *Ri. rickettsii* and *Ri. monacensis*, respectively (32, 33). However, little is known about the influence of these endosymbionts in tick vector competence (34). Based on the results of the present work, these bacteria should be somehow related to the transmission of several pathogens by one single tick species.

Although geographically distinct populations of a given tick species have microbiomes with different composition, functional profile is conserved and redundant within tick species (35). The results from the functional analysis showed a higher predicted regulation of mycothiol biosynthesis in PCV ticks which is associated with reactive oxygen species (ROS) detoxification as well as protection against electrophilic compounds (36). Such a defensive mechanism in PCV ticks against ROS and electrophilic compounds might be contributing with the broader vector competence of these tick species as it facilitates pathogens replication and transmission (37).

Biosynthesis of dTDP-L-rhamnose is an important pathway for viability or virulence of many bacterial pathogens such as Group B *Streptococcus*, *Enterococcus faecalis* and *Pseudomonas spp.* (38). This pathway was associated with PCV ticks and since it is absent in mammals (39), it might be an interesting aim to modulate vector competence of this tick group in order to make it less competent to transmit many pathogens.

It has been shown that *Rickettsia* and *Corynebacterium* genera were related to penicillin degradation pathways (40). Thus, these genera might be related to the higher β-lactam resistance predicted by PICRUSt2 analysis in PCV ticks, i.e. higher peptidoglycan biosynthesis V β-lactam (MetaCyc). Both of these genera have been associated with PCV ticks and together with the higher β-lactam resistance might represent an important mechanism to promote many pathogens development.

Most of the studies evaluating functional differences in the host-pathogen relationship tests one single pathogen versus one single host. Our results alongside these one-to-one host-pathogen interactions from previous studies have shown important shared pathways that contribute with higher β-lactam resistance regulation and tick's redox balance mechanisms. Altogether these pathways might be related to the competence to transmit several pathogens.

After selecting the bacterial genera associated with any of the tested groups beta diversity results differed in terms of R^2 being about 1.5 times higher after picking taxa with LinDA's $\log_2 > 0.5$ and BH adjusted p-value < 0.05. This demonstrates that these bacteria are better able to distinguish MCV and PCV ticks' groups as by PhILR and Euclidean distance. However, this performance should be applicable only to other tick samples that had sequenced the 16S rRNA V3-V4 region as it had high accuracy, 0.95 (95%CI 0.90-0.98) when validated with another set of samples from this same variable region. Validation with V1-V2 dataset could not be tested as none of the 24 genera indicated by LinDA analysis were present in this dataset. This difference in bacterial composition should be associated with the lower comparability between datasets from different 16S variable regions due to specificities in bacteria classification of each region (41).

Several bacteria groups related to PCV tick species are commonly found in the environment which might be related to the sequenced tick tissue and pre-washing step. Samples herein analyzed had the tick's whole body sequenced and were surface washed with ethanol. Such a cleaning method is known to be the least effective for surface decontamination when compared to other methods, such as 5% sodium hypochlorite (42). However, if samples that had been previously washed with 5% sodium hypochlorite were chosen as selection criteria for the analysis, most of the tick samples would have been Australian ticks which sequenced the V1-V2 16S rRNA region. This criteria would have decreased the influence of environmental bacteria in the analysis, nevertheless, it would have inserted a geographical bias as well as diminished the number of tick species from both groups.

It is noteworthy that the relationship of environmental bacteria with tick microbiota has been proposed affecting its composition (43, 44). Thus, these bacteria may play a role in the tick microbiota indirectly affecting the tick's vectorial competence for many pathogens. Silva's 16S rRNA database presents limitations to accurately identify bacteria to the species level (45). Therefore, the analysis of the present study was limited to bacteria family and genera level. Such accuracy level bewilders the separation of species that could be actually part of the environment from those that had already established a co-evolutionary relationship in the tick microbiome and thus, play a role in the vector competence of PCV tick species. Although

environmental bacteria could not be removed from our analysis, assuming the influence of such bacteria in microbial composition of ticks, changes in the environment could help us modulate the tick microbiome (46). Thus, the bacteria genera herein appointed as associated with the MCV group, such as *Escherichia-Shigella* and *Francisella* could help us, until certain point, diminish the vectorial competence for several pathogens of a PCV tick species. As an example, such an approach can be performed as recently proposed by Mazuecos and collaborators (19) where modified symbiont *Sphingomonas* promoted a reduction of *An. phagocytophilum* in *Ix. scapularis* ticks. Thus, changing the tick's microbiota using the above-mentioned bacteria associated with mono competent vector ticks could be a method to reduce such a broad vector competence. Naturally, due to the above-mentioned limitations regarding the samples, predictions and mechanisms, the results of the present work should be empirically validated in the future for better comprehension of the mechanisms related to PCVs.

Conclusion

Based on our exploratory approach on the microbiome of uninfected PCV versus MCV ticks, the bacteria genera *Rickettsia*, *Staphylococcus* and *Corynebacterium* and *Arsenophonus* among others are related to a broader vector competence. Additionally, pathways related to ROS detoxification, dTDP-L-rhamnose biosynthesis and β-lactam resistance regulation could be participating in the infection of several tick-borne pathogens in ticks. These findings could be used in the future to regulate the permissiveness of PCV ticks in order to reduce their vector competence or even block the transmission of several pathogens by one single tick species. As we used and integrated publicly available data, it was not possible to determine the species level for all ASVs. Due to this limitation, the genus level was used for the major analysis. Deeper insights would be found evaluating each bacteria species on those ticks. Despite these limitations, we were able to provide understanding on the bacteria community role on the vector competence of tick species studied.

Methods

Vector competence literature review

For this analysis, tick species that have 16S rRNA gene sequencing data in the SRA (Sequence Read Archive) and NCBI's BioProject, between January and April 2023, were selected. Following, a literature review was performed, assessing studies which evaluated the tick species' vector competence for one or more pathogens. Thus, all 58 tick species with 16S rRNA microbiota data deposited in SRA were reviewed for their vector competence for

pathogens of medical and veterinary importance. Reviews were carried out for each species through systematic searches, using the species name and terms related to vector competence (i.e. "Vectorial competence" OR "Vector competence" OR "Vector capability") as well as pathogen transmission pathways (vertical OR horizontal OR transstadial OR transovarial) AND transmi*). The searches were performed on the NCBI's PubMed. When less than 15 studies were found, only the species name was used for the search and all results titles and abstracts were filtered to find works that evaluated the vector competence of the species in a laboratory setting.

To assess the vector competence of each species, tick species and pathogen pairs were classified according to the degree of evidence presented, which used adaptations of the criteria previously outlined in the literature (8). Thus, the criteria to determine vector competence were experimental demonstration of:

i - acquisition of pathogens when feeding uninfected ticks on experimental infective hosts;

ii - maintenance of pathogens during the seedlings of experimentally infected tick life stages (vertical transmission and/or transstadial passage);

iii - transmission of pathogens to a susceptible host in a subsequent blood meal.

Each study was evaluated, focused on demonstration of any of these criteria and a tick/pathogen pair was classified according to the level of evidence presented by the applied method. The criteria was as follows:

- grade 1 when there was only detection of the pathogen in the tick collected in endemic areas;

- grade 2 when ticks collected from the field were evaluated in the laboratory for transmission of pathogens to susceptible hosts or to other life stages (vertical or transstadial transmission) as well as laboratory experiments using artificial infections;

- grade 3 when there was experimental demonstration of at least one of the aforementioned criteria using colony reared ticks;

- grade 4 when all vector competence criteria have been demonstrated in the laboratory.

Species were classified as competent to transmit a given pathogen only when there were studies that evaluated vector competence with evidence degrees 3 or 4 and empirically demonstrated such competence. Despite existing works declaring vector competence for certain pathogens, if they were based only on pathogen detection in ticks or on citations of another published work without enough vector competence evidence, the tick species was still classified

as an incompetent vector for such pathogens. When tick species presented vector competence for more than one pathogen with a grade 3 or 4 evidence degree it was classified as being PCV. On the other hand, if it showed vector competence for only one or any pathogen with grade 3 or 4 evidence degree, it was classified as MCV. A table summarizing the findings and classification of the review can be found in supplementary material.

Data selection and tick classification

From the data on each tick sample with bacterial 16S-rRNA sequencing deposited at the NCBI, a metadata was assembled containing information such as life stage, pre-sequencing washing method, variable region of the 16S gene, tick collection location and tick species. A new variable was added regarding the number of pathogens that each tick species is competent to transmit as the aforementioned classification. In order to reduce the heterogeneity between samples and retain the highest number of tick species, only samples from datasets containing field collected ticks by flagging, ticks' whole body, ticks without previous detection of infection of any pathogen and ticks with prewash using alcohol or alcohol plus some other reagent in the methodology were included. In addition, the sequencing was filtered for V3-V4 or V4 regions with paired-end layout and 250 or 300 bases as a higher number of samples was attained in comparison with other variable regions.

Data processing

All samples were downloaded using SRA toolkit's fastq-dump (github.com/ncbi/sra-tools) and quality checked by Trimmomatic v0.32 (47) to remove sequencing adapter, small and unspecific reads as well as those with Phred quality score lower than 30. After performing the data quality control, all forward and reverse reads have been joined and processed on QIIME2 2022.11 (48) pipeline, removing chimeric sequences and building ASVs (Amplicon Sequence Variant) using the Deblur algorithm (49). Then, all ASVs have been classified using the SILVA v138.1 16S database (50) assuming 97% identity. Before usage, the database was filtered to remove sequences that could be associated with fungi, filtering out Eukaryota, plants, removing Mitochondria and Chloroplast, and sequences with unspecified species classification such as *Unkown*, *uncultured*, NA, *metagenome* and *unidentified*. After classification, a phylogenetic tree was built using MAFFT (Multiple Alignment using Fast Fourier Transform) (51) and fasttree (52) methods. For the analysis, all of these data generated on QIIME2, i.e. ASV table, ASV taxonomic classification and phylogenetic tree, have been imported in R

environment v4.2.3 using Qiime2R package (53). This package creates a phyloseq object that can be further analyzed by the Phyloseq package (54) to measure diversity indices.

Batch effect correction

Before any analysis, a batch correction has been performed to reduce the technical bias variation that could have been caused by batch, e.g. each project being sequenced and performed by different research groups and sequencing machines. Such correction was performed by the ConQuR_libsize function (55), from the ConQuR package v2.0. After batch correction, the corrected ASV table was incorporated in a phyloseq object. For future analysis, samples with less than 100 reads and taxa with less than 10 counts have been removed.

Compositional and diversity analysis

To visualize the microbial composition of MCV and PCV tick species, heatmaps using the batch corrected mean counts transformed by centered log-ratio, CLR, were created. CLR transformed counts were scaled using Z-scores in order to reduce the range of values for better visualization of differences between groups. As the alpha diversity metric could not be measured using CLR transformed counts, the rarefied batch corrected counts have been used for such analysis, rarefying for 1000 counts and using Shannon and Inverse Simpson indices. Beta diversity was assessed by means of ASV count transformation using Phylogenetic Isometric Log-Ratio Transform, PhILR (56), followed by an Euclidean distance matrix. Such an approach takes into account the data's compositionality characteristic together with the phylogenetic distance from taxa present in samples. Principal coordinate analysis was applied to evaluate group distances in terms of beta diversity followed by PERMANOVA test (57), in order to determine statistically significant differences in each group centroid according to the Euclidean distance matrix. The multivariate dispersion has been tested through the vegan's package v2.6.4 betadisper function to assess the homogeneity of each sample distance in relation to its group centroid. If groups are equally homogeneous, then *p*-value from the PERMANOVA test is not inflated.

Co-occurrence network

Co-occurrence networks have been built using Sparse Correlations for Compositional data, SparCC, between samples from each group, MCV and PCV. In order to compare networks from both groups, 100 bootstrap replicates of SparCC correlation matrices were made to extract network performance metrics such as betweenness centrality, modularity and density. After

collecting these metrics from 100 bootstrapped networks, they were compared between MCV and PCV groups with Mann-Whitney test. The package ggClusterNet v0.1.0 has been used to measure SparCC correlation matrices and networks were built using igraph v1.4.1.

Functional analysis

In order to further describe PCV ticks, a functional analysis was performed using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), predicting metagenome functions that are regulated by a given bacterial community based on 16S-rRNA sequencing (58). Predicted pathways were described according to the MetaCyc database and further hierarchically classified into superclasses as provided by the file2meco R package v0.5.1. Differently abundant pathways were identified using Linear models for Differential Abundance, LinDA (59) with a cut-off of 1 $\log_2$ fold change and Benjamini-Hochberg false discovery rate (BH) adjusted *p*-value < 0.05.

Differential analysis and validation of differentially abundant bacteria

To determine groups of bacteria with higher probability to explain differences among groups (MCV vs PCV), LinDA has been applied as it takes into account the data's compositionality. Differently abundant genera related to MCV or PCV were determined when $\log_2$ fold change was > 0.5 with an BH adjusted *p*-value < 0.05. Tick life stages have been used in the LinDA model to adjust the vector competence variable.

The bacteria identified as differentially abundant between MCV and PCV ticks in LinDA were used in a principal component regression approach to assess if these bacterial genera accurately separate tick species as MCV or PCV. Thus, only bacterial genera with the above mentioned fold change and *p*-value criteria were used to run a PCA. The first two components were modeled in a logistic regression to determine if samples belonged to any of the studied tick groups. A validation was performed on a dataset, composed of other tick samples which sequenced V1-V2 and V3-V4 16S rRNA regions and were unused in the differential analysis as well as samples from other tick species in both groups. The performance of the logistic model built from the train dataset was measured using the above mentioned validation datasets containing only the differently abundant bacterial genera. Receiver operating characteristic (ROC) curves, package pROC v1.18.0, and the confusion matrix method, package caret v6.0.94, have been used to measure the accuracy, non informative rate and concordance kappa between train and test datasets.

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Data availability

Raw sequences were obtained from NCBI's SRA under the following BioProjects: PRJEB36903, PRJNA574713, PRJNA577275, PRJNA631062, PRJNA661974, PRJNA664219, PRJNA732915, PRJNA733831, PRJNA766341, PRJNA801881, PRJNA352452, PRJNA401547, PRJNA438789, PRJNA494526, PRJNA523509, PRJNA530927, PRJNA548395, PRJEB46006, PRJNA640465 and PRJNA559059. Metadata and code are available upon request.

References

1. WHO. 2017. Global vector control response 2017–2030. Geneva: World Health Organization.

2. Franklinos LHV, Jones KE, Redding DW, Abubakar I. 2019. The effect of global change on mosquito-borne disease. Lancet Infect Dis 19:e302–e312.

3. Eisen L. 2022. Tick species infesting humans in the United States. Ticks Tick Borne Dis 13:102025.

4. Singh K, Kumar S, Sharma AK, Jacob SS, RamVerma M, Singh NK, Shakya M, Sankar M, Ghosh S. 2022. Economic impact of predominant ticks and tick-borne diseases on Indian dairy production systems. Exp Parasitol 243:108408.

5. Johnson N, Phipps LP, Hansford KM, Folly AJ, Fooks AR, Medlock JM, Mansfield KL. 2022. One Health Approach to Tick and Tick-Borne Disease Surveillance in the United Kingdom. Int J Environ Res Public Health 19.

6. Moutailler S, Valiente Moro C, Vaumourin E, Michelet L, Tran FH, Devillers E, Cosson J-F, Gasqui P, Van VT, Mavingui P, Vourc'h G, Vayssier-Taussat M. 2016. Co-infection of Ticks: The Rule Rather Than the Exception. PLoS Negl Trop Dis 10:e0004539.

7. Wu VY, Chen B, Christofferson R, Ebel G, Fagre AC, Gallichotte EN, Sweeny AR, Carlson CJ, Ryan SJ. 2022. A minimum data standard for vector competence experiments. Sci Data 9:634.

8. Eisen L. 2020. Vector competence studies with hard ticks and Borrelia burgdorferi sensu lato spirochetes: A review. Ticks Tick Borne Dis 11.

9. Hajnická V, Kúdelová M, Štibrániová I, Slovák M, Bartíková P, Halásová Z, Pančík P, Belvončíková P, Vrbová M, Holíková V, Hails RS, Nuttall PA. 2017. Tick-Borne Transmission of Murine Gammaherpesvirus 68. Front Cell Infect Microbiol 7:458.

10. Król N, Militzer N, Stöbe E, Nijhof AM, Pfeffer M, Kempf VAJ, Obiegala A. 2021. Evaluating Transmission Paths for Three Different Bartonella spp. in Ixodes ricinus Ticks Using Artificial Feeding. Microorganisms 9.

11. Levin ML, Stanley HM, Hartzer K, Snellgrove AN. 2021. Incompetence of the Asian Longhorned Tick (Acari: Ixodidae) in Transmitting the Agent of Human Granulocytic Anaplasmosis in the United States. J Med Entomol 58:1419–1423.

12. Sharma R, Cozens DW, Armstrong PM, Brackney DE. 2021. Vector competence of human-biting ticks Ixodes scapularis, Amblyomma americanum and Dermacentor variabilis for Powassan virus. Parasit Vectors 14:466.

13. Smith DJW. 1942. Studies in the epidemiology of q fever. Aust J Exp Biol Med Sci 20:213–217.

14. Piesman J, Stone BF. 1991. Vector competence of the Australian paralysis tick, Ixodes holocyclus, for the Lyme disease spirochete Borrelia burgdorferi. Int J Parasitol 21:109–111.

15. Soares JF, Soares HS, Barbieri AM, Labruna MB. 2012. Experimental infection of the tick Amblyomma cajennense, Cayenne tick, with Rickettsia rickettsii, the agent of Rocky Mountain spotted fever. Med Vet Entomol 26:139–151.

16. Horta MC, Sabatini GS, Moraes-Filho J, Ogrzewalska M, Canal RB, Pacheco RC, Martins TF, Matushima ER, Labruna MB. 2010. Experimental infection of the opossum Didelphis aurita by Rickettsia felis, Rickettsia bellii, and Rickettsia parkeri and evaluation of the transmission of the infection to ticks Amblyomma cajennense and Amblyomma dubitatum. Vector Borne Zoonotic Dis 10:959–967.

17. Hussain S, Perveen N, Hussain A, Song B, Aziz MU, Zeb J, Li J, George D, Cabezas-Cruz A, Sparagano O. 2022. The Symbiotic Continuum Within Ticks: Opportunities for Disease Control. Front Microbiol 13:854803.

18. de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof AM, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancova M, Golovchenko M, Grubhoffer L, Caracappa S, Fooks AR, Gortazar C, Rego ROM. 2017. Tick-Pathogen Interactions and Vector Competence: Identification of Molecular Drivers for Tick-Borne Diseases. Front Cell Infect Microbiol 7.

19. Mazuecos L, Alberdi P, Hernández-Jarguín A, Contreras M, Villar M, Cabezas-Cruz A, Simo L, González-García A, Díaz-Sánchez S, Neelakanta G, Bonnet SI, Fikrig E, de la Fuente J. 2023. Frankenbacteriosis targeting interactions between pathogen and symbiont to control infection in the tick vector. iScience 26:106697.

20. Gil JC, Helal ZH, Risatti G, Hird SM. 2020. microbiome correlates with life stage, not the presence of human pathogens, in ticks submitted for diagnostic testing. PeerJ 8:e10424.

21. Kumar D, Sharma SR, Adegoke A, Kennedy A, Tuten HC, Li AY, Karim S. 2022. Recently Evolved -Like Endosymbiont Outcompetes an Ancient and Evolutionarily Associated -Like Endosymbiont in the Lone Star Tick () Linked to the Alpha-Gal Syndrome. Front Cell Infect Microbiol 12:787209.

22. Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao CC, Ching WM, Karim S.

2014. An Insight Into the Microbiome of the Amblyomma maculatum (Acari: Ixodidae). J Med Entomol 51:119–129.

23. Choubdar N, Karimian F, Koosha M, Oshaghi MA. 2021. An integrated overview of the bacterial flora composition of hyalomma anatolicum, the main vector of cchf. PLoS Negl Trop Dis 15.

24. Krawczyk AI, Röttjers S, Coimbra-Dores MJ, Heylen D, Fonville M, Takken W, Faust K, Sprong H. 2022. Tick microbial associations at the crossroad of horizontal and vertical transmission pathways. Parasit Vectors 15:380.

25. Epis S, Sassera D, Beninati T, Lo N, Beati L, Piesman J, Rinaldi L, McCoy KD, Torina A, Sacchi L, Clementi E, Genchi M, Magnino S, Bandi C. 2008. Midichloria mitochondrii is widespread in hard ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species. Parasitology 135:485–494.

26. Li S-S, Zhang X-Y, Zhou X-J, Chen K-L, Masoudi A, Liu J-Z, Zhang Y-K. 2022. Bacterial microbiota analysis demonstrates that ticks can acquire bacteria from habitat and host blood meal. Exp Appl Acarol 87:81–95.

27. Duron O, Binetruy F, Noël V, Cremaschi J, McCoy KD, Arnathau C, Plantard O, Goolsby J, Pérez de León AA, Heylen DJA, Van Oosten AR, Gottlieb Y, Baneth G, Guglielmone AA, Estrada-Peña A, Opara MN, Zenner L, Vavre F, Chevillon C. 2017. Evolutionary changes in symbiont community structure in ticks. Mol Ecol 26:2905–2921.

28. Olivieri E, Epis S, Castelli M, Varotto Boccazzi I, Romeo C, Desirò A, Bazzocchi C, Bandi C, Sassera D. 2019. Tissue tropism and metabolic pathways of Midichloria mitochondrii suggest tissue-specific functions in the symbiosis with Ixodes ricinus. Ticks Tick Borne Dis 10:1070–1077.

29. Nhan T-X, Parienti J-J, Badiou G, Leclercq R, Cattoir V. 2012. Microbiological investigation and clinical significance of Corynebacterium spp. in respiratory specimens. Diagn Microbiol Infect Dis 74:236–241.

30. Maitre A, Wu-Chuang A, Mateos-Hernández L, Foucault-Simonin A, Moutailler S, Paoli J-C, Falchi A, Díaz-Sánchez AA, Banović P, Obregón D, Cabezas-Cruz A. 2022. Rickettsia helvetica infection is associated with microbiome modulation in Ixodes ricinus collected from humans in Serbia. Sci Rep 12:11464.

31. Bonnet SI, Binetruy F, Hernández-Jarguín AM, Duron O. 2017. The Tick Microbiome: Why Non-pathogenic Microorganisms Matter in Tick Biology and Pathogen Transmission. Front Cell Infect Microbiol 7:236.

32. Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ, Munderloh UG. 2002. Rickettsia monacensis sp. nov., a spotted fever group Rickettsia, from ticks (Ixodes ricinus) collected in a European city park. Appl Environ Microbiol 68:4559–4566.

33. Kurtti TJ, Felsheim RF, Burkhardt NY, Oliver JD, Heu CC, Munderloh UG. 2015. Rickettsia buchneri sp. nov., a rickettsial endosymbiont of the blacklegged tick Ixodes scapularis. Int J Syst Evol Microbiol 65:965–970.

34. Hodosi R, Kazimirova M, Soltys K. 2022. What do we know about the microbiome of ?

Front Cell Infect Microbiol 12:990889.

35. Estrada-Peña A, Cabezas-Cruz A, Obregón D. 2020. Behind Taxonomic Variability: The Functional Redundancy in the Tick Microbiome. Microorganisms 8.

36. Hand CE, Honek JF. 2005. Biological chemistry of naturally occurring thiols of microbial and marine origin. J Nat Prod 68:293–308.

37. Hernandez EP, Talactac MR, Fujisaki K, Tanaka T. 2019. The case for oxidative stress molecule involvement in the tick-pathogen interactions -an omics approach. Dev Comp Immunol 100:103409.

38. van der Beek SL, Zorzoli A, Çanak E, Chapman RN, Lucas K, Meyer BH, Evangelopoulos D, de Carvalho LPS, Boons G-J, Dorfmueller HC, van Sorge NM. 2019. Streptococcal dTDP-L-rhamnose biosynthesis enzymes: functional characterization and lead compound identification. Mol Microbiol 111:951–964.

39. Adibekian A, Stallforth P, Hecht M-L, Werz DB, Gagneux P, Seeberger PH. 2011. Comparative bioinformatics analysis of the mammalian and bacterial glycomes. Chem Sci 2:337–344.

40. Chigwada AD, Mapholi NO, Ogola HJO, Mbizeni S, Masebe TM. 2022. Pathogenic and Endosymbiotic Bacteria and Their Associated Antibiotic Resistance Biomarkers in and Ticks Infesting Nguni Cattle (spp.). Pathogens 11.

41. Willis C, Desai D, LaRoche J. 2019. Influence of 16S rRNA variable region on perceived diversity of marine microbial communities of the Northern North Atlantic. FEMS Microbiol Lett 366.

42. Hoffmann A, Fingerle V, Noll M. 2020. Analysis of Tick Surface Decontamination Methods. Microorganisms 8.

43. Rynkiewicz EC, Hemmerich C, Rusch DB, Fuqua C, Clay K. 2015. Concordance of bacterial communities of two tick species and blood of their shared rodent host. Mol Ecol 24:2566–2579.

44. Zolnik CP, Prill RJ, Falco RC, Daniels TJ, Kolokotronis S-O. 2016. Microbiome changes through ontogeny of a tick pathogen vector. Mol Ecol 25:4963–4977.

45. Hoffman C, Siddiqui NY, Fields I, Gregory WT, Simon HM, Mooney MA, Wolfe AJ, Karstens L. 2021. Species-Level Resolution of Female Bladder Microbiota from 16S rRNA Amplicon Sequencing. mSystems 6:e0051821.

46. Aivelo T, Norberg A, Tschirren B. 2019. Bacterial microbiota composition of ticks: the role of environmental variation, tick characteristics and microbial interactions. PeerJ 7:e8217.

47. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

48. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Titus Brown C, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener

C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Gregory Caporaso J. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857.

49. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R. 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems 2.

50. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: D590–6.

51. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780.

52. Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 26:1641–1650.

53. jbisanz. jbisanz/qiime2R. https://github.com/jbisanz/qiime2R. Retrieved 18 March 2021.

54. McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8:e61217.

55. Ling W, Lu J, Zhao N, Lulla A, Plantinga AM, Fu W, Zhang A, Liu H, Song H, Li Z, Chen J, Randolph TW, Koay WLA, White JR, Launer LJ, Fodor AA, Meyer KA, Wu MC. 2022. Batch effects removal for microbiome data via conditional quantile regression. Nat Commun 13:5418.

56. Silverman JD, Washburne AD, Mukherjee S, David LA. 2017. A phylogenetic transform enhances analysis of compositional microbiota data. Elife 6.

57. Tang Z-Z, Chen G, Alekseyenko AV. 2016. PERMANOVA-S: association test for microbial community composition that accommodates confounders and multiple distances. Bioinformatics 32:2618–2625.

58. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688.

59. Zhou H, He K, Chen J, Zhang X. 2022. LinDA: linear models for differential abundance analysis of microbiome compositional data. Genome Biol 23:95.

4.2 **Manuscrito 2** - The Evil that bacteria do in ZIKV susceptibility in mosquitoes.

O manuscrito foi escrito para ser submetido na revista ***Microbiology Spectrum*** (Fator de impacto = 9.043) como artigo científico e corresponde aos objetivos específicos da tese:

Comparar a composição microbiana de mosquitos com perfil de susceptibilidade ao vírus Zika com os refratários;

Apontar possíveis marcadores microbianos de mosquitos associados à susceptibilidade ou refratariedade ao vírus Zika.

The Evil that bacteria do in ZIKV susceptibility in mosquitoes

Running head: ZIKV mosquito susceptibility microbiota

Tiago F. Mota[a], Vanessa R. Salgado[b], Deborah B. M. Fraga[a], Artur T. L. Queiroz[a]#

[a]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Bahia, Brazil
[b]Faculdade de Medicina Veterinária da União Metropolitana de Educação e Cultura (UNIME), Lauro de Freitas, BA 42700-000, Brazil

#Address correspondence to Artur T. L. Queiroz, artur.queiroz@fiocruz.br

Abstract

Vector competence for Zika virus (ZIKV) was demonstrated for several mosquito species mostly belonging to the *Aedes* genus. In contrast, *Culex* and *Anopheles* mosquito species were refractory for ZIKV infections showing lower infection rates and viral loads. The microbiome plays an essential role in the mosquito basal immune response which can promote a refractoriness for arboviral infections. Thus, the present work aimed to compare publicly available 16S rRNA data from ZIKV refractory mosquito species with ZIKV susceptible ones in order to assess the microbiome profile of mosquito species associated with ZIKV susceptibility. Alpha and beta diversity, differential abundance analysis, co-occurrence networks and functional profiles were analyzed. Although no differences have been observed in the microbial community of ZIKV susceptible and refractory mosquitoes, several bacteria genera were differently abundant in both larva and adult mosquitoes. Genera such as *Pseudomonas*, *Halomonas, Wolbachia* and *Serratia* were more abundant in ZIKV susceptible adult mosquitoes while for the susceptible larvae only *Variovorax* and JG30-KF-CM45 were differentially abundant. As for the functional profile, metabolism pathways were enriched in ZIKV susceptible adults showing a more active microbiome when compared with the refractory mosquitoes. The structure of the co-occurrence network showed a less dense and connected bacterial community in ZIKV susceptible adults. These findings illuminate our comprehension of the differences in the bacteria community in face of mosquito species' vector competence for ZIKV while pointing us out for new directions in vector borne disease control.

Importance

Mosquito vector competence for ZIKV may be affected by differences in their microbiome. Such an influence of the microbiota on pathogen susceptibility has been shown for several pathogens such as Dengue virus and *Plasmodium falciparum*. Therefore,

understanding how the bacterial community of mosquitoes relates to the ZIKV susceptibility or refractoriness could help us identify new possibilities for control measures. Employing a meta analysis on publicly available 16S rRNA of ZIKV refractory and susceptible mosquitoes, bacterial genera such as *Pseudomonas*, *Halomonas*, *Wolbachia* and *Serratia* seemed to be related with ZIKV susceptible mosquitoes. Our results bring us light into a microbial profile that could be shifting mosquito species towards a susceptibility to ZIKV infections. The results of the present work are helpful to understand how the mosquito microbiota is associated with its ZIKV susceptibility phenotype.

Introduction

Mosquito borne diseases are a major public health problem worldwide representing a threat to half of the world's population and accounting for about 17% of all infectious diseases (1). Especially in tropical countries where its climate favors year-round transmission (2). These diseases are prone to spread throughout the globe as global warming keeps changing weather conditions which favors new mosquito populations to be established in places where they were unlikely to be present (3). Many mosquito species from different genera such as *Aedes*, *Culex* and *Anopheles* are responsible for the transmission of these diseases and their blood meal preferences put in danger not only human but animal health as well (4). Among the pathogens transmitted by mosquitoes, Zika virus (ZIKV) is one of great concern as its infection can lead to Congenital Zika Syndrome causing brain abnormalities and/or microcephaly in newborns (5, 6). It belongs to the same family as Dengue virus (DENV), Flaviviridae, and together with Chikungunya virus (CHIKV), Togaviridae, they share mosquito vectors with similar clinical signs in humans (7).

Since the 2016-2018 ZIKV outbreak, several studies have been published regarding the vector competence of different mosquito species of *Aedes*, *Anopheles* and *Culex* genera (6). The species *Ae. aegypti* and *Ae. albopictus* were the most tested ones for ZIKV vector competence and both were incriminated as being competent to get infected, disseminate infection and transmit as well (8). Besides *Cx. quinquefasciatus*, all tested *Culex sp.* together with *Anopheles sp.* and some *Aedes sp.* were refractory (9). Studies on *Cx. quinquefasciatus* susceptibility to ZIKV have shown different results. However, due to few studies demonstrating infection and dissemination in this mosquito species and such results not being reproduced in most of the studies, it is highly unlikely that *Cx. quinquefasciatus* is susceptible to ZIKV (10). Differences in mosquito susceptibility might be related to their microbiome as it has been shown that a microbiota reintroduction has led to lower DENV-2 viral load in *Ae. aegypti* (11). Such

an effect of the microbiota in viral infection is associated with the co-evolutionary relationship between the mosquito and its microbiome which can modulate vector competence towards either susceptibility or refractoriness (12). Thus, the mosquito microbiome in ZIKV infection circumstances can help us identify means to modulate the mosquito vector competence.

Biological control methods are becoming more popular as mosquito resistance for insecticide impairs the efficacy of classical chemical control measures (13). Thus, the screening for new possibilities of biological control with different bacteria are important for the development of novel mosquito-borne diseases control measures (14). The use of bacteria to control mosquito-borne diseases has been extensively evaluated in the literature. For instance, bacteria of the genus *Wolbachia* have shown an ability to diminish arboviral infections in mosquitoes blocking transmission as well as limiting the vectorial capacity of *Ae. aegypti* (15). The introduction of *Serratia ureilytica* previously to *Plasmodium falciparum* infection in *Anopheles sinensis* has led to 98-100% reduction in oocyst count in the mosquito midgut (16). Similarly, *Chromobacterium spp.* has shown interesting results being able to inhibit *Pl. faciparum* and DENV-2 infections in *An. gambiae* and mosquito C6/36 cell line, respectively (17, 18). These findings highlight novel possibilities derived from the mosquito microbiome that could play an important role in the development of new biological control measures.

The present work aimed to compare the microbiota of ZIKV susceptible and refractory mosquito species in order to identify bacteria of interest that might be related to both phenotypes. An approach using high throughput microbiome data of mosquitoes could be helpful to identify bacteria of interest to be further evaluated on its use for ZIKV susceptibility modulation.

Results

After batch effect correction and life stage stratification the ZIKV refractory group was represented by 70 larva and 89 adult samples while the ZIKV susceptible group by 86 and 97 larva and adult samples, respectively. Unfortunately, there were not enough samples matching the selection criteria to build a validation dataset. Bacterial composition analysis showed differences in the bacterial community's profile between mosquito ZIKV susceptibility groups, figure 1. Genera such as *Flavobacterium, Leucobacter*, *Enterobacter*, *Wolbachia* and *Pedobacter* seemed to be more abundant in ZIKV susceptible larva mosquitoes. On the other hand, *Streptococcus*, *Hydroegenophaga*, *Corynebacterium*, *Dechloromonas*, *Novosphingobium* and *Rhodobacter* seemed to be more prevalent in the ZIKV refractory group in larvae as seen in the heatmap, figure 1. As for the adult mosquitoes, similar abundance

differences have been seen for *Wolbachia*, *Corynebacterium* and *Dechloromonas*, i.e., *Wolbachia* being more abundant in ZIKV susceptible mosquitoes while the others being less abundant in this group. Interestingly, the bacterial genus *Hydroegenophaga* seemed to be more abundant in ZIKV refractory larvae as opposed to its higher abundance in ZIKV susceptible adults. Additionally, *Methylobacterium-Methylorubium*, *Aeromonas*, *Chryseobacterium* and *Cutibacterium* were also more abundant in ZIKV susceptible adult mosquitoes while *Acidovorax*, *Clostridium sensu stricto 1*, *Comamonas*, *Bacillus*, *Delftia*, *Stenotrophomonas* and *Serratia* were less abundant in this same group, figure 1.

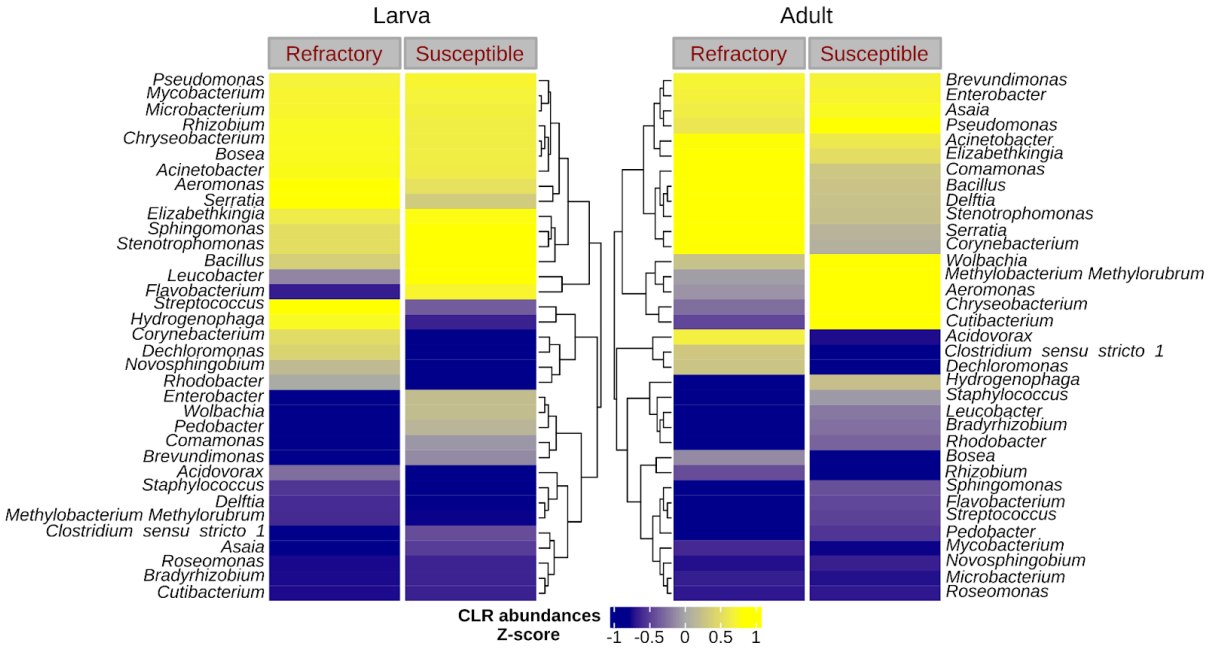

Fig 1. Microbial composition highlighting differences in the bacterial community of ZIKV refractory and susceptible mosquito species for larvae and adults. A heatmap using mean CLR transformed abundances by group is represented. Z-score has been applied in order to reduce color scale range.

Regarding the alpha diversity in the ZIKV susceptible adult group presented a higher diversity only in the inverse Simpson index, figure 2A. Such a difference has not been observed in larva mosquitoes for both indices. Groups of larval ZIKV susceptible and refractory mosquitoes presented an almost overlapping bacterial community as seen in figure 3C. Such a result has also been observed for adult mosquitoes. Only the comparison between groups in adult mosquitoes showed a statistically significant difference with the PERMANOVA test, $F = 7.64$, $R^2 = 0.04$ and BH adjusted P-value < 0,01. The multivariate dispersion analysis showed that, within groups, samples were heterogeneously dispersed with statistically significant

differences between pairwise comparisons of adult mosquitoes ANOVA's F = 6.78 and P = 0.012.

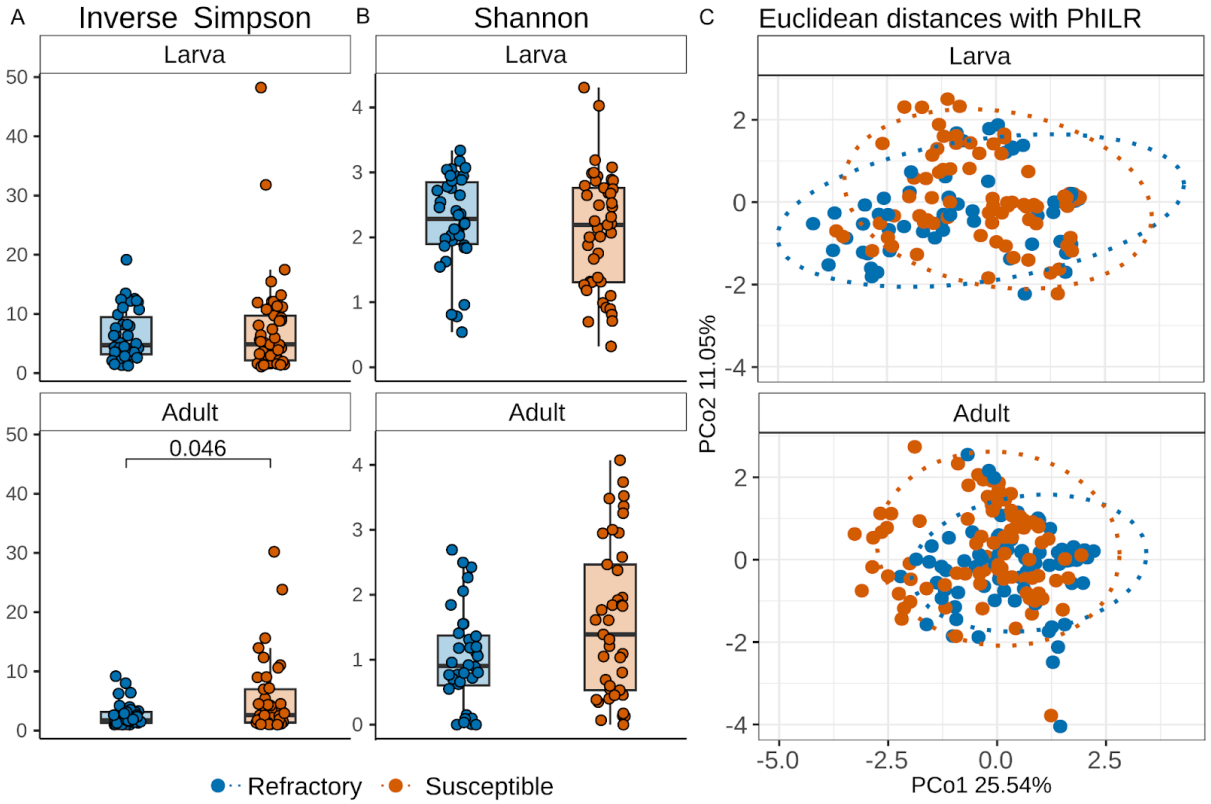

Fig 2. Bacterial community's alpha and beta diversity of ZIKV refractory and susceptible mosquito species for larvae and adults. In A and B, alpha diversity's Inverse Simpson and Shannon metrics, respectively, in two development stages, larvae and adults. Mann-Whitney test has been applied to compare the alpha diversity between groups. Only statistically significant differences are shown, P < 0.05. In C, a PCoA from an Euclidean matrix of PhILR transformed counts between ZIKV refractory and susceptible groups is represented for larva and adult mosquitos.

According to LinDA analysis, *Variovorax* and JG30-KF-CM45 were more abundant and most related to larva mosquitoes susceptible to ZIKV, figure 3. On the other hand, 36 bacterial genera were associated to the refractory group in larvae being *Dechloromonas*, *Rhizobium*, *Acintobacter*, *Acidovorax* and *Rhodobacter* more than four times more abundant and *Bosea* and *Chryseobacterium* about eight times more abundant in this group. For the adult mosquitoes, *Pseudomonas*, *Serratia*, *Wolbachia*, *Stenotrophomonas*, *Staphylococcus*, *Halomonas*, *Raoultella*, *Micrococcus* and *Shewanella* were more abundant and associated to the ZIKV susceptible group. In contrast, UKL13-1, *Methylocystis* and *Dechloromonas* were less abundant in this same group of adult mosquitoes. Using only the differently abundant

bacterial Genera or Family to classify the mosquito samples into being refractory or susceptible to ZIKV, low areas under the curve (AUC), < 0.7, were achieved in the ROC analysis. A moderate AUC, between 0.7 and 0.8, was only obtained for the adults using the differentially abundant bacterial genera.

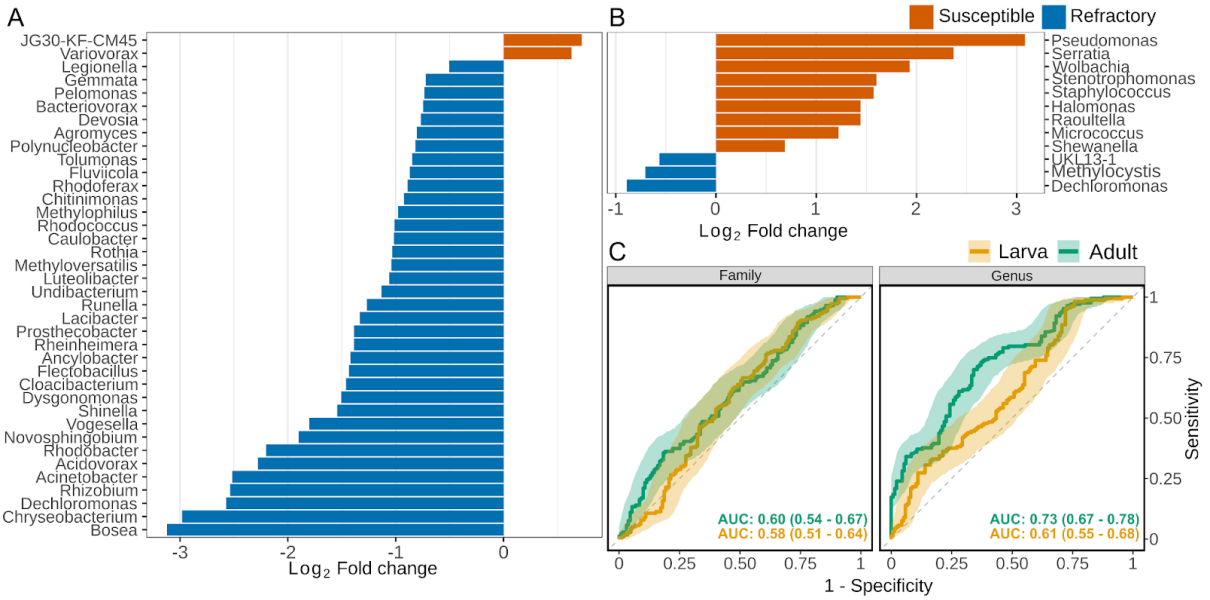

Fig 3. Differential analysis to identify to which group bacteria genera were associated with, and assessment of accuracy to classify mosquitos into ZIKV refractory or susceptible for both larvae and adults using these bacteria genera. In A, results from LinDA analysis of the microbiota from both ZIKV refractory and susceptible groups in larvae are shown. In B, the results for adults are exhibited. Only taxa with $\log_2$ fold change > 0.5 and Benjamini-Hochberg adjusted P < 0.05 are shown. In C, receiver operating curves (ROC) and the area under the curve (AUC) with 95% confidence interval within parentheses in the train data set based on principal component regression.

Differently abundant KOs, predicted by PICRUSt2, were enriched, demonstrating the majority of pathways, 40 out of 50, enriched in the ZIKV susceptible adult mosquitoes group. Most of these pathways, 31 out of 40, were related to the metabolism of different compounds, figure 4. Other pathways unrelated to metabolism such as Cationic AntiMicrobial Peptide (CAMP) resistance, sulfur relay, Phosphotransferase, bacterial secretion and Two-component systems as well as bacterial chemotaxis and pathways related to biofilm formation were also enriched in adult ZIKV susceptible mosquitoes. In contrast, adult ZIKV refractory mosquitoes presented only purine, sulfur, glyoxylate and dicarboxylate metabolisms enriched. Similarly as observed in adult mosquitoes, in ZIKV susceptible larvae more enriched pathways have been observed, 10 out of 50 in comparison with 2 out of 50 for ZIKV refractory larvae. In comparison

with adult ZIKV susceptible mosquitoes, the larvae shared only two pathways, lipopolysaccharide biosynthesis and Two-component system.

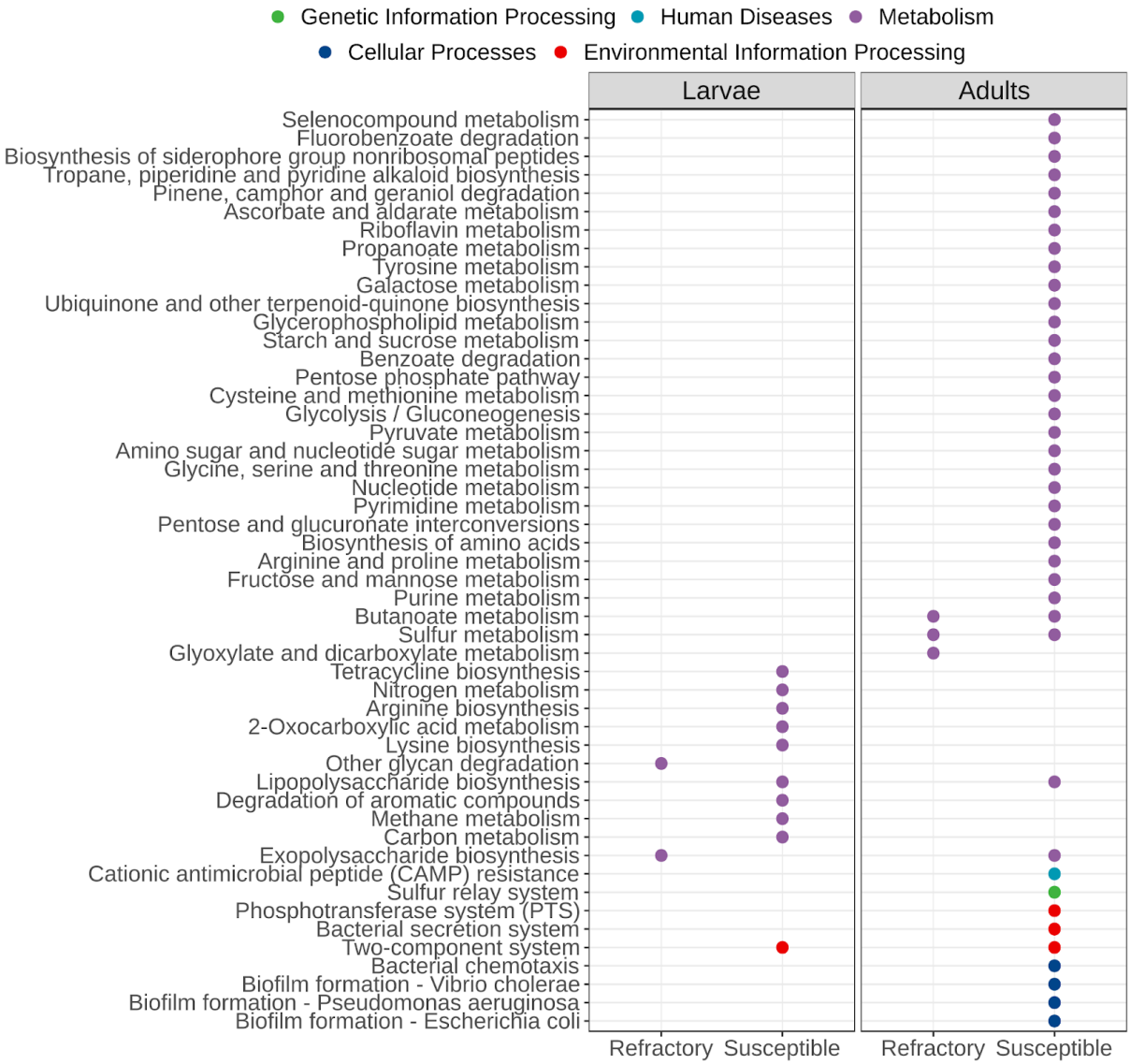

Fig 4. Dotplot of enriched pathways based on differently abundant KEGG Orthology IDs predicted by PICRUSt2 in ZIKV refractory and susceptible mosquitoes followed by LinDA analysis. Pathways are sorted according to the KEGG BRITE level 1 hierarchy which are represented by different colors.

Networks of correlations between bacterial genera can be seen in figure 5A. Networks from ZIKV refractory larvae mosquitoes seemed to be more connected and denser. Such a difference has been confirmed when the density of 100 bootstrapped networks were compared, figure 5B. The average degree and density of this group's networks was higher in comparison

with ZIKV susceptible larvae mosquitoes. Similarly, in the adult mosquitoes the ZIKV susceptible ones demonstrated a less dense network with lower average degree as well.

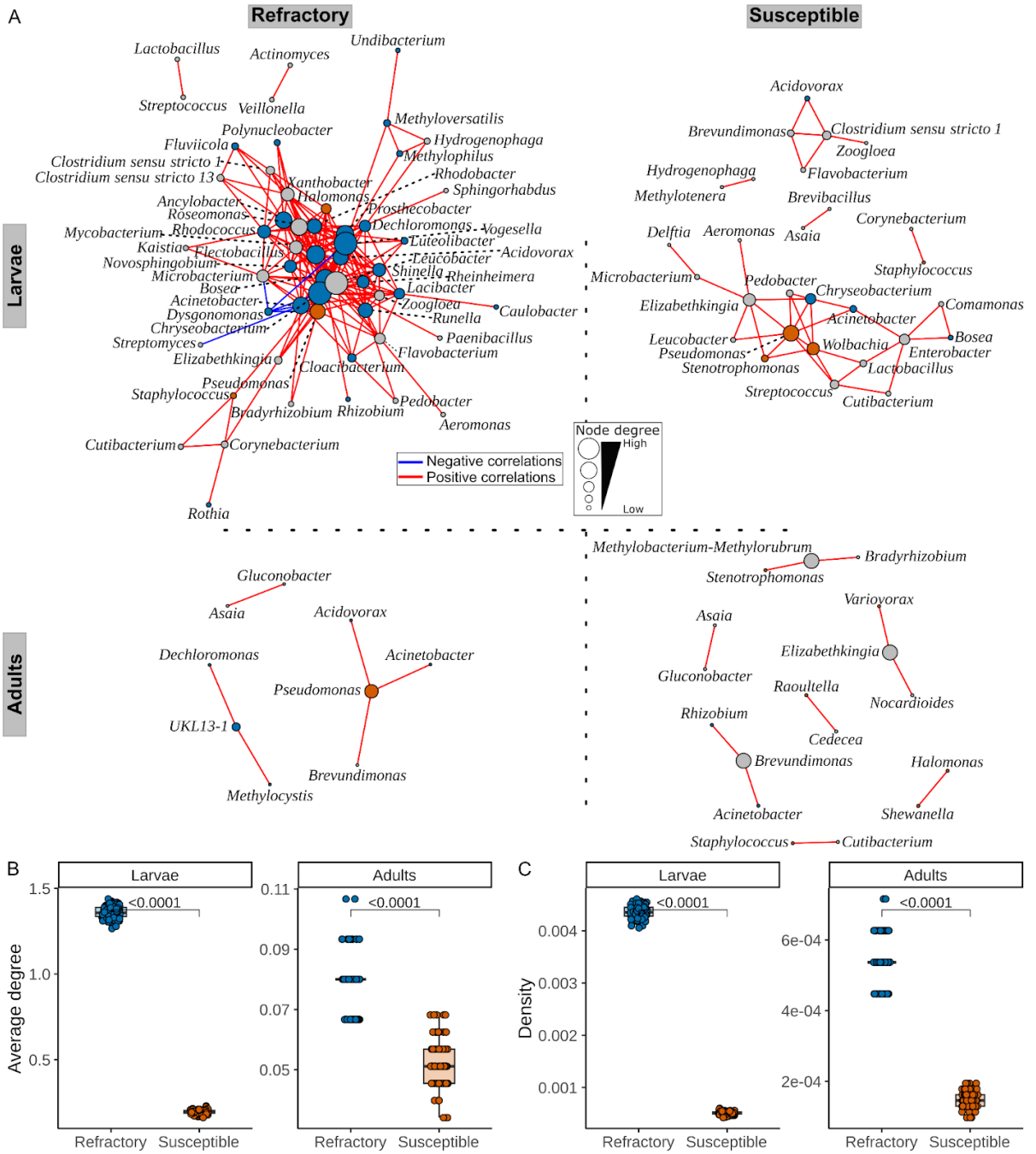

Fig 5. Co-occurrence networks of ZIKV refractory or susceptible mosquitoes at larval and adult development stages. In A, networks of each group and development stage pair are presented. Connection between bacteria genera is shown for correlations > 0.4 with $P < 0.05$ being red and blue for positive and negative correlations, respectively. Node size was measured by node degree, i.e. number of connections. Node colors represent the bacteria that have been assigned as differently abundant for each

group, blue for ZIKV refractory mosquitoes and orange for the susceptible ones. In B and C the network's average degree and density are compared in a boxplot from 100 bootstrapped networks.

Discussion

Studying the microbial composition of both larval and adult ZIKV susceptible or refractory mosquitoes, differences could be observed in terms of bacterial genera composition. However, no significant difference has been observed in terms of alpha and beta diversities. The bacterial community of ZIKV susceptible adult mosquitoes seemed to be higher only for the inverse Simpson metric. Such a difference was not replicated with the Shannon index. Additionally, together with an almost non-significant P-value, 0.046, the idea of no difference in alpha diversity between ZIKV susceptible and refractory mosquitoes is more probable. Similar result in alpha diversity has been observed in field collected *Ae. aegypti* naturally infected with ZIKV (34). When the alpha diversity of ZIKV resistant *Ae. aegypti* population was compared with the ZIKV susceptible population, no statistically significant differences have also been observed (35). For another Flavivirus, DENV-2, a reduction in microbial diversity using antibiotic treated mosquitoes has not shown any difference in viral load and prevalence in *Ae. aegypti* (36).

As for the beta diversity, a statistically significant difference has only been observed in the comparison between ZIKV susceptible and refractory adult mosquitoes. However, due to the heterogeneity seen in these groups, the PERMANOVA's P-value might be inflated in this comparison. Thus, it is more reasonable to believe that there is no difference for the beta diversity between ZIKV susceptible and refractory mosquitoes. An absence in beta diversity difference of ZIKV susceptible and refractory *Ae. aegypti* has also been observed (35). Such results corroborate what has been found in the present work and indicate that microbial diversity does not affect both ZIKV infection or refractoriness.

Although, herein associated with ZIKV susceptible adult mosquitoes, *Wolbachia* presence in mosquito species have been shown to diminish infection rates or even block arboviral transmission (37). However, such an impact in mosquito-borne pathogens is mostly related to the use of interspecies-transferred *Wolbachia* (14). It is believed that due to co-evolution between native *Wolbachia* and mosquitoes, its pathogen-blocking trait is limited in comparison with interspecies-transferred *Wolbachia (37)*. In some studies with other arboviruses, native *Wolbachia* infection did not affect DENV-2, DENV-3 and WNV infections in terms of viral load and infection rate (38, 39). Thus, despite being present in natural populations of mosquitoes, the role of native *Wolbachia* should still be assessed in order to

evaluate its impact in vector competence (40). Due to limitations in the identification to the species level of the SILVA database used in the present work (41), the *Wolbachia* genus herein associated with ZIKV susceptible adult mosquitoes could be related to native symbiont *Wolbachia*. Therefore, for ZIKV infection in mosquitoes these *Wolbachia* might be somehow beneficial.

The genus *Serratia* has been associated with ZIKV susceptible adult mosquitoes in the present work. Interestingly, it has been shown an increase in viral load of DENV-2, ZIKV and Sindbis virus when *Se. marcescens* was introduced in *Ae. aegypti* four days post infection (42). Such a positive effect in these viral infections was related to SmEnhancin, secreted by *Se. marcescens*, which digests mucins in the mosquito's gut epithelia (42). Similar increase in mosquito susceptibility to arboviruses has been observed in other *Serratia sp.* For instance, *Se. odorifera* has been associated with an increase in permissiveness of DENV-1 and CHIKV infections, as it downregulates the immune response by means of P40 protein production (43, 44). Thus, based on the results of the present work and the above mentioned mechanisms, the genus *Serratia* could be favoring ZIKV infections in adult mosquitoes.

In contrast with findings in the literature, *Pseudomonas has* been associated with ZIKV susceptible adult mosquitoes in the present work. It has been recently demonstrated that *Ps. alcaligenes* favors *Plasmodium berghei* infection catabolizing 3-hydroxykynurenine which impairs the peritrophic matrix's structure (45). The influence of *Pseudomonas* in mosquito's arboviral infections has not been evaluated yet. However, its role in protection of the peritrophic matrix's structure suggests that it should be rather blocking viral infection than shifting the mosquito towards a susceptibility for ZIKV. Nevertheless, a less structured peritrophic matrix, via HPx1 silencing, was associated with higher ZIKV load and infection intensity in *Ae. aegypti* contrasting the results with DENV-2 (46). Therefore, the impact of the peritrophic matrix in arboviral infection differs among closely related viruses, both DENV-2 and ZIKV belonging to Flaviridae, and it seems to be positive for ZIKV infection in the mosquito. It is worth highlighting that the impact of *Pseudomonas* on ZIKV infection in mosquitoes should be further evaluated in the future.

The bacterial genus *Halomonas* has been associated with the ZIKV susceptible adults. A bacteria of this genus, *Ha. venusta*, has been demonstrated to synthesize selenium nanoparticles which has an anti biofilm activity (47). Thus, *Halomonas sp*. might be favoring a ZIKV susceptibility by reducing biofilm formation favoring virus infection in mosquitoes. However, little is known about the impact of biofilm on mosquito vector competence for arboviruses. The majority of the literature regarding the effects of biofilm reduction in

mosquitoes are related to its application as larvicide (48–50). Interestingly, our functional analysis predicted pathways related to biofilm formation in ZIKV susceptible adults. Nonetheless, it is not clear how biofilm would be associated with ZIKV infection susceptibility in mosquitoes. It is possible that it is favoring bacteria such as *Staphylococcus*, *Pseudomonas* and *Serratia*, which have been also related to ZIKV susceptible adult mosquitoes and known to produce biofilm (51–53). Thus, such biofilm formation might be indirectly promoting a microbial community that favors ZIKV infection in mosquitoes. The biofilm could also be related to a physical barrier blocking the ZIKV-mosquito interaction (54). However, such a barrier role for biofilms has not been demonstrated for arboviral infections in mosquitos thus far. Therefore, the mechanisms through which *Halomonas* is favoring a ZIKV susceptibility should be further evaluated.

Nothing has been found on the association between most of the herein differently abundant bacterial genera and ZIKV infection in mosquitoes. Thus, based on the results of the present work, they are probably related to ZIKV refractoriness or susceptibility. However, such a statement should be empirically tested to elucidate the mechanisms through which they impact the ZIKV-mosquito relationship.

The functional prediction analysis result shows that the microbiota changes influenced by ZIKV susceptibility promote a higher expression of genes associated with metabolism pathways by the microbiota. The higher number of enriched pathways related to metabolism might be important for ZIKV susceptibility. Metabolic alterations such as pentose phosphate and glycolysis, enriched in ZIKV susceptible adult mosquitoes, are known to favor ZIKV infection in mosquito cells (55). Mosquito pathogen tolerance is associated with these metabolic alterations (56) which might be triggered by the microbiota of ZIKV susceptible mosquitoes as seen in the present work. The metabolism enriched pathways were related to the energy, amino acid, carbohydrate, nucleotide and lipid metabolism as well as glycan biosynthesis. Such a wide metabolic profile enriched in ZIKV susceptible mosquitoes may be related to the microbiota's response in the mosquito host in order to promote a homeostasis (57) which in return might be promoting a ZIKV susceptibility. However, it is worth mentioning that such analysis using bacterial 16S-rRNA sequences is a prediction based on the pathways that known bacteria express, adjusted by the 16S gene expression. Thus, this enrichment result should be further investigated to better evaluate their higher predicted expression under the condition of ZIKV susceptibility.

The lower density and average degree in the ZIKV susceptible mosquito co-occurrence networks might be related to a less complex microbiota community (58). Thus, a less connected

microbial community may be related to a mosquito's susceptibility to ZIKV infections. Comparing the co-occurrence networks of several mosquito species microbial communities, lower density has been observed in *Cx. quinquefasciatus* in comparison with *Ae. aegypti* (59). Differences in network densities have also been observed when eight silvatic mosquito species in Brazil (60). Despite these differences in co-occurrence network densities between mosquito species, little is known about the impact of viral infection or susceptibility on the network connections and density. Based on the results of the present work, a less connected bacterial community should be related to ZIKV susceptibility.

Conclusion

The present work has shown a lack of differences in bacterial diversity between ZIKV susceptible and refractory mosquitoes. Additionally, there were low AUCs when the differently abundant bacteria were used to understand how well these groups would be classified. These findings indicate that probably instead of microbiome another aspect of these mosquito species such as gene expression or genetic similarities might be more useful to distinguish them in terms of ZIKV susceptibility. Nonetheless, interesting insights could be taken from the bacteria present in ZIKV susceptible mosquitoes. For instance, native *Wolbachia* could be related to a susceptible phenotype opposing what is known when interspecies-transferred *Wolbachia* are used in a ZIKV infection context. Furthermore, other bacterial genera such as *Pseudomonas*, *Halomonas* and *Serratia* could also be favoring the susceptibility of mosquitoes to ZIKV. These results open up possibilities for future work on bacteria that can modulate the ZIKV susceptibility in mosquitoes.

Methodology

Data selection and mosquito classification

Mosquito species were selected based on a previous review that evaluated ZIKV vector competence of mosquitoes (9). Then, 16S rRNA gene sequencing data were searched in the NCBI's SRA (Sequence Read Archive) and BioProject, in August 2023. Data sets were searched using "microbiome", "microbiota", "16S" and the mosquito species as a search query. From the data on each mosquito sample with bacterial 16S-rRNA sequencing deposited at the NCBI, a metadata was assembled containing information such as origin of the mosquito, i.e., colony reared or field collected, life stage, pre-sequencing washing method, 16S rRNA variable region, collection location and mosquito species. A new variable was added regarding the

vector competence to ZIKV of each mosquito species as recently reviewed in the literature (9). In order to reduce the heterogeneity between samples and retain the highest number of mosquito species, only samples from data sets containing mosquitoes' whole body and without previous detection of infection of any pathogen. In addition, the sequencing was filtered for V3-V4 or V4 regions with a paired-end layout and 250 or 300 bases as a higher number of samples was attained in comparison with other variable regions. After the above mentioned filters 190 samples of *Ae. aegypti*, *Ae. albopictus* and *Ae. japonicus* were selected for the ZIKV susceptible group while 172 samples of *An. gambiae*, *Ae. triseriatus* and *Cx. pipiens* were selected for the ZIKV refractory group.

Data processing

The download of all samples has been performed using SRA toolkit's fastq-dump (github.com/ncbi/sra-tools) and the quality check in order to remove the sequencing adapter, small and unspecific reads, and those with Phred quality score lower than 30 has been done using Trimmomatic v0.32 (19). Due to the low quality of the reverse sequences in some data sets, only the forward sequence has been used in the downstream analysis. After data's quality control, all reads have been processed on the QIIME2 2023.5 (20) pipeline, removing chimeric sequences and building ASVs using the Deblur algorithm (21). All ASV bacterial classification has been done using the SILVA v138.1 16S database (22) assuming 97% identity. The SILVA database was filtered to remove sequences associated with fungi and plants, filtering out Eukaryota as well as *Mitochondria* and *Chloroplast*. Sequences with unspecified species classification such as unkown, uncultured, NA, metagenome, and unidentified have also been removed from the database. After classification, a phylogenetic tree was built using MAFFT (multiple alignment using fast Fourier transform) (23) and fasttree (24) methods. For the analysis in R environment v4.3.1, all generated on QIIME2, i.e., ASV table, ASV taxonomic classification, and phylogenetic tree, have been imported using Qiime2R package (25). This package creates a phyloseq object that can be further analyzed by the Phyloseq package (26) to measure diversity indices.

Batch effect correction

Before any analysis, a batch correction has been performed to reduce the technical bias variation that could have been caused by batch, e.g., each project being sequenced and performed by different research groups and sequencing machines. To diminish microbiota differences due to mosquito origin, this variable has been used as a variable for batch correction

as well. Such correction was performed by the ComBat_seq function (27), from the sva package v3.48.0. After batch correction, the corrected ASV table was incorporated in a phyloseq object. For downstream analysis, samples with less than 100 reads and taxa with less than 10 counts have been removed and the data set was divided into adult and larva data sets as well as separately analyzed.

Compositional and diversity analysis

To visualize the microbial composition of mosquito species refractory and susceptible to ZIKV, heatmaps using the batch-corrected mean counts transformed by centered log-ratio were created. CLR transformed counts were scaled using Z-scores in order to reduce the range of values for better visualization of differences between groups. Since the functions to measure the alpha diversity metrics only accept integers and not CLR transformed counts, the rarefied batch-corrected counts have been used for such analysis, rarefying for 1,000 counts and using Shannon and Inverse Simpson indices. Beta diversity was assessed by means of ASV count transformation using PhILR (28), followed by a Euclidean distance matrix. Such an approach takes into account the data's compositional characteristics together with the phylogenetic distance from taxa present in samples. Principal coordinate analysis was applied to evaluate group distances in terms of beta diversity followed by PERMANOVA test (29), in order to determine statistically significant differences in each group centroid according to the Euclidean distance matrix. The multivariate dispersion has been tested through the vegan's package v2.6.4 betadisper function to assess the homogeneity of each sample distance in relation to its group centroid. If groups are equally homogeneous, then the P-value from the PERMANOVA test is not inflated.

Co-occurrence network

Co-occurrence networks have been built using Sparse Correlations for Compositional data (SparCC) between samples from each group of mosquito species, refractory and susceptible within the larva and adult mosquitoes data sets. In order to compare networks from both groups, 100 bootstrap replicates of SparCC correlation matrices were made to extract network performance metrics such as density and average degree. After collecting these metrics from 100 bootstrapped networks, they were compared between groups with the Mann-Whitney test. The package ggClusterNet v0.1.0 has been used to measure SparCC correlation matrices, and networks were built using igraph v1.4.1.

Functional analysis

In order to further describe susceptible mosquito species, a functional analysis was performed using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), predicting metagenome functions that are regulated by a given bacterial community based on 16S-rRNA sequencing (30). Predicted pathways were described according to the KEGG Orthology database and further hierarchically classified into the BRITE Hierarchy. Differently abundant pathways were identified using Linear models for Differential Abundance analysis (31) with a cut-off of 1.5 $\log_2$ fold change and Benjamini-Hochberg false discovery rate (BH) adjusted $P < 0.05$. Then, the differently abundant KEGG Orthology IDs were used in an enrichment analysis as described in the package MicrobiomeProfiler v1.6.1 (32) using the functions compareCluster and enrichKO from clusterProfiler v4.8.2 (33).

Differential analysis and validation of differentially abundant bacteria

To determine groups of bacteria with higher probability to explain differences among groups (refractory vs susceptible), LinDA has been applied as it takes into account the data's compositionality. Differently abundant genera related to refractory or susceptible groups were determined when $\log_2$ fold change was > 0.5 with a Benjamini-Hochberg false discovery rate (BH) adjusted $P < 0.05$. Pre-wash method applied before DNA extraction has been used in the LinDA model to adjust the variable for ZIKV susceptibility.

The bacteria identified as differentially abundant between ZIKV refractory or susceptible mosquito species in LinDA were used in a principal component regression approach to assess if these bacterial genera or families accurately separate mosquito species as refractory or susceptible for ZIKV. Thus, only bacterial genera and families with the abovementioned fold change and P-value criteria were used to run a PCA. The first two components were modeled in a logistic regression to determine if samples belonged to any of the studied groups. The performance of the logistic model built from the train data set was measured using the abovementioned validation data sets containing only the differently abundant bacterial genera. Receiver operating characteristic curves, package pROC v1.18.0, and the confusion matrix method, package caret v6.0.94, have been used to measure the accuracy and non-informative rate.

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Data availability

Raw sequences were obtained from NCBI's SRA under the following BioProjects: PRJEB40885, PRJNA422599, PRJNA477711, PRJNA523634, PRJNA572589, PRJNA606895, PRJNA625381, PRJNA672031, PRJNA702783, PRJNA750810, PRJNA767109, PRJNA873190, PRJNA919511 and PRJNA943216. Metadata and code are available upon request.

References

1. Eder M, Cortes F, Teixeira de Siqueira Filha N, Araújo de França GV, Degroote S, Braga C, Ridde V, Turchi Martelli CM. 2018. Scoping review on vector-borne diseases in urban areas: transmission dynamics, vectorial capacity and co-infection. Infect Dis Poverty 7:90.

2. Colón-González FJ, Sewe MO, Tompkins AM, Sjödin H, Casallas A, Rocklöv J, Caminade C, Lowe R. 2021. Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. Lancet Planet Health 5:e404–e414.

3. Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, Yi D, Johnson K, Earl L, Marczak LB, Shirude S, Davis Weaver N, Bisanzio D, Perkins TA, Lai S, Lu X, Jones P, Coelho GE, Carvalho RG, Van Bortel W, Marsboom C, Hendrickx G, Schaffner F, Moore CG, Nax HH, Bengtsson L, Wetter E, Tatem AJ, Brownstein JS, Smith DL, Lambrechts L, Cauchemez S, Linard C, Faria NR, Pybus OG, Scott TW, Liu Q, Yu H, Wint GRW, Hay SI, Golding N. 2019. Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol 4:854–863.

4. Kent RJ. 2009. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. Mol Ecol Resour 9:4–18.

5. Freitas DA, Souza-Santos R, Carvalho LMA, Barros WB, Neves LM, Brasil P, Wakimoto MD. 2020. Congenital Zika syndrome: A systematic review. PLoS One 15:e0242367.

6. Gregory CJ, Oduyebo T, Brault AC, Brooks JT, Chung K-W, Hills S, Kuehnert MJ, Mead P, Meaney-Delman D, Rabe I, Staples E, Petersen LR. 2017. Modes of Transmission of Zika Virus. J Infect Dis 216:S875–S883.

7. Bhat EA, Ali T, Sajjad N, Kumar R, Bron P. 2023. Insights into the structure, functional perspective, and pathogenesis of ZIKV: an updated review. Biomed Pharmacother 165:115175.

8. Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux A-B. 2018. An overview of mosquito vectors of Zika virus. Microbes Infect 20:646–660.

9. Bisia M, Montenegro-Quinoñez CA, Dambach P, Deckert A, Horstick O, Kolimenakis A, Louis VR, Manrique-Saide P, Michaelakis A, Runge-Ranzinger S, Morrison AC. 2023. Secondary vectors of Zika Virus, a systematic review of laboratory vector competence studies. PLoS Negl Trop Dis 17:e0011591.

10. MacLeod HJ, Dimopoulos G. 2020. Detailed Analyses of Zika Virus Tropism in Culex quinquefasciatus Reveal Systemic Refractoriness. MBio 11.

11. Ramirez JL, Souza-Neto J, Torres Cosme R, Rovira J, Ortiz A, Pascale JM, Dimopoulos G. 2012. Reciprocal tripartite interactions between the Aedes aegypti midgut microbiota, innate immune system and dengue virus influences vector competence. PLoS Negl Trop Dis 6:e1561.

12. Cansado-Utrilla C, Zhao SY, McCall PJ, Coon KL, Hughes GL. 2021. The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. Microbiome 9:111.

13. Haddi K, Tomé HVV, Du Y, Valbon WR, Nomura Y, Martins GF, Dong K, Oliveira EE. 2017. Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of Aedes aegypti: a potential challenge for mosquito control. Sci Rep 7:46549.

14. Shi H, Yu X, Cheng G. 2023. Impact of the microbiome on mosquito-borne diseases. Protein Cell https://doi.org/10.1093/procel/pwad021.

15. Bliman P-A, Aronna MS, Coelho FC, da Silva MAHB. 2018. Ensuring successful introduction of Wolbachia in natural populations of Aedes aegypti by means of feedback control. J Math Biol 76:1269–1300.

16. Gao H, Bai L, Jiang Y, Huang W, Wang L, Li S, Zhu G, Wang D, Huang Z, Li X, Cao J, Jiang L, Jacobs-Lorena M, Zhan S, Wang S. 2021. A natural symbiotic bacterium drives mosquito refractoriness to Plasmodium infection via secretion of an antimalarial lipase. Nat Microbiol 6:806–817.

17. Saraiva RG, Huitt-Roehl CR, Tripathi A, Cheng Y-Q, Bosch J, Townsend CA, Dimopoulos G. 2018. Chromobacterium spp. mediate their anti-Plasmodium activity through secretion of the histone deacetylase inhibitor romidepsin. Sci Rep 8:6176.

18. Saraiva RG, Fang J, Kang S, Angleró-Rodríguez YI, Dong Y, Dimopoulos G. 2018. Aminopeptidase secreted by Chromobacterium sp. Panama inhibits dengue virus infection by degrading the E protein. PLoS Negl Trop Dis 12:e0006443.

19. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

20. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Author

Correction: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:1091.

21. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R. 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems 2.

22. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–6.

23. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780.

24. Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 26:1641–1650.

25. Bisanz J. qiime2R: Import qiime2 artifacts to R. Github. https://github.com/jbisanz/qiime2R. Retrieved 28 September 2023.

26. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217.

27. Sva. Bioconductor. https://bioconductor.org/packages/release/bioc/html/sva.html. Retrieved 21 September 2023.

28. Silverman JD, Washburne AD, Mukherjee S, David LA. 2017. A phylogenetic transform enhances analysis of compositional microbiota data. Elife 6.

29. Tang Z-Z, Chen G, Alekseyenko AV. 2016. PERMANOVA-S: association test for microbial community composition that accommodates confounders and multiple distances. Bioinformatics 32:2618–2625.

30. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688.

31. Zhou H, He K, Chen J, Zhang X. 2022. LinDA: linear models for differential abundance analysis of microbiome compositional data. Genome Biol 23:95.

32. Meijun Chen GY. 2021. MicrobiomeProfiler. Bioconductor. https://bioconductor.org/packages/MicrobiomeProfiler.

33. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G. 2021. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation (Camb) 2:100141.

34. Arévalo-Cortés A, Damania A, Granada Y, Zuluaga S, Mejia R, Triana-Chavez O. 2022. Association of Midgut Bacteria and Their Metabolic Pathways with Zika Infection and Insecticide Resistance in Colombian Populations. Viruses 14.

35. Coatsworth H, Caicedo PA, Van Rossum T, Ocampo CB, Lowenberger C. 2018. The

Composition of Midgut Bacteria in Aedes aegypti (Diptera: Culicidae) That Are Naturally Susceptible or Refractory to Dengue Viruses. J Insect Sci 18.

36. Audsley MD, Ye YH, McGraw EA. 2017. The microbiome composition of Aedes aegypti is not critical for Wolbachia-mediated inhibition of dengue virus. PLoS Negl Trop Dis 11:e0005426.

37. Lindsey ARI, Bhattacharya T, Newton ILG, Hardy RW. 2018. Conflict in the Intracellular Lives of Endosymbionts and Viruses: A Mechanistic Look at Wolbachia-Mediated Pathogen-blocking. Viruses 10.

38. Micieli MV, Glaser RL. 2014. Somatic Wolbachia (Rickettsiales: Rickettsiaceae) levels in Culex quinquefasciatus and Culex pipiens (Diptera: Culicidae) and resistance to West Nile virus infection. J Med Entomol 51:189–199.

39. Silva JBL, Magalhães Alves D, Bottino-Rojas V, Pereira TN, Sorgine MHF, Caragata EP, Moreira LA. 2017. Wolbachia and dengue virus infection in the mosquito Aedes fluviatilis (Diptera: Culicidae). PLoS One 12:e0181678.

40. Inácio da Silva LM, Dezordi FZ, Paiva MHS, Wallau GL. 2021. Systematic Review of Wolbachia Symbiont Detection in Mosquitoes: An Entangled Topic about Methodological Power and True Symbiosis. Pathogens 10.

41. Hoffman C, Siddiqui NY, Fields I, Gregory WT, Simon HM, Mooney MA, Wolfe AJ, Karstens L. 2021. Species-Level Resolution of Female Bladder Microbiota from 16S rRNA Amplicon Sequencing. mSystems 6:e0051821.

42. Wu P, Sun P, Nie K, Zhu Y, Shi M, Xiao C, Liu H, Liu Q, Zhao T, Chen X, Zhou H, Wang P, Cheng G. 2018. A Gut Commensal Bacterium Promotes Mosquito Permissiveness to Arboviruses. Cell Host Microbe 25:101–112.e5.

43. Apte-Deshpande A, Paingankar M, Gokhale MD, Deobagkar DN. 2012. Serratia odorifera a midgut inhabitant of Aedes aegypti mosquito enhances its susceptibility to dengue-2 virus. PLoS One 7:e40401.

44. Apte-Deshpande AD, Paingankar MS, Gokhale MD, Deobagkar DN. 2014. Serratia odorifera mediated enhancement in susceptibility of Aedes aegypti for chikungunya virus. Indian J Med Res 139:762–768.

45. Feng Y, Peng Y, Song X, Wen H, An Y, Tang H, Wang J. 2022. Anopheline mosquitoes are protected against parasite infection by tryptophan catabolism in gut microbiota. Nat Microbiol 7:707–715.

46. Talyuli OAC, Oliveira JHM, Bottino-Rojas V, Silveira GO, Alvarenga PH, Barletta ABF, Kantor AM, Paiva-Silva GO, Barillas-Mury C, Oliveira PL. 2023. The Aedes aegypti peritrophic matrix controls arbovirus vector competence through HPx1, a heme-induced peroxidase. PLoS Pathog 19:e1011149.

47. Vaigankar DC, Mujawar SY, Mohanty AK, Dubey SK. 2022. Biogenesis of selenium nanospheres using Halomonas venusta strain GUSDM4 exhibiting potent environmental applications. Arch Microbiol 204:372.

48. Raju P, Arivalagan P, Natarajan S. 2020. One-pot fabrication of multifunctional catechin@ZIF-L nanocomposite: Assessment of antibiofilm, larvicidal and photocatalytic activities. J Photochem Photobiol B 203:111774.

49. Yazhiniprabha M, Vaseeharan B. 2019. In vitro and in vivo toxicity assessment of selenium nanoparticles with significant larvicidal and bacteriostatic properties. Mater Sci Eng C Mater Biol Appl 103:109763.

50. Abinaya M, Vaseeharan B, Divya M, Sharmili A, Govindarajan M, Alharbi NS, Kadaikunnan S, Khaled JM, Benelli G. 2018. Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity and larvicidal toxicity against malaria and Zika virus vectors. J Trace Elem Med Biol 45:93–103.

51. Pompilio A, Ranalli M, Piccirilli A, Perilli M, Vukovic D, Savic B, Krutova M, Drevinek P, Jonas D, Fiscarelli EV, Tuccio Guarna Assanti V, Tavío MM, Artiles F, Di Bonaventura G. 2020. Biofilm Formation among Stenotrophomonas maltophilia Isolates Has Clinical Relevance: The ANSELM Prospective Multicenter Study. Microorganisms 9.

52. Rice SA, Koh KS, Queck SY, Labbate M, Lam KW, Kjelleberg S. 2005. Biofilm formation and sloughing in Serratia marcescens are controlled by quorum sensing and nutrient cues. J Bacteriol 187:3477–3485.

53. Thi MTT, Wibowo D, Rehm BHA. 2020. Pseudomonas aeruginosa Biofilms. Int J Mol Sci 21.

54. do Nascimento RM, Campolina TB, Chaves BA, Delgado JLF, Godoy RSM, Pimenta PFP, Secundino NFC. 2022. The influence of culture-dependent native microbiota in Zika virus infection in Aedes aegypti. Parasit Vectors 15:57.

55. Thaker SK, Chapa T, Garcia G Jr, Gong D, Schmid EW, Arumugaswami V, Sun R, Christofk HR. 2019. Differential Metabolic Reprogramming by Zika Virus Promotes Cell Death in Human versus Mosquito Cells. Cell Metab 29:1206–1216.e4.

56. Oliveira JH, Bahia AC, Vale PF. 2020. How are arbovirus vectors able to tolerate infection? Dev Comp Immunol 103:103514.

57. Sterkel M, Oliveira JHM, Bottino-Rojas V, Paiva-Silva GO, Oliveira PL. 2017. The Dose Makes the Poison: Nutritional Overload Determines the Life Traits of Blood-Feeding Arthropods. Trends Parasitol 33:633–644.

58. Zhou J, Liao Z, Liu Z, Guo X, Zhang W, Chen Y. 2022. Urbanization increases stochasticity and reduces the ecological stability of microbial communities in amphibian hosts. Front Microbiol 13:1108662.

59. Hegde S, Khanipov K, Albayrak L, Golovko G, Pimenova M, Saldaña MA, Rojas MM, Hornett EA, Motl GC, Fredregill CL, Dennett JA, Debboun M, Fofanov Y, Hughes GL. 2018. Microbiome Interaction Networks and Community Structure from Laboratory-Reared and Field-Collected, and Mosquito Vectors. Front Microbiol 9:2160.

60. da Silva H, Oliveira TMP, Sallum MAM. 2022. Bacterial Community Diversity and Bacterial Interaction Network in Eight Mosquito Species. Genes 13.

5 **DISCUSSÃO**

Ao analisar a microbiota de artrópodes vetores associada à competência vetorial foi possível observar gêneros de bactéria que podem estar modulando a competência vetorial do artrópode. Sob a ótica de um artrópode competente para transmitir diversos patógenos, bactérias dos gêneros *Rickettsia*, *Staphylococcus* e *Corynebacterium* foram associadas à competência vetorial para transmitir diversos patógenos em carrapatos. Por outro lado, sob a ótica de um patógeno e seus vetores, os mosquitos tiveram bactérias dos gêneros *Pseudomonas*, *Serratia*, *Halomonas* e *Wolbachia* associadas a um perfil de susceptibilidade à infecção por ZIKV.

Tanto *Staphylococcus* quanto *Micrococcus* foram associados ao perfil amplo de competência vetorial de carrapatos e ao perfil susceptível de mosquitos para ZIKV. Ambas ainda não foram avaliadas quanto a sua influência na infecção de patógenos em artrópodes. No entanto, os resultados do presente trabalho sugerem que tais bactérias estejam de alguma forma associadas à competência vetorial de artrópodes. Possivelmente estejam contribuindo à manutenção de condições favoráveis a infecções por patógenos como no caso de endossimbiontes que contribuem a homeostase e atenuação da resposta imune do artrópode (Sassera et al., 2013). Por outro lado, não houve bactérias em comum entre mosquitos refratários ao ZIKV e carrapatos mono competentes.

Um comparativo mais fidedigno seria a classificação de mosquitos de forma similar ao que foi feita com carrapatos, ou seja, mono ou pluri competentes. Foi realizada uma revisão de literatura sobre a competência vetorial de mosquitos com os mesmo métodos aplicados em carrapatos no presente trabalho. No entanto, o entendimento de competência vetorial em carrapatos difere de como esta questão foi avaliada em mosquitos. Para a literatura de carrapatos, a competência vetorial deve ser avaliada demonstrando a transmissão horizontal do patógeno a um hospedeiro vertebrado suscetível (Eisen, 2020). Entretanto, apesar da questão ter sido debatida e ser similar na literatura de mosquitos, a maioria dos trabalhos determinaram transmissão somente pela detecção do patógeno na saliva do mosquito (Bisia Et al., 2023). A falta de padronização de métodos e conceitos como no termo “transmissão” na determinação da competência vetorial de mosquitos influenciou na classificação dos mosquitos em mono ou pluri competentes (Drouin et al., 2022).

Recentemente foi publicada uma tentativa de padronização para que futuros trabalhos possam empregar métodos e conceitos mais comparáveis (Wu et al., 2022). Devido a essas questões a grande maioria das amostras de microbioma de mosquitos foram classificadas como pluri competentes, deixando desbalanceado o comparativo entre os grupos. Dessa forma, tal

análise não pode ser conduzida em mosquitos. Entretanto, foi possível observar com a revisão de literatura da competência vetorial de mosquitos que no caso do ZIKV somente espécies do gênero *Aedes* eram competentes enquanto espécies de outros gêneros não mantinham a infecção. Tal achado foi corroborado com a revisão recentemente publicada que avaliou especificamente a competência vetorial de mosquitos para o ZIKV (Bisia et al., 2023).

Independente destas questões supracitadas, diferenças no microbioma entre carrapatos mono e pluri competentes bem como mosquitos suscetíveis ou refratários ao ZIKV puderam ser observadas. Nas duas situações foram encontradas bactérias simbiontes, comumente encontradas na microbiota de carrapatos ou mosquitos. Esse tipo de bactéria é interessante para o desenvolvimento de novas abordagens de controle biológico como a modulação da microbiota pela introdução de bactérias específicas (Ferreira et al., 2023). Além disso, algumas bactérias simbiontes sofrem transmissão vertical no artrópode permitindo que modificações nessas bactérias seja passada para futuras gerações do artrópode (Mendiola; Civitello; Gerardo, 2020). Em carrapatos, bactérias do gênero *Candidatus Midichloria* foram associadas a uma competência vetorial mais restrita, vetores mono competentes. Esse gênero de bactéria é um simbionte em carrapatos que participa na homeostase reduzindo o estresse oxidativo bem como disponibilização de nutrientes contribuindo na digestão e biodisponibilização (Olivieri et al., 2019). Em mosquitos, alguns gêneros de bactérias como a *Serratia*, *Wolbachia* e *Pseudomonas*, conhecidas por serem simbiontes, foram associadas ao grupo de mosquitos suscetíveis ao ZIKV (Alomar et al., 2023; Bai et al., 2019; Khaligh; Vahedi; Chavshin, 2020). Assim, de acordo com os resultados do presente trabalho, esses gêneros de bactéria poderiam ser candidatos para futuros testes que venham a de alguma forma modular a competência vetorial de artrópodes vetores.

Em relação à diversidade da comunidade microbiana de carrapatos mono e pluri competentes, uma maior diversidade foi observada em carrapatos com ampla competência vetorial, pluri competentes. Esta diferença na diversidade contrasta o que foi observado na literatura com carrapatos da espécie *Ix. scapularis* infectados ou co-infectados por múltiplos patógenos (Gil et al., 2020). Dado que utilizamos amostras não infectadas, a competência vetorial para múltiplos patógenos pode estar associada a uma maior diversidade microbiana. No comparativo entre mosquitos suscetíveis ou refratários ao ZIKV, não houve diferenças significantes em termos de diversidade. Tal ausência de diferença na diversidade da comunidade bacteriana também foi observada em *Ae. aegypti* naturalmente infectados por ZIKV (Arévalo-Cortés et al., 2022). Em mosquitos *Ae. aegypti* de populações refratárias ao DENV-2 também não foi observada uma diferença na diversidade microbiana em comparação

à mosquitos da mesma espécie, porém suscetíveis à infecção (Coatsworth et al., 2018). Assim, diferenças na diversidade microbiana pode não estar associado à competência vetorial em mosquitos, contudo à competência vetorial ampla ao menos em carrapatos.

A análise funcional do microbioma de carrapatos e mosquitos no presente trabalho sugere uma maior abundância na expressão de algumas vias metabólicas relacionadas à competência vetorial de artrópodes. Para carrapatos pluri competentes, vias relacionadas à regulação do estresse oxidativo, biossíntese de dTDP-L-rhamnose e resistência a β-Lactam foram mais abundantes. Estas vias podem estar relacionadas a uma maior sobrevivência de patógenos no carrapato bem como uma manutenção da homeostase da microbiota (Chigwada et al., 2022; Hernandez et al., 2019; Van Der Beek et al., 2019). Foi observado um enriquecimento de diversas vias associadas ao metabolismo em mosquitos suscetíveis ao ZIKV. Em especial as vias das pentoses-fosfato e glicólise estavam enriquecidas nos mosquitos deste grupo. Tais vias metabólicas estão relacionadas à infecção por ZIKV em culturas de células de mosquito (Thaker et al., 2019). Adicionalmente, alterações metabólicas são importantes em artrópodes para a tolerância patógenos (Oliveira; Bahia; Vale, 2020). Dessa forma, a alteração em vias metabólicas associadas a uma homeostase no artrópode, por exemplo via controle do estresse oxidativo, e suprimento de nutrientes podem estar associados à competência vetorial em artrópodes.

Redes de co-ocorrência menos densas e conectadas foram observadas tanto em mosquitos suscetíveis ao ZIKV quanto em carrapatos pluri-competentes. Diferenças na densidade das redes de co-ocorrência foram observadas na comparação entre diferentes espécies de mosquito (Da Silva; Oliveira; Sallum, 2022; Hegde et al., 2018). No entanto, pouco se sabe sobre o impacto de uma infecção patogênica ou susceptibilidade a um ou mais patógenos na estrutura da rede de co-ocorrência microbiana de artrópodes. Redes menos densas e conectadas estão relacionadas a uma menor complexidade da comunidade microbiana (Zhou et al., 2022). Assim, os resultados do presente trabalho sugerem que uma comunidade microbiana menos complexa e estruturada pode estar contribuindo para a competência vetorial de um ou mais patógenos.

É importante ressaltar que o banco de dados 16S rRNA de Silva apresenta limitações para identificar com precisão as bactérias ao nível da espécie (Hoffman et al., 2021). Portanto, a análise do presente estudo limitou-se ao nível de família e gênero de bactérias. Tal nível de precisão impossibilita a identificação de espécies que realmente poderiam ter estabelecido uma relação co-evolutiva no microbioma do artrópode e, portanto, desempenham um papel na competência vetorial de artrópodes. Conseguimos identificar gêneros diferentemente

abundantes associados, mas não sabemos quais espécies desses gêneros estão de fato associadas à modulação da competência vetorial. Entretanto, os gêneros de bactérias aqui apontados como associados aos grupos de VMC ou VPC, suscetíveis ou refratários ao ZIKV, podem nos indicar um ponto inicial para futuros estudos na tentativa de modular a competência vetorial de artrópodes e desenvolver novos métodos de controle. Além disso, devido ao tecido dos artrópodes ter sido o corpo inteiro e os métodos de lavagem prévia à extração de DNA, algumas bactérias diferentemente abundantes podem ter sido oriundas do ambiente. Tais limitações são importantes de serem debatidas para que futuros trabalhos possam melhor avaliar o impacto das bactérias identificadas no presente estudo na competência vetorial de artrópodes.

6 **CONSIDERAÇÕES FINAIS**

Nosso trabalho demonstrou diferenças no microbioma de artrópodes vetores associados à competência vetorial. As bactérias dos gêneros *Rickettsia*, *Staphylococcus* e *Corynebacterium* foram associadas aos carrapatos com pluri competência vetorial. Enquanto *Cadidatus Midichloria*, *Escherichia-Shigella* e *Francisella* foram associados a um perfil de vetores mono competentes. Em relação aos mosquitos, *Pseudomonas*, *Serratia*, *Halomonas* e *Wolbachia* bem como *Variovorax* e JG30-KF-CM4 foram associadas a um perfil de susceptibilidade ao ZIKV em adultos e larvas, respectivamente.

Em vista do crescente aumento de resistência dos artrópodes aos métodos de controle baseados em inseticidas e/ou repelentes, os gêneros de bactérias identificados no presente trabalho podem servir para o desenvolvimento de novos métodos de controle biológico. Tais métodos podem ser estruturados para agir de forma mais focada na relação entre patógeno e hospedeiro invertebrado evitando o impacto ecológico dos métodos químicos de controle em outras espécies de artrópodes importantes para a sustentabilidade de biomas.

Naturalmente, devido às limitações mencionadas em relação às amostras, previsões e mecanismos, os resultados do presente trabalho deverão ser validados empiricamente no futuro para melhor compreensão dos mecanismos relacionados a relação da microbiota de artrópodes e sua competência vetorial. Independente das limitações apresentadas, o presente trabalho provê pontos de partida para a compreensão do papel da microbiota na competência vetorial de artrópodes.

REFERÊNCIAS

ABBAS-AGHABABAZADEH, F.; LI, Q.; FRIDLEY, B. L. Comparison of Normalization Approaches for Gene Expression Studies Completed with High-Throughput Sequencing. **PloS One**, v. 13, n. 10, p. e0206312, 31 out. 2018.

ABRAHAM, N. M. *et al.* Pathogen-Mediated Manipulation of Arthropod Microbiota to Promote Infection. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 5, p. E781–E790, 31 jan. 2017.

ADEGOKE, A. *et al*. Laboratory Colonization by Dirofilaria Immitis Alters the Microbiome of Female Aedes Aegypti Mosquitoes. **Parasites & Vectors**, v. 13, n. 1, p. 349, 13 jul. 2020.

AITCHISON, J. The Statistical Analysis of Compositional Data. **Journal of the Royal Statistical Society. Series B, Statistical methodology**, v. 44, n. 2, p. 139–177, 1982.

ALKAN, C. et al. Sandfly-Borne Phleboviruses of Eurasia and Africa: Epidemiology, Genetic Diversity, Geographic Range, Control Measures. **Antiviral Research**, v. 100, n. 1, p. 54–74, out. 2013.

ALOMAR, A. A. *et al.* Native Wolbachia Infection and Larval Competition Stress Shape Fitness and West Nile Virus Infection in Culex Quinquefasciatus Mosquitoes. **Frontiers in Microbiology**, v. 14, p. 1138476, 15 mar. 2023.

ANAGNOSTOU, V. *et al.* Novel Phlebovirus in Febrile Child, Greece. **Emerging Infectious Diseases**, v. 17, n. 5, p. 940–941, maio 2011.

ANDERSON, M. J.; WALSH, D. C. I. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? **Ecological monographs**, v. 83, n. 4, p. 557–574, nov. 2013.

ANDREWS, T. S. *et al.* Tutorial: Guidelines for the Computational Analysis of Single-Cell RNA Sequencing Data. **Nature Protocols**, v. 16, n. 1, p. 1–9, jan. 2021.

AÑEZ, N. et al. Update on Chagas Disease in Venezuela during the Period 2003-2018. A Review. **Acta Tropica**, v. 203, p. 105310, mar. 2020.

ANTINORI, S. et al. Chagas Disease in Europe: A Review for the Internist in the Globalized World. **European Journal of Internal Medicine**, v. 43, p. 6–15, set. 2017.

ANTUNES, S. *et al.* Functional Genomics Studies of Rhipicephalus (Boophilus) Annulatus Ticks in Response to Infection with the Cattle Protozoan Parasite, Babesia Bigemina. **International Journal for Parasitology**, v. 42, n. 2, p. 187–195, fev. 2012.

ARÉVALO-CORTÉS, A. *et al.* Association of Midgut Bacteria and Their Metabolic Pathways with Zika Infection and Insecticide Resistance in Colombian Aedes Aegypti Populations. **Viruses**, v. 14, n. 10, 6 out. 2022.

ASSHAUE, K. P. *et al.* Tax4Fun: Predicting Functional Profiles from Metagenomic 16S rRNA Data. **Bioinformatics** , v. 31, n. 17, p. 2882–2884, 1 set. 2015.

ATHNI, T. S. *et al.* The Influence of Vector-Borne Disease on Human History: Socio-Ecological Mechanisms. **Ecology Letters**, v. 24, n. 4, p. 829–846, abr. 2021.

AZAR, S. R.; WEAVER, S. C. Vector Competence Analyses on Aedes Aegypti Mosquitoes Using Zika Virus. **Journal of Visualized Experiments: JoVE**, n. 159, 31 maio 2020.

BAI, L. *et al.* A Gut Symbiotic Bacterium Serratia Marcescens Renders Mosquito Resistance to Plasmodium Infection Through Activation of Mosquito Immune Responses. **Frontiers in Microbiology**, v. 10, p. 1580, 18 jul. 2019.

BALVOČIŪTĖ, M.; HUSON, D. H. SILVA, RDP, Greengenes, NCBI and OTT - How Do These Taxonomies Compare? **BMC Genomics**, v. 18, n. Suppl 2, p. 114, 14 mar. 2017.

BANOVIĆ, P. *et al.* Humans Infested with Ixodes Ricinus Are Exposed to a Diverse Array of Tick-Borne Pathogens in Serbia. **Ticks and Tick-Borne Diseases**, v. 12, n. 2, p. 101609, mar. 2021.

BENT, S. J.; FORNEY, L. J. The Tragedy of the Uncommon: Understanding Limitations in the Analysis of Microbial Diversity. **The ISME Journal**, v. 2, n. 7, p. 689–695, jul. 2008.

BISIA, M. et al. Secondary Vectors of Zika Virus, a Systematic Review of Laboratory Vector Competence Studies. **PLoS Neglected Tropical Diseases**, v. 17, n. 8, p. e0011591, ago. 2023.

BLAKELY, B. N.; HANSON, S. F.; ROMERO, A. Survival and Transstadial Persistence of Trypanosoma Cruzi in the Bed Bug (Hemiptera: Cimicidae). **Journal of Medical Entomology**, v. 55, n. 3, p. 742–746, 4 maio 2018.

BOULANGER, N. et al. Ticks and Tick-Borne Diseases. **Medecine et Maladies Infectieuses**, v. 49, n. 2, p. 87–97, mar. 2019.

BREUNER, N. E. et al. Failure of the Asian Longhorned Tick, Haemaphysalis Longicornis, to Serve as an Experimental Vector of the Lyme Disease Spirochete, Borrelia Burgdorferi Sensu Stricto. **Ticks and Tick-Borne Diseases**, v. 11, n. 1, p. 101311, jan. 2020.

_____. Transmission of the Relapsing Fever Spirochete, Borrelia Miyamotoi, by Single Transovarially-Infected Larval Ixodes Scapularis Ticks. **Ticks and Tick-Borne Diseases**, v. 9, n. 6, p. 1464–1467, set. 2018.

BRUNEEL, S. *et al.* Assessing the Drivers behind the Structure and Diversity of Fish Assemblages Associated with Rocky Shores in the Galapagos Archipelago. **Journal of Marine Science and Engineering**, v. 9, n. 4, p. 375, 1 abr. 2021.

BUCKNER, E. A.; ALTO, B. W.; LOUNIBOS, L. P. Vertical Transmission of Key West Dengue-1 Virus by Aedes Aegypti and Aedes Albopictus (Diptera: Culicidae) Mosquitoes from Florida. **Journal of Medical Entomology**, v. 50, n. 6, p. 1291–1297, nov. 2013.

BUKIN, Y. S. *et al.* The Effect of 16S rRNA Region Choice on Bacterial Community Metabarcoding Results. **Scientific Data**, v. 6, p. 190007, 5 fev. 2019.

CALLAHAN, B. J.; MCMURDIE, P. J.; HOLMES, S. P. Exact Sequence Variants Should

Replace Operational Taxonomic Units in Marker-Gene Data Analysis. **The ISME Journal**, v. 11, n. 12, p. 2639–2643, dez. 2017.

CAMINADE, C.; MCINTYRE, K. M.; JONES, A. E. Impact of Recent and Future Climate Change on Vector-Borne Diseases. **Annals of the New York Academy of Sciences**, v. 1436, n. 1, p. 157–173, jan. 2019.

CAMMAROTA, G. *et al.* Gut Microbiome, Big Data and Machine Learning to Promote Precision Medicine for Cancer. **Nature Reviews. Gastroenterology & Hepatology**, v. 17, n. 10, p. 635–648, out. 2020.

CHAO, A.; CHIU, C.-H.; JOST, L. Phylogenetic Diversity Measures and Their Decomposition: A Framework Based on Hill Numbers. In: PELLENS, R.; GRANDCOLAS, P. (Org.). **Biodiversity Conservation and Phylogenetic Systematics: Preserving our evolutionary heritage in an extinction crisis**. Cham: Springer International Publishing, 2016. p. 141–172.

CHAO, A.; LEE, S.-M. Estimating the Number of Classes via Sample Coverage. **Journal of the American Statistical Association**, v. 87, n. 417, p. 210–217, 1 mar. 1992.

CHEN, L. et al. Gut Microbial Co-Abundance Networks Show Specificity in Inflammatory Bowel Disease and Obesity. **Nature Communications**, v. 11, n. 1, p. 4018, 11 ago. 2020.

CHIGWADA, A. D. *et al.* Pathogenic and Endosymbiotic Bacteria and Their Associated Antibiotic Resistance Biomarkers in Amblyomma and Hyalomma Ticks Infesting Nguni Cattle (Bos Spp.). **Pathogens**, v. 11, n. 4, 2 abr. 2022.

CHITIMIA-DOBLER, L. *et al.* Imported Hyalomma Ticks in Germany in 2018. **Parasites & Vectors**, v. 12, n. 1, p. 1–9, 26 mar. 2019. Acesso em: 27 mar. 2021.

COATSWORTH, H. et al. The Composition of Midgut Bacteria in Aedes Aegypti (Diptera: Culicidae) That Are Naturally Susceptible or Refractory to Dengue Viruses. **Journal of Insect Science** , v. 18, n. 6, 1 nov. 2018.

DAFLON-TEIXEIRA, N. F. *et al.* Multiple approaches to address potential risk factors of chagas disease transmission in northeastern Brazil. **The American Journal of Tropical Medicine and Hygiene**, v. 100, n. 2, p. 296–302, fev. 2019.

DA SILVA, H.; OLIVEIRA, T. M. P.; SALLUM, M. A. M. Bacterial community diversity and bacterial interaction network in eight mosquito species. **Genes**, v. 13, n. 11, 7 nov. 2022.

DAVIS, N. M. et al. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. **Microbiome**, v. 6, n. 1, p. 226, 17 dez. 2018.

DE LA CUESTA-ZULUAGA, J.; ESCOBAR, J. S. Considerations for optimizing microbiome analysis using a marker gene. **Frontiers in Nutrition**, v. 3, p. 26, 8 ago. 2016.

DE LA FUENTE, J. *et al.* Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. **Frontiers in Cellular and Infection Microbiology**, v. 7, n. apr, 2017.

DENNISON, N. J.; JUPATANAKUL, N.; DIMOPOULOS, G. The mosquito microbiota influences vector competence for human pathogens. **Current Opinion in Insect Science**, v. 3, p. 6–13, 1 set. 2014.

DE OLIVEIRA, E. F. *et al.* Vector competence of lutzomyia cruzi naturally demonstrated for leishmania infantum and suspected for leishmania amazonensis. **The American Journal of Tropical Medicine and Hygiene**, v. 96, n. 1, p. 178–181, 11 jan. 2017.

DOS SANTOS, S. O. et al. Incrimination of Lutzomyia Cruzi as a Vector of American Visceral Leishmaniasis. **Medical and Veterinary Entomology**, v. 12, n. 3, p. 315–317, jul. 1998.

DOUGLAS, G. M. *et al.* PICRUSt2 for prediction of metagenome functions. **Nature Biotechnology**, v. 38, n. 6, p. 685–688, jun. 2020.

DROUIN, A. et al. Vector competence of mediterranean mosquitoes for rift valley fever virus: a meta-analysis. **Pathogens**, v. 11, n. 5, 23 abr. 2022.

EDER, M. *et al.* Scoping review on vector-borne diseases in urban areas: transmission dynamics, vectorial capacity and co-infection. **Infectious Diseases of Poverty**, v. 7, n. 1, p. 1–24, 3 set. 2018. Acesso em: 27 mar. 2021.

EGOZCUE, J. J. *et al.* Isometric logratio transformations for compositional data analysis. **Mathematical geology**, v. 35, n. 3, p. 279–300, 1 abr. 2003.

EISEN, Lars. Vector competence studies with hard ticks and Borrelia burgdorferi sensu lato spirochetes: A review. **Ticks and tick-borne diseases**, v. 11, n. 3, p. 101359, 1 maio 2020. Acesso em: 28 mar. 2021.

EISEN, R. J.; EISEN, L. The blacklegged tick, ixodes scapularis: an increasing public health concern. **Trends in Parasitology**, v. 34, n. 4, p. 295–309, abr. 2018.

FAITH, D. P. Conservation evaluation and phylogenetic diversity. **Biological conservation**, v. 61, n. 1, p. 1–10, 1 jan. 1992.

FANG, H. et al. CCLasso: correlation inference for compositional data through lasso. **Bioinformatics** , v. 31, n. 19, p. 3172–3180, 1 out. 2015.

FARES, W. et al. Co-circulation of toscana virus and leishmania infantum in a focus of zoonotic visceral leishmaniasis from central tunisia. **Acta Tropica**, v. 204, p. 105342, abr. 2020.

FERREIRA, Q. R. *et al.* Role of the microbiome in aedes spp. vector competence: what do we know? **Viruses**, v. 15, n. 3, 17 mar. 2023.

FRANKLINOS, L. H. V. *et al.* The effect of global change on mosquito-borne disease. **The Lancet infectious diseases**, v. 19, n. 9, p. e302–e312, set. 2019.

FRIEDMAN, J.; ALM, E. J. Inferring correlation networks from genomic survey data. **PLoS Computational Biology**, v. 8, n. 9, p. e1002687, 20 set. 2012.

GALLOWAY-PEÑA, J.; HANSON, B. Tools for analysis of the microbiome. **Digestive**

Diseases and Sciences, v. 65, n. 3, p. 674–685, mar. 2020.

GAO, C. et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. **Nature Communications**, v. 13, n. 1, p. 3867, 5 jul. 2022.

GASSER, R. B. *et al.* Major prospects for exploring canine vector borne diseases and novel intervention methods using 'omic technologies. **Parasites & Vectors**, v. 4, p. 53, 13 abr. 2011.

GHAHVECHI KHALIGH, F.; VAHEDI, M.; CHAVSHIN, A. R. Identification of symbiotic bacteria in the midgut of the medically important mosquito, culiseta longiareolata (diptera: culicidae). **BMC Research Notes**, v. 13, n. 1, p. 378, 10 ago. 2020.

GIL, J. C. *et al.* Ixodes scapularis microbiome correlates with life stage, not the presence of human pathogens, in ticks submitted for diagnostic testing. **PeerJ**, v. 8, p. e10424, 2 dez. 2020.

giordano, b. v. *et al.* discovery of an aedes (stegomyia) albopictus population and first records of aedes (Stegomyia) aegypti in Canada. **Medical and Veterinary Entomology**, v. 34, n. 1, p. 10–16, mar. 2020.

GLOOR, G. B. *et al.* It's All relative: analyzing microbiome data as compositions. **Annals of Epidemiology**, v. 26, n. 5, p. 322–329, maio 2016.

_____. Microbiome datasets are compositional: and this is not optional. **Frontiers in Microbiology**, v. 8, p. 2224, 15 nov. 2017.

GLOOR, G. B.; REID, G. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. **Canadian Journal of Microbiology**, v. 62, n. 8, p. 692–703, ago. 2016.

GOBERNA, M.; VERDÚ, M. Cautionary notes on the use of co-occurrence networks in soil ecology. **Soil biology & biochemistry**, v. 166, p. 108534, 1 mar. 2022.

GODDARD, L. B. *et al.* Vector competence of california mosquitoes for west nile virus. **Emerging Infectious Diseases**, v. 8, n. 12, p. 1385–1391, dez. 2002.

GOULD, E. *et al.* Emerging arboviruses: why today? **One Health (Amsterdam, Netherlands)**, v. 4, p. 1–13, dez. 2017.

GREENACRE, M. Compositional data analysis. **Annual Review of Statistics and Its Application**, v. 8, n. 1, p. 271–299, 7 mar. 2021.

GREENACRE, M.; MARTÍNEZ-ÁLVARO, M.; BLASCO, A. compositional data analysis of microbiome and any-omics datasets: a validation of the additive logratio transformation. **Frontiers in Microbiology**, v. 12, p. 727398, 11 out. 2021.

GRISI, L. et al. Reassessment of the potential economic impact of cattle parasites in Brazil. **Revista Brasileira de Parasitologia Veterinaria = Brazilian Journal of Veterinary Parasitology: Orgao Oficial Do Colegio Brasileiro de Parasitologia Veterinaria**, v. 23, n. 2, p. 150–156, abr. 2014.

GUEDES, D. R. *et al.* Zika virus replication in the mosquito culex quinquefasciatus in brazil. **Emerging Microbes & Infections**, v. 6, n. 8, p. e69, 9 ago. 2017.

GUO, X. *et al.* Vector competence and vertical transmission of zika virus in (diptera: culicidae). **Vector Borne and Zoonotic Diseases** , v. 20, n. 5, p. 374–379, maio 2020.

GUO, X.-X. *et al.* Culex pipiens quinquefasciatus: a potential vector to transmit zika virus. **Emerging Microbes & Infections**, v. 5, n. 9, p. e102, 7 set. 2016.

HEGDE, S. *et al.* Microbiome interaction networks and community structure from laboratory-reared and field-collected aedes aegypti, aedes albopictus, and culex quinquefasciatus mosquito vectors. **Frontiers in Microbiology**, v. 9, p. 2160, 10 set. 2018.

HERNANDEZ, E. P. *et al.* The case for oxidative stress molecule involvement in the tick-pathogen interactions -an omics approach. **Developmental and Comparative Immunology**, v. 100, p. 103409, nov. 2019.

HIRANO, H.; TAKEMOTO, K. Difficulty in inferring microbial community structure based on co-occurrence network approaches. **BMC Bioinformatics**, v. 20, n. 1, p. 1–14, 13 jun. 2019.

HOFFMAN, C. *et al.* Species-level resolution of female bladder microbiota from 16s rrna amplicon sequencing. **mSystems**, v. 6, n. 5, p. e0051821, 26 out. 2021.

HOFFMANN, A. A. et al. Stability of the wmel wolbachia infection following invasion into aedes aegypti populations. **PLoS Neglected Tropical Diseases**, v. 8, n. 9, p. e3115, set. 2014.

HOFFMANN, A. A. *et al.* Successful establishment of wolbachia in aedes populations to suppress dengue transmission. **Nature**, v. 476, n. 7361, p. 454–457, 24 ago. 2011.

HUNTINGTON, M. K.; ALLISON, J.; NAIR, D. Emerging vector-borne diseases. **American Family Physician**, v. 94, n. 7, p. 551–557, 1 out. 2016.

IWAI, S. *et al.* Piphillin: improved prediction of metagenomic content by direct inference from human microbiomes. **PloS One**, v. 11, n. 11, p. e0166104, 7 nov. 2016.

JI, F.; SADREYEV, R. I. RNA-seq: basic bioinformatics analysis. **Current Protocols in Molecular Biology**, v. 124, n. 1, p. e68, out. 2018.

KARIM, S. *et al.* A study of ticks and tick-borne livestock pathogens in pakistan. **PLoS Neglected Tropical Diseases**, v. 11, n. 6, p. e0005681, jun. 2017.

KELLY, P. H. *et al.* The gut microbiome of the vector lutzomyia longipalpis is essential for survival of leishmania infantum. **mBio**, v. 8, n. 1, 17 jan. 2017.

KERRIGAN, Z.; KIRKPATRICK, J. B.; D'HONDT, S. Influence of 16s rrna hypervariable region on estimates of bacterial diversity and community composition in seawater and marine sediment. **Frontiers in Microbiology**, v. 10, p. 1640, 16 jul. 2019.

KHAN, J. *et al.* Epidemiological trends and risk factors associated with dengue disease in

pakistan (1980-2014): a systematic literature search and analysis. **BMC Public Health**, v. 18, n. 1, p. 745, 15 jun. 2018.

KIM, B.-R. *et al*. Deciphering diversity indices for a better understanding of microbial communities. **Journal of Microbiology and Biotechnology**, v. 27, n. 12, p. 2089–2093, 28 dez. 2017.

KIM, H.; KIM, S.; JUNG, S. Instruction of microbiome taxonomic profiling based on 16s rrna sequencing. **Journal of Microbiology** , v. 58, n. 3, p. 193–205, mar. 2020.

KISHORE, D. *et al.* Inferring microbial co-occurrence networks from amplicon data: a systematic evaluation. **mSystems**, v. 8, n. 4, p. e0096122, 31 ago. 2023.

KLEMETSEN, T.; WILLASSEN, N. P.; KARLSEN, C. R. Full-length 16s rrna gene classification of atlantic salmon bacteria and effects of using different 16s variable regions on community structure analysis. **MicrobiologyOpen**, v. 8, n. 10, p. e898, out. 2019.

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

KRAUSE, P. J. Human babesiosis. **International Journal for Parasitology**, v. 49, n. 2, p. 165–174, fev. 2019.

KRISHNAVAJHALA, A.; ARMSTRONG, B. A.; LOPEZ, J. E. Vector competence of geographical populations of ornithodoros turicata for the tick-borne relapsing fever spirochete borrelia turicatae. **Applied and Environmental Microbiology**, v. 84, n. 21, 1 nov. 2018.

LANGILLE, M. G. I. *et al.* Predictive functional profiling of microbial communities using 16s rrna marker gene sequences. **Nature Biotechnology**, v. 31, n. 9, p. 814–821, set. 2013.

LAYEGHIFARD, M.; HWANG, D. M.; GUTTMAN, D. S. Disentangling interactions in the microbiome: a network perspective. **Trends in Microbiology**, v. 25, n. 3, p. 217–228, mar. 2017.

LI, M. *et al.* What can we learn about the bias of microbiome studies from analyzing data from mock communities? **Genes**, v. 13, n. 10, 28 set. 2022.

LIU, G. *et al.* Structural and functional characteristics of the microbiome in deep-dentin caries. **Journal of Dental Research**, v. 99, n. 6, p. 713–720, jun. 2020.

LIU, Q. *et al.* Trends of the global, regional, and national incidence of malaria in 204 countries from 1990 to 2019 and implications for malaria prevention. **Journal of Travel Medicine**, 24 mar. 2021.

LIU, Y.-X. *et al.* A practical guide to amplicon and metagenomic analysis of microbiome data. **Protein & Cell**, 11 maio 2020.

LÓPEZ-ALADID, R. et al. Determining the most accurate 16s rrna hypervariable region for taxonomic identification from respiratory samples. **Scientific Reports**, v. 13, n. 1, p. 3974, 9 mar. 2023.

LÓPEZ-GARCÍA, E. *et al.* Application of compositional data analysis to study the relationship between bacterial diversity in human faeces and sex, age, and weight. **Biomedicines**, v. 11, n. 8, 28 jul. 2023.

LÓPEZ, M. S.; MÜLLER, G. V.; SIONE, W. F. Analysis of the spatial distribution of scientific publications regarding vector-borne diseases related to climate variability in South America. **Spatial and Spatio-Temporal Epidemiology**, v. 26, p. 35–93, ago. 2018.

LOURADOUR, I. *et al.* The midgut microbiota plays an essential role in sand fly vector competence for leishmania major. **Cellular Microbiology**, v. 19, n. 10, out. 2017.

LOVELL, D. *et al.* Proportionality: A valid alternative to correlation for relative data. **PLoS Computational Biology**, v. 11, n. 3, p. e1004075, mar. 2015.

LOVELL, D. R.; CHUA, X.-Y.; MCGRATH, A. Counts: an outstanding challenge for log-ratio analysis of compositional data in the molecular biosciences. **NAR Genomics and Bioinformatics**, v. 2, n. 2, p. lqaa040, jun. 2020.

LOZUPONE, C. A. *et al.* Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. **Applied and Environmental Microbiology**, v. 73, n. 5, p. 1576–1585, mar. 2007.

LUTZ, K. C. *et al.* A survey of statistical methods for microbiome data analysis. **Frontiers in applied mathematics and statistics**, v. 8, 14 jun. 2022.

MACLEOD, H. J.; DIMOPOULOS, G. Detailed analyses of zika virus tropism in culex quinquefasciatus reveal systemic refractoriness. **mBio**, v. 11, n. 4, 18 ago. 2020.

MANN, S. *et al.* A Review of leishmaniasis: current knowledge and future directions. **Current Tropical Medicine Reports**, p. 1–12, 17 mar. 2021.

MARCHESI, J. R.; RAVEL, J. The vocabulary of microbiome research: a proposal. **Microbiome**, v. 3, p. 31, 30 jul. 2015.

MAROLI, M. *et al.* Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. **Medical and Veterinary Entomology**, v. 27, n. 2, p. 123–147, jun. 2013.

MASSAD, E. *et al.* On the origin and timing of Zika Virus introduction in Brazil. **Epidemiology and Infection**, v. 145, n. 11, p. 2303–2312, ago. 2017.

MATCHADO, M. S. *et al.* Network analysis methods for studying microbial communities: a mini review. **Computational and Structural Biotechnology Journal**, v. 19, p. 2687–2698, 4 maio 2021.

MA, Y.; LUO, Y.; JIANG, H. A novel normalization and differential abundance test framework for microbiome data. **Bioinformatics** , v. 36, n. 13, p. 3959–3965, 1 jul. 2020.

MCART, S. H.; COOK-PATTON, S. C.; THALER, J. S. Relationships between arthropod richness, evenness, and diversity are altered by complementarity among plant genotypes.

Oecologia, v. 168, n. 4, p. 1013–1021, abr. 2012.

MCMURDIE, P. J.; HOLMES, S. Waste not, want not: why rarefying microbiome data is inadmissible. **PLoS Computational Biology**, v. 10, n. 4, p. e1003531, abr. 2014.

MENDIOLA, S. Y.; CIVITELLO, D. J.; GERARDO, N. M. An integrative approach to symbiont-mediated vector control for agricultural pathogens. **Current Opinion in Insect Science**, v. 39, p. 57–62, jun. 2020.

MINWUYELET, A. *et al.* Prevalence and risk factors of asymptomatic infection in gondar zuria district, northwest ethiopia. **Infection and Drug Resistance**, v. 13, p. 3969–3975, 4 nov. 2020.

MIZRAHI-MAN, O.; DAVENPORT, E. R.; GILAD, Y. Taxonomic classification of bacterial 16s rrna genes using short sequencing reads: evaluation of effective study designs. **PloS One**, v. 8, n. 1, p. e53608, 7 jan. 2013.

MONDOT, S. *et al.* Structural robustness of the gut mucosal microbiota is associated with crohn's disease remission after surgery. **Gut**, v. 65, n. 6, p. 954–962, jun. 2016.

MUHAMAD RIZAL, N. S. *et al.* Advantages and limitations of 16s rrna next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. **Diagnostics (Basel, Switzerland)**, v. 10, n. 10, 14 out. 2020.

MUTHENENI, S. R. *et al.* Influence of Socioeconomic Aspects on Lymphatic Filariasis: A Case-Control Study in Andhra Pradesh, India. **Journal of Vector Borne Diseases**, v. 53, n. 3, p. 272–278, jul. 2016.

NAUCKE, T. J. *et al.* Sandflies and leishmaniasis in germany. **Parasitology Research**, v. 103 Suppl 1, p. S65–8, dez. 2008.

NAUCKE, T. J.; LORENTZ, S. First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in germany. **Parasites & Vectors**, v. 5, n. 1, p. 1–5, 1 abr. 2012. Acesso em: 27 mar. 2021.

NEARING, J. T. *et al.* Microbiome differential abundance methods produce different results across 38 datasets. **Nature Communications**, v. 13, n. 1, p. 342, 17 jan. 2022.

NIANG, E. H. A. *et al.* Biological control of mosquito-borne diseases: the potential of -based interventions in an ivm framework. **Journal of Tropical Medicine**, v. 2018, p. 1470459, 15 nov. 2018.

NIU, S.-Y. *et al.* Bioinformatics tools for quantitative and functional metagenome and metatranscriptome data analysis in microbes. **Briefings in Bioinformatics**, v. 19, n. 6, p. 1415–1429, 27 nov. 2018.

NOOKAEW, I. *et al.* A Comprehensive comparison of rna-seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in saccharomyces cerevisiae. **Nucleic Acids Research**, v. 40, n. 20, p. 10084–10097, 1 nov. 2012.

NORMAN, F. F.; LÓPEZ-VÉLEZ, R. Chagas disease: comments on the 2018 paho guidelines for diagnosis and management. **Journal of Travel Medicine**, v. 26, n. 7, 14 out. 2019.

NÚÑEZ, A. I. *et al.* Alteration in the culex pipiens transcriptome reveals diverse mechanisms of the mosquito immune system implicated upon rift valley fever phlebovirus exposure. **PLoS neglected tropical diseases**, v. 14, n. 12, p. e0008870, 10 dez. 2020. Acesso em: 23 mar. 2021.

OGUNLADE, S. T. *et al*. A review: borne arboviral infections, controls and based strategies. **Vaccines**, v. 9, n. 1, 8 jan. 2021.

OLIVEIRA, F. *et al.* Sand Flies, Leishmania, and Transcriptome-Borne Solutions. **Parasitology International**, v. 58, n. 1, p. 1–5, mar. 2009.

OLIVEIRA, J. H.; BAHIA, A. C.; VALE, P. F. How are arbovirus vectors able to tolerate infection? **Developmental and Comparative Immunology**, v. 103, p. 103514, fev. 2020.

OLIVIERI, E. *et al.* Tissue Tropism and Metabolic Pathways of Midichloria Mitochondrii Suggest Tissue-Specific Functions in the Symbiosis with Ixodes Ricinus. **Ticks and Tick-Borne Diseases**, v. 10, n. 5, p. 1070–1077, ago. 2019.

ONYICHE, T. E. *et al.* Global distribution of species in questing ticks: a systematic review and meta-analysis based on published literature. **Pathogens**, v. 10, n. 2, 19 fev. 2021.

ORKUN, Ö. Molecular Investigation of the Natural Transovarial Transmission of Tick-Borne Pathogens in Turkey. **Veterinary Parasitology**, v. 273, p. 97–104, set. 2019.

PALIY, O.; SHANKAR, V. Application of multivariate statistical techniques in microbial ecology. **Molecular Ecology**, v. 25, n. 5, p. 1032–1057, mar. 2016.

PALMER, R. J. *et al.* Analysis of oral bacterial communities: comparison of homi with a tree-based approach implemented in QIIME. **Journal of Oral Microbiology**, v. 11, n. 1, p. 1586413, 1 abr. 2019.

PERMUTATIONAL MULTIVARIATE ANALYSIS OF VARIANCE (PERMANOVA). In: ANDERSON, Marti J. **wiley statsref**: statistics reference online. Chichester, UK: John Wiley & Sons, Ltd, 15 nov. 2017. p. 1–15.

PETERSEN, C.; ROUND, J. L. Defining dysbiosis and its influence on host immunity and disease. **Cellular Microbiology**, v. 16, n. 7, p. 1024–1033, jul. 2014.

PIOTROWSKI, M.; RYMASZEWSKA, A. Expansion of tick-borne rickettsioses in the world. **Microorganisms**, v. 8, n. 12, 30 nov. 2020.

PULKKINEN, L. I. A.; BUTCHER, S. J.; ANASTASINA, M. Tick-borne encephalitis virus: a structural view. **Viruses**, v. 10, n. 7, 28 jun. 2018.

RANDOLPH, S. E. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. **Parasitology**, v. 129 Suppl, p. S37–65, 2004.

RANJAN, R. *et al.* Analysis of the microbiome: advantages of whole genome shotgun versus

16s amplicon sequencing. **Biochemical and Biophysical Research Communications**, v. 469, n. 4, p. 967–977, 22 jan. 2016.

REIS, L. L. D. *et al.* Changes in the epidemiology of visceral leishmaniasis in Brazil from 2001 to 2014. **Revista Da Sociedade Brasileira de Medicina Tropical**, v. 50, n. 5, p. 638–645, set. 2017.

RINKER, D. C.; PITTS, R. J.; ZWIEBEL, L. J. Disease vectors in the era of next generation sequencing. **Genome Biology**, v. 17, n. 1, p. 95, 6 maio 2016.

RODINO, K. G.; THEEL, E. S.; PRITT, B. S. Tick-borne diseases in the united states. **Clinical Chemistry**, v. 66, n. 4, p. 537–548, 1 abr. 2020.

SALDAÑA, M. A.; HEGDE, S.; HUGHES, G. L. Microbial Control of Arthropod-Borne Disease. **Memorias Do Instituto Oswaldo Cruz**, v. 112, n. 2, p. 81–93, 2017. Acesso em: 1 ago. 2020.

SARAIVA, R. G. et al. Mosquito gut antiparasitic and antiviral immunity. **Developmental and Comparative Immunology**, v. 64, p. 53–64, nov. 2016.

SASSERA, D. *et al.* Microbial Symbiosis and the Control of Vector-Borne Pathogens in Tsetse Flies, Human Lice, and Triatomine Bugs. **Pathogens and Global Health**, v. 107, n. 6, p. 285–292, set. 2013.

SCHRIEFER, A. E. *et al.* A multi-amplicon 16S rRNA sequencing and analysis method for improved taxonomic profiling of bacterial communities. **Journal of Microbiological Methods**, v. 154, p. 6–13, nov. 2018.

SEGATA, N. *et al.* Metagenomic biomarker discovery and explanation. **Genome Biology**, v. 12, n. 6, p. 1–18, 24 jun. 2011. Acesso em: 18 mar. 2021.

SEMENZA, J. C.; SUK, J. E. Vector-borne diseases and climate change: a european perspective. **FEMS Microbiology Letters**, v. 365, n. 2, 1 fev. 2018.

SHI, J. et al. Tick-borne viruses. **Virologica Sinica**, v. 33, n. 1, p. 21–43, fev. 2018.

SILVERMAN, J. D. *et al.* A phylogenetic transform enhances analysis of compositional microbiota data. **eLife**, v. 6, 15 fev. 2017.

_____. Naught All Zeros in Sequence Count Data Are the Same. **Computational and Structural Biotechnology Journal**, v. 18, p. 2789–2798, 28 set. 2020.

SLATKO, B. E.; GARDNER, A. F.; AUSUBEL, F. M. Overview of next-generation sequencing technologies. **Current Protocols in Molecular Biology**, v. 122, n. 1, p. e59, abr. 2018.

SMARTT, C. T. *et al.* Culex quinquefasciatus (diptera: culicidae) from florida transmitted Zika Virus. **Frontiers in Microbiology**, v. 9, p. 768, 26 abr. 2018.

SOUZA-NETO, J. A.; POWELL, J. R.; BONIZZONI, M. Aedes Aegypti Vector Competence

Studies: A Review. **Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases**, v. 67, p. 191–209, jan. 2019.

SPIES, D. *et al.* Comparative analysis of differential gene expression tools for rna sequencing time course data. **Briefings in Bioinformatics**, v. 20, n. 1, p. 288–298, 6 out. 2017. Acesso em: 12 set. 2023.

SREENIVASAMURTHY, S. K. *et al.* Mosquito-borne diseases and omics: tissue-restricted expression and alternative splicing revealed by transcriptome profiling of anopheles stephensi. **Omics: A Journal of Integrative Biology**, v. 21, n. 8, p. 488–497, ago. 2017.

STALEY, C.; KAISER, T.; KHORUTS, A. Clinician Guide to Microbiome Testing. **Digestive Diseases and Sciences**, v. 63, n. 12, p. 3167–3177, dez. 2018.

STANEK, G. *et al.* Lyme Borreliosis. **The Lancet**, v. 379, n. 9814, p. 461–473, 4 fev. 2012.

STEINER, F. E. *et al.* Infection and co-infection rates of anaplasma phagocytophilum variants, babesia spp., borrelia burgdorferi, and the rickettsial endosymbiont in ixodes scapularis (acari: ixodidae) from sites in indiana, maine, pennsylvania, and wisconsin. **Journal of Medical Entomology**, v. 45, n. 2, p. 289–297, mar. 2008.

SUDARIKOV, K.; TYAKHT, A.; ALEXEEV, D. Methods for The Metagenomic Data Visualization and Analysis. **Current Issues in Molecular Biology**, v. 24, p. 37–58, 6 jul. 2017.

TEICH, V.; ARINELLI, R.; FAHHAM, L. Aedes aegypti e sociedade: o impacto econômico das arboviroses no Brasil. **Jornal Brasileiro de Economia da Saúde**, v. 9, n. 3, p. 267–276, dez. 2017.

THAKER, S. K. *et al.* Differential Metabolic Reprogramming by Zika Virus Promotes Cell Death in Human versus Mosquito Cells. **Cell Metabolism**, v. 29, n. 5, p. 1206–1216.e4, 7 maio 2019.

TRENTELMAN, J. J. A. *et al.* A Combined Transcriptomic Approach to Identify Candidates for an Anti-Tick Vaccine Blocking B. Afzelii Transmission. **Scientific Reports**, v. 10, n. 1, p. 1–14, 18 nov. 2020. Acesso em: 23 mar. 2021.

URAKI, R. *et al.* Altered Vector Competence in an Experimental Mosquito-Mouse Transmission Model of Zika Infection. **PLoS Neglected Tropical Diseases**, v. 12, n. 3, p. e0006350, mar. 2018.

VAN DER BEEK, S. L. *et al.* Streptococcal dTDP-L-Rhamnose Biosynthesis Enzymes: Functional Characterization and Lead Compound Identification. **Molecular Microbiology**, v. 111, n. 4, p. 951–964, abr. 2019.

VARGAS-ALBORES, F. *et al.* Size-Variable Zone in V3 Region of 16S rRNA. **RNA Biology**, v. 14, n. 11, p. 1514–1521, 2 nov. 2017.

VÁZQUEZ-BAEZA, Y. *et al.* EMPeror: A Tool for Visualizing High-Throughput Microbial Community Data. **GigaScience**, v. 2, n. 1, p. 16, 26 nov. 2013.

VEDURURU, R. K. *et al.* Whole Transcriptome Analysis of Mosquito Head and Thorax Post-Chikungunya Virus Infection. **Pathogens**, v. 8, n. 3, 27 ago. 2019.

VIEIRA, C. B. *et al.* Triatomines: Trypanosomatids, Bacteria, and Viruses Potential Vectors? **Frontiers in Cellular and Infection Microbiology**, v. 8, 2018.

VIEIRA, C. S. *et al.* Impact of Trypanosoma Cruzi on Antimicrobial Peptide Gene Expression and Activity in the Fat Body and Midgut of Rhodnius Prolixus. **Parasites & Vectors**, v. 9, p. 119, 1 mar. 2016.

WEISS, S. *et al.* Correlation Detection Strategies in Microbial Data Sets Vary Widely in Sensitivity and Precision. **The ISME Journal**, v. 10, n. 7, p. 1669–1681, jul. 2016.

_____. Normalization and Microbial Differential Abundance Strategies Depend upon Data Characteristics. **Microbiome**, v. 5, n. 1, p. 27, 3 mar. 2017.

WILKE, André B. B.; BEIER, John C; BENELLI, Giovanni. Complexity of the relationship between global warming and urbanization – an obscure future for predicting increases in vector-borne infectious diseases. **Current Opinion in Insect Science**, v. 35, p. 1-9, 1 out. 2019. Acesso em: 27 mar. 2021.

WHO. **Global vector control response 2017–2030**. [S.l.]: World Health Organization, Geneva, 2017a.

_____. **Weekly epidemiological record: Global leishmaniasis update, 2006 - 2015, a turning point in leishmaniasis surveillance**. [S.l.]: World Health organization, Geneva, 2017b.

_____. **World malaria report 2020: 20 years of global progress and challenges**. [S.l.]: World Health Organization, Geneva, 2020.

WILLIS, A. D. Rarefaction, Alpha Diversity, and Statistics. **Frontiers in Microbiology**, v. 10, p. 2407, 23 out. 2019.

WILSON, A. L. *et al.* The Importance of Vector Control for the Control and Elimination of Vector-Borne Diseases. **PLoS Neglected Tropical Diseases**, v. 14, n. 1, p. e0007831, jan. 2020.

WU, T. *et al.* clusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data. **Innovation (Cambridge (Mass.))**, v. 2, n. 3, p. 100141, 28 ago. 2021.

WU, V. Y. *et al.* A Minimum Data Standard for Vector Competence Experiments. **Scientific Data**, v. 9, n. 1, p. 634, 19 out. 2022.

WU, Y.; CHEN, Y.; SHEN, T.-J. A likelihood framework for modeling pairwise beta diversity patterns based on the tradeoff between colonization and extinction. **Frontiers in ecology and evolution**, v. 8, 3 abr. 2020.

XIA, Y.; SUN, J. Hypothesis Testing and Statistical Analysis of Microbiome. **Genes & Diseases**, v. 4, n. 3, p. 138–148, set. 2017.

XUE, Z.; KABLE, M. E.; MARCO, M. L. Impact of DNA Sequencing and Analysis Methods on 16S rRNA Gene Bacterial Community Analysis of Dairy Products. **mSphere**, v. 3, n. 5, 17 out. 2018.

yalamanchili, H. K.; WAN, Y. W.; LIU, Z. Data analysis pipeline for rna-seq experiments: from differential expression to cryptic splicing. **Current Protocols in Bioinformatics / Editoral Board, Andreas D. Baxevanis [et Al.]**, v. 59, p. 11.15.1–11.15.21, 13 set. 2017.

YANG, L.; CHEN, J. A Comprehensive evaluation of microbial differential abundance analysis methods: current status and potential solutions. **Microbiome**, v. 10, n. 1, p. 130, 19 ago. 2022.

YAN, J. *et al.* Network approaches to systems biology analysis of complex disease: integrative methods for multi-omics data. **Briefings in Bioinformatics**, v. 19, n. 6, p. 1370–1381, 30 jun. 2017. Acesso em: 23 mar. 2021.

ZHOU, H. *et al.* LinDA: Linear models for differential abundance analysis of microbiome compositional data. **Genome Biology**, v. 23, n. 1, p. 95, 14 abr. 2022.

ZHOU, J. *et al.* Urbanization increases stochasticity and reduces the ecological stability of microbial communities in amphibian hosts. **Frontiers in Microbiology**, v. 13, p. 1108662, 2022.

ZHOU, Y. H.; GALLINS, P. A Review and tutorial of machine learning methods for microbiome host trait prediction. **Frontiers in Genetics**, v. 10, p. 579, 25 jun. 2019.

ZINSZER, K. *et al.* Reconstruction of Zika virus introduction in brazil. **Emerging Infectious Diseases journal - CDC,** v. 3, n. 1, Jan. 2017.

ZULFIQAR, H.; MALIK, A. Bancroftian Filariasis. **StatPearls**. Treasure Island (FL): StatPearls Publishing, 2021.

Apêndice A - Artigos produzidos em colaborações durante o período do doutorado.

Natural infection by *Leishmania infantum* in the *Lutzomyia longipalpis* population of an endemic coastal area to visceral leishmaniasis in Brazil is not associated with bioclimatic factors.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Natural infection by *Leishmania infantum* in the *Lutzomyia longipalpis* population of an endemic coastal area to visceral leishmaniasis in Brazil is not associated with bioclimatic factors

Tiago Feitosa Mota[1☯], Orlando Marcos Farias de Sousa[1☯], Yuri de Jesus Silva[1], Lairton Souza Borja[1], Bruna Martins Macedo Leite[1], Manuela da Silva Solcà[2], Djalma Alves de Melo[3], Claudia Ida Brodskyn[1], Edelberto Santos Dias[4], Patrícia Sampaio Tavares Veras[1,5], Deborah Bittencourt Mothé Fraga[1,2,5]*

1 Instituto Gonçalo Moniz—Fundação Oswaldo Cruz, Salvador, Brazil, **2** Escola de Medicina Veterinária e Zootecnia—Universidade Federal da Bahia, Salvador, Brazil, **3** Centro de Controle de Zoonoses de Camaçari, Camaçari, Brazil, **4** Instituto René Rachou—Fundação Oswaldo Cruz, Belo Horizonte, Brazil, **5** Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais—INCT-DT, Salvador, Brazil

☯These authors contributed equally to this work.
* deborah.fraga@bahia.fiocruz.br

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Abstract

Visceral leishmaniasis (VL) is a zoonosis caused by the protozoan *Leishmania infantum* and in Brazil is transmitted mainly by the bite of *Lutzomuyia longipalpis* sand flies. Data about the presence, distribution, natural infection rate, seasonal and monthly dynamics of the vector population are important for optimizing the measures to control VL in endemic areas. This study aimed to identify sand fly fauna in an endemic area for VL to detect the prevalence of *L. infantum* infection in the *Lu. longipalpis* population and to elucidate the influence of bioclimatic factors on the monthly fluctuations of this vector. HP light traps were monthly set in the intradomicile and peridomicile of residences located in the central and beachfront areas of Camaçari, a VL endemic area. The sand fly collection was conducted in two periods: i) period 1—between December 2011 and November 2012 and ii) period 2—August 2014 and July 2015. Sand fly species were identified and detection of *L. infantum* infection by qPCR was performed in pools of female *Lu. longipalpis*. For the first time, the parasite load of positive pools was correlated with the number of *Lu. longipalpis* captured per month in both periods. Correlation analyses between the monthly fluctuation of the sand fly population and bioclimatic indices of the municipality in both collection periods were also performed. In both evaluated periods, more than 98% of the collected sand flies were *Lu. longipalpis*, confirming the predominance of this species in the region. It was captured mostly in the beachfront area in all months evaluated (99%). For the period 1, *Leishmania* DNA was detected in 81% of tested pools representing a minimal infection rate of 9.6%. In the period 2, 40% of the pools were positive with a minimal infection rate of 10.2%. Infected sand flies were only detected in the beachfront area in both periods. The parasite load was low and did not vary in the evaluated months despite the number of collected sand flies. No

Seroepidemiological survey of canine visceral leishmaniasis in a continental island of northeast Brazil.

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Seroepidemiological survey of canine visceral leishmaniasis in a cont nental island of northeast Brazil

Invest gação soroepidemiológica da leishmaniose visceral canina em uma ilha cont nental do Nordeste do Brasil

Enice Fonseca Klein[1], Roseclea Chagas[1], Tiago Feitosa Mota[1], Jairo Torres Magalhães-Junior[2], Adriano Figueiredo Monte-Alegre[3*], Daniela Farias dos Santos Laranjeira[4], Stella Maria Barrouin-Melo[5], Carlos Roberto Franke[6]

[1] Médica Veterinária, Laboratório de Infectologia Veterinária da Universidade Federal da Bahia (UFBA); [2] Doutor em Ciência Animal nos Trópicos. Docente do Centro Mult disciplinar Campus da Barra da Universidade Federal do Oeste da Bahia; [3] Doutor em Parasitologia. Docente do Departamento de Biointeração do Inst tuto de Ciências da Saúde da UFBA; [4] Doutora em Patologia Experimental e Comparada. Docente do Curso de Medicina Veterinária da UFBA; [5] Doutora em Imunologia. Docente do Curso de Medicina Veterinária da UFBA; [6] Doutor em Parasitologia. Docente do Curso de Medicina Veterinária da UFBA.

Abstract

Introduct on: *Visceral leishmaniasis (VL) is endemic disease in the neighboring municipalit es of the cont nental island Ilha de Maré, Salvador, Bahia, Brazil. Nevertheless, VL has not been reported in the island itself.* ***Object ve:*** *the present study aimed to invest gate the seroprevalence and clinical signs of Canine Visceral Leishmaniasis (CVL) and to ident fy the sand f y populat on present in the village of Botelho, Ilha de Maré.* ***Methodology:*** *sera of local dogs were tested for ant -Leishmania IgG by immunoassays (screening with TR DPP ™ rapid test and conf rmatory with indirect ELISA, Bio-Manguinhos/Fiocruz) and an entomological survey was conducted to est mate and ident fy the phlebotomine fauna of the region.* ***Results:*** *seven out of 106 samples (6.6%) were posit ve using rapid test. These posit ve samples were sent to the Central Laboratory of Bahia for conf rmat on by indirect ELISA. However, all samples presentednegat ve results. Nine specimens of Pressat a chot , subfamily Phlebotominae were ident f ed, being this species frequently found in areas with cutaneous leishmaniasis transmission in Brazil.* ***Conclusion:*** *although this work did not conf rm the presence of CVL in Ilha de Maré, new serological and entomological studies in a larger area are required for the maintenance of the epidemiological surveillance in the emphasized insular area.*

Keywords: *Leishmania. Pressat a chot . Phlebotomines*

Resumo

Introdução: Leishmaniose Visceral (LV) é uma doença endêmica em municípios vizinhos à Ilha de Maré, situada na plataforma cont nental do município de Salvador, Bahia, Brasil. Entretanto, casos de LV não tem sido not f cados nesta Ilha. **Objet vo**: O presente trabalho objet vou invest gar a soroprevalência e sinais clínicos de Leishmaniose Visceral Canina (LVC) e ident f car a população de f ebótomos presentes no povoado de Botelho, Ilha de Maré. **Metodologia:** soro de cães locais foram testados para IgG ant -*Leishmania* por imunoensaios (triagem com teste rápido TR DPP™ e conf rmatório com Elisa Indireto, Bio-Manguinhos/Fiocruz) e uma invest gação entomológica foi conduzida para est mar e ident f car a fauna f ebotomínea da região. **Resultados:** sete de 106 amostras (6,6%) foram posit vas usando o teste rápido. As amostras posit vas foram encaminhadas ao Laboratório Central da Bahia para a conf rmação por ELISA indireto. Entretanto, essas amostras apresentaram resultado negat vo. Foram encontrados nove exemplares da espécie *Pressat a chot ,* subfamília Phlebotominae, espécie frequentemente encontrada em áreas de transmissão de leishmaniose cutânea no Brasil. **Conclusão:** apesar deste trabalho não ter conf rmado a presença de LVC na Ilha de Maré, novos inquéritos sorológicos e analises entomológicas em uma maior área são necessários para a manutenção de uma vigilância epidemiológica na região insular em destaque.

Palavras-chave: *Leishmania. Pressat a chot .* Flebotomíneos.

INTRODUCTION

Leishmaniasis are a group of zoonotic parasitic diseases, present in many countries and known in two clinical forms: visceral and cutaneous, depending on the Leishmania species involved in the infect on (ALVAR et al., 2012; CONTI et al., 2016; THAKUR et al., 2018). The World Health Organizat on include these diseases among the main zoonosis of the present t me, due to its high morbidity and mortality registered in humans(WHO, 2017).

Domest c dog is considered the main urban reservoir of visceral leishmaniasis (VL), which is caused in the Americas by Leishmania infantum, due to its high suscept bility to the parasite and intense cutaneous parasit sm (SILVA et

Correspondente/Corresponding: *Adriano Figueiredo Monte Alegre – Inst tuto de Ciências da Saúde, Universidade Federal da Bahia – End: Av. Miguel Calmon, s.n. Valo do Canela Salvador-BA CEP: 40110.100 – Tel: (71) 99913-7189 – E-mail: adriano.monte@uf a.br

Longitudinal profiling of the vaccination coverage in Brazil reveals a recent change in the patterns hallmarked by differential reduction across regions.

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Longitudinal profiling of the vaccination coverage in Brazil reveals a recent change in the patterns hallmarked by differential reduction across regions

Náthaly Césare[a,1], Tiago F. Mota[b,1], Fernanda F.L. Lopes[a], Ana Claudia M. Lima[a], Ricardo Luzardo[a], Luiz Fernando Quintanilha[a], Bruno B. Andrade[a,b,c,d,e,2,*], Artur T.L. Queiroz[b,e,2], Kiyoshi F. Fukutani[a,b,e,2]

[a] Curso de Medicina, Faculdade de Tecnologia e Ciências (UniFTC), Salvador, Bahia, Brazil
[b] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil
[c] Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Bahia, Brazil
[d] Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Bahia, Brazil
[e] Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER), Salvador, Bahia, Brazil

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ABSTRACT

Objective: Vaccination coverage is decreasing worldwide, favoring the potential reemergence of vaccine-preventable diseases. In this study, we performed a longitudinal characterization of vaccination coverage in Brazil and compared the profiles between the distinct regions in the country to test whether there has been a substantial change over the last 5 years.
Methods: De-identified publicly available data were retrieved from the repository of the Brazilian Ministry of Health, comprising detailed information on vaccination coverage in all age groups between 1994 and 2019. The vaccination coverage for the whole country and for each Brazilian region, by year, was examined, and a time-series pattern analysis was performed.
Results: A significant decrease in overall vaccination coverage across the country regions was observed between 2017 and 2019, especially in childhood immunization. A reduction in BCG, hepatitis B, influenza, and rotavirus vaccine coverage was observed. Conversely, vaccines against measles, mumps, rubella, varicella, and meningococcus showed an increase in coverage. Region-specific changes in vaccination patterns within the study period were observed.
Conclusions: A substantial reduction in vaccination coverage was detected in Brazil, a country already highly susceptible to the emergence of epidemic infectious diseases. Continuing evaluation of the immunization program actions may help to improve vaccination coverage and prevent new epidemics.

Introduction

Over recent decades, humanity has seen the control or eradication of several diseases due to the development of vaccines (Hotez et al., 2020). Some generations have never experienced an epidemic situation of certain diseases such as measles and polio (Greenlee and Newton, 2018). Recently, however, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been affecting countries worldwide, and the current absence of an effective vaccination against the virus has had a devastating impact on public health, with millions of casualties (Luan et al., 2020; Velavan and Meyer, 2020), reinforcing the importance of vaccines. Unfortunately, vaccination coverage has been decreasing worldwide. This reduction could lead to the potential reemergence of vaccine-preventable diseases (VPDs) (Siani, 2019).

The Brazilian National Immunization Program (Programa Nacional de Imunizações, PNI) and the Unified Health System (Sistema Único de Saúde, SUS) have been providing free vaccines against several diseases to people of all age groups, reaching a national coverage higher than 90% (Sato, 2018). Nevertheless,

* Corresponding author at: Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, no. 121, Candeal, Salvador, Bahia 40296-710, Brazil.
E-mail address: Bruno.andrade@fiocruz.br (B.B. Andrade).
[1] Náthaly Césare and Tiago F. Mota contributed equally to this work.
[2] Bruno B. Andrade, Artur T.L. Queiroz, and Kiyoshi F. Fukutani contributed equally to this work.

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Effects of larval rearing substrates on some life-table parameters of *Lutzomyia longipalpis* sand flies.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Effects of larval rearing substrates on some life-table parameters of *Lutzomyia longipalpis* sand flies

Kelsilandia Aguiar Martins[1¤]*, Maria Helena de Athayde Meirelles[1,2], Tiago Feitosa Mota[1], Ibrahim Abbasi[3], Artur Trancoso Lopo de Queiroz[1], Claudia Ida Brodskyn[1,2], Patrícia Sampaio Tavares Veras[1], Deborah Bittencourt Mothé Fraga[1,2‡], Alon Warburg[3‡]

1 Instituto Gonçalo Moniz-Fundação Oswaldo Cruz, Salvador, Brazil, **2** Escola de Medicina Veterinária e Zootecnia-Universidade Federal da Bahia, Salvador, Brazil, **3** Kuvin Center for the Study of Infectious & Tropical Diseases, Department of Microbiology and Molecular Genetics, Institute of Medical Research, Israel-Canada, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

¤ Current address: The Royal Veterinary College, University of London, Hawkshead Lane, London, United Kingdom
‡ These authors contributed equally as senior authors.
* kaguiarmartins@rvc.ac.uk

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Abstract

Sand flies are the insects responsible for transmitting *Leishmania* parasites, the causative agents of leishmaniasis in humans. However, the effects of sand fly breeding sites on their biology and ecology remain poorly understood. Herein, we studied how larval nutrition associated with putative breeding sites of the sand fly *Lutzomyia longipalpis* affects their oviposition, development, microbiome, and susceptibility to *Leishmania* by rearing *L. longipalpis* on substrates collected from an endemic area for leishmaniasis in Brazil. The results showed that female *L. longipalpis* select the oviposition site based on its potential to promote larval maturation and while composting cashew leaf litter hindered the development, larvae reared on chicken feces developed rapidly. Typical gut microbial profiles were found in larvae reared upon cashew leaf litter. Adult females from larvae reared on substrate collected in chicken coops were infected with *Leishmania infantum*, indicating that they were highly susceptible to the parasite. In conclusion, the larval breeding sites can exert an important role in the epidemiology of leishmaniasis.

Author summary

Sand flies are the insect vectors involved in the transmission of many pathogens, however, the transmission of parasites to humans leading to visceral leishmaniasis is currently the most critical threat caused by this insect. Despite the importance of the vector, many aspects of the biology of sand flies are poorly understood, especially their breeding sites. This study was designed to evaluate the oviposition, life span, microbiome, and parasite infections in the main species of sand fly responsible for visceral leishmaniasis in America. Insects were reared on substrates collected from different putative habitats of sand flies in

Immune response dynamics and *Lutzomyia longipalpis* exposure characterize a biosignature of visceral leishmaniasis susceptibility in a canine cohort.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Immune response dynamics and *Lutzomyia longipalpis* exposure characterize a biosignature of visceral leishmaniasis susceptibility in a canine cohort

Manuela da Silva Solcà[1,2☯], Maiara Reis Arruda[1☯], Bruna Martins Macedo Leite[1], Tiago Feitosa Mota[1], Miriam Flores Rebouças[1], Matheus Silva de Jesus[1], Leila Denise Alves Ferreira Amorim[3], Valéria Matos Borges[1], Jesus Valenzuela[4], Shaden Kamhawi[4], Patrícia Sampaio Tavares Veras[1,5], Deborah Bittencourt Mothé Fraga[1,2,5‡], Claudia Ida Brodskyn[1,6‡*]

1 Instituto Gonçalo Moniz—Fundação Oswaldo Cruz, Bahia, Brazil, **2** Escola de Medicina Veterinária e Zootecnia—Universidade Federal da Bahia, Bahia, Brazil, **3** Instituto de Matemática e Estatística—Universidade Federal da Bahia, Bahia, Brazil, **4** Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, United States of America, **5** Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais / INCT-DT, Bahia, Brazil, **6** Instituto Nacional de Ciência e Tecnologia-Investigação em Imunologia /INCT-III, São Paulo, Brazil

☯ These authors contributed equally to this work.
‡ DBMF and CIB also contributed equally to this work.
* claudia.brodskyn@fiocruz.br, cbrodskyn2009@gmail.com

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Abstract

Background

Reports have shown correlations between the immune response to vector saliva and Leishmaniasis outcome. We followed dogs in an endemic area for two years characterizing resistance or susceptibility to canine visceral leishmaniasis (CVL) according to *Leishmania infantum* diagnosis and clinical development criteria. Then, we aimed to identify a biosignature based on parasite load, serum biological mediators' interactions, and vector exposure intensity associated with CVL resistance and susceptibility.

Methodology/Principal findings

A prospective two-year study was conducted in an area endemic for CVL. Dogs were evaluated at 6-month intervals to determine infection, clinical manifestations, immune profile, and sandfly exposure. CVL resistance or susceptibility was determined upon the conclusion of the study. After two years, 78% of the dogs were infected with *L. infantum* (53% susceptible and 47% resistant to CVL). Susceptible dogs presented higher splenic parasite load as well as persistence of the parasite during the follow-up, compared to resistant ones. Susceptible dogs also displayed a higher number of correlations among the investigated biological mediators, before and after infection diagnosis. At baseline, anti-saliva antibodies, indicative of exposure to the vector, were detected in 62% of the dogs, reaching 100% in one year. Higher sandfly exposure increased the risk of susceptibility to CVL by 1.6 times (CI: 1.11–

Identification of Bioactive Compounds against Aedes aegypti (Diptera: Culicidae) by Bioassays and in Silico Assays.

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FULL PAPER

CHEMISTRY & BIODIVERSITY

Identification of Bioactive Compounds against *Aedes aegypti* (Diptera: Culicidae) by Bioassays and *in Silico* Assays

Geisiane Amorim de Brito,[a] Paulo Fernando Rocha de Oliveira,[a] Caliene Melo de Andrade Silva,[a] Moysés Fagundes de Araújo Neto,[b] Franco Henrique Andrade Leite,[b] Paulo Roberto Ribeiro Mesquita,[c, d] Tiago Feitosa Mota,[e] and Jairo Torres Magalhães-Junior*[a]

[a] Centro Multidisciplinar de Barra, Universidade Federal do Oeste da Bahia, Bahia, Brasil, e-mail: jairo.magalhaes@ufob.edu.br

[b] Departamento de Saúde, Laboratório de Modelagem Molecular, Universidade Estadual de Feira de Santana, Bahia, Brasil

[c] Centro Tecnológico Agropecuário do Estado da Bahia (CETAB), Bahia, Brasil

[d] Faculdade Maria Milza, Bahia, Brasil

[e] Doutorando em Biotecnologia em Saúde e Medicina Investigativa, Instituto Gonçalo Moniz – FIOCRUZ, Bahia, Brasil

Most of the hematophagous insects act as disease vectors, including *Aedes aegypti*, responsible for transmitting some of the most critical arboviruses globally, such as Dengue. The use of repellents based on natural products is a promising alternative for personal protection compared to industrial chemical repellents. In this study, the repellent effect of essential oils extracted from *Lippia thymoides*, *Lippia alba*, *Cymbopogon winterianus*, *and Eucalyptus globulus* leaves was evaluated. Essential oils used showed repellent activity against *Ae. aegypti* in laboratory bioassays, obtaining protection rates above 70% from 3.75 mg/mL and higher concentration for all analyzed oils. GC/MS identified 57 constituents, which were used in the ligand-based pharmacophore model to expose compounds with requirements for repellents that modulate mosquitoes behavior through odorant-binding protein 1 *Ae. aegypti.* Ligand-based pharmacophore model approach results suggested that repellent activity from *C. winterianus*, *L. alba*, and *L. thymoides* essential oils' metabolites is related to Citronelal (QFIT = 26.77), Citronelol (QFIT = 11.29), Citronelol acetate (QFIT = 52.22) and Geranil acetate (QFIT = 10.28) with synergistic or individual activity. *E. globulus* essential oil's repellent activity is associated with Ledol (0.94%; QFIT = 41.95). Molecular docking was applied to understand the binding mode and affinity of the essential oils' data set at the protein binding site. According to molecular docking, Citronelol (ChemPLP = 60.98) and geranyl acetate (ChemPLP = 60.55) were the best-classified compounds compared to the others and they can be explored to develop new repellents.

Keywords: mosquito, essential oils, pharmacophore model, bioactive compounds, molecular docking.

Introduction

Aedes aegypti is a hematophagous arthropod of great epidemiological importance. This vector is responsible for arboviruses transmission such as urban yellow fever, Dengue, Chikungunya, and Zika virus, being distributed mainly in tropical and subtropical regions of the globe.[1]

Despite the epidemiological impact of arboviruses transmitted by *Ae. aegypti* in terms of public health, the control of *Ae. aegypti* has been a significant challenge since the indiscriminate use of chemical agents has favored the growth of resistant insect populations and negatively impacted the environment, considering its toxicity.[2] Aspects related to infrastructure problems in cities, especially in develop-

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Multiplex qPCR assay to determine *Leishmania infantum* load in *Lutzomyia longipalpis* sandfly samples.

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Multiplex qPCR assay to determine Leishmania infantum load in Lutzomyia longipalpis sandfly samples

Tiago Feitosa Mota[1] | Claudia Ida Brodskyn[1] | Luis Gustavo Morello[2,3] | Fabricio Klerynton Marchini[2,3] | Marco Aurelio Krieger[2] | Rita de Cássia Pontello Rampazzo[3] | Deborah Bittencourt Mothé Fraga[1,4]

[1]Laboratorio de Interaçao Parasito-Hospedeiro e Epidemiologia (LaIPHE), Instituto Gonçalo Moniz (IGM), FIOCRUZ-BA, Rua Waldemar Falcao, Salvador, Brazil

[2]Instituto Carlos Chagas (ICC), FIOCRUZ-PR, Rua Prof. Algacyr Munhoz Mader, Curitiba, Brazil

[3]Instituto de Biologia Molecular do Paraná (IBMP), Rua Professor Algacyr Munhoz Mader, Curitiba, Brazil

[4]Departamento de Medicina Veterinária Preventiva e Produçao Animal, Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia (UFBA), Salvador, Brazil

Correspondence
Deborah Bittencourt Mothé Fraga, Laboratorio de Interaçao Parasito-Hospedeiro e Epidemiologia (LaIPHE), Instituto Gonçalo Moniz (IGM), FIOCRUZ-BA, Rua Waldemar Falcao, 121 Candeal, 40296-710, Salvador, Bahia, Brazil.
Email: deborah.fraga@fiocruz.br

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Abstract

The study aimed to develop a multiplex qPCR to detect Leishmania infantum load in different sandfly sample settings using Leishmania kDNA and sandfly vacuolar ATPase (VATP) subunit C as internal control gene. The amplification of Lutzomyia longipalpis VATP gene was evaluated together with Leishmania infantum kDNA in a multiplex reaction. The concentration of VATP gene oligonucleotides was adjusted until no statistically significant difference was observed between all multiplex standard curves and singleplex curves, that is, only kDNA amplification. Limit of detection (LoD) was measured using a probit model and a cut-off defined by receiver operating characteristic analysis. Limit of quantification (LoQ) was assessed by a linear model using the coefficient of variation threshold of 25%. After assuring VATP gene amplification, its primer–probe concentrations were best at 100 nM/10 nM, respectively. The cut-off C_q value for L. infantum kDNA was defined as 35.46 with 100% of sensitivity and specificity. A total of 95% LoD was determined to be of 0.162 parasites while LoQ was 5.858. Our VATP/kDNA multiplex qPCR assay shows that it can be used to evaluate both DNA integrity and determine L. infantum load in L. longipalpis even for low yielded samples, that is, individual midguts.

KEYWORDS
insect control gene, multiplex qPCR, phlebotomine, visceral leishmaniasis

INTRODUCTION

Sandflies are responsible for the transmission of Leishmania in the Americas, where the main vector species responsible to transmit the etiologic agent for visceral leishmaniasis (VL), Leishmania infantum, is Lutzomyia longipalpis. Despite recent VL cases in Santa Catarina State, L. longipalpis was not found, thus far (Borges et al., 2017). Other sandfly species such as Nyssomyia neivai have been collected in this state and have been detected with L. infantum DNA when evaluated by molecular techniques (Saraiva et al., 2009; Grott et al., 2015). In other states in Brazil, other sandfly species such as L. cruzi, Pintomyia fischeri or Migonemyia migonei have been proved or suspected to be susceptible to L. infantum infection (Guimaraes et al., 2016; Falcao de Oliveira et al., 2017; Galvis-Ovallos et al., 2021). This entomological survey together with L. infantum DNA detection is a crucial information for the development of control strategies and detection of new vector species candidates.

Screening organic repellent compounds against *Lutzomyia longipalpis* (Diptera: Psychodidae) present in plant essential oils: Bioassay plus an in-silico approach.

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Screening organic repellent compounds against *Lutzomyia longipalpis* (Diptera: Psychodidae) present in plant essential oils: Bioassay plus an in silico approach

Tiago F. Mota[a,*], Caliene M. de A. Silva[c], Maurício dos S. Conceição[c], Deborah B.M. Fraga[a,b], Claudia I. Brodskyn[a], Moysés F. de A. Neto[d], Isis B. Santana[d], Paulo R.R. Mesquita[e,f], Franco H. A. Leite[d], Jairo T. Magalhães-Júnior[c]

[a] Laboratório de Interação Parasito-Hospedeiro e Epidemiologia (LaIPHE), Instituto Gonçalo Moniz (IGM), FIOCRUZ-BA, Rua Waldemar Falcão, 121 Candeal, 40296-710, Salvador, Bahia, Brazil
[b] Departamento de Medicina Veterinária Preventiva e Produção Animal, Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia (UFBA), Av. Adhemar de Barros, 500 Ondina, 40170-110, Salvador, Bahia, Brazil
[c] Centro Multidisciplinar do Campus de Barra da Universidade Federal do Oeste da Bahia, Bahia, Brasil
[d] Laboratório de Modelagem Molecular, Departamento de Saúde, Universidade Estadual de Feira de Santana, Bahia, Brasil
[e] Centro Tecnológico Agropecuário do Estado da Bahia (CETAB), Bahia, Brazil
[f] Faculdade Maria Milza, Bahia, Brazil

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ABSTRACT

In the Americas, *Lutzomyia longipalpis* is the most relevant sand f y species for the transmission of visceral leishmaniasis. For its vector control in Brazil, insecticide spraying has not shown persistent reduction in disease prevalence while some sand f y populations are reported resistant to the insecticides used in spraying. The usage of repellents and personal protection behavior can reduce vector borne diseases prevalence. Therefore, the search for new repellent compounds is needed to use together with insecticide spraying, especially from natural sources to overcome the resistance developed by some sand f y populations to the compounds commercially used. In silico strategies have been applied together with repellency bioassays successfully identifying new bioactive compounds from natural sources. Thus, the present study aimed to screen repellent potential of neem (*Azadirachta indica*), citronella (*Cymbopogon winterianus*), bushy matgrass (*Lippia alba*) and 'alecrim do mato' (*Lippia thymoides*) essential oils against L. *longipalpis* and to identify potential repellent compounds by chemical analysis and in silico approach. Plant essential oils were extracted from leaves and repellency bioassays were performed on volunteers using colony reared L. *longipalpis*. Aside from neem oil, all other tested essential oil has shown a reduced number of sand f y bites using higher concentrations. Chemical composition from oils was assessed and its compounds were screened on a pharmacophore model using odorant binding protein 1 (OBP1). All essential oils were majorly composed of either oxygenated monoterpenes, except for the oil extracted from neem which was composed of sesquiterpene hydrocarbons. Molecular docking was performed with the compounds that best superimposed in the OBP1 pharmacophore model, identifying those binding to OBP4, which is associated with insect repellency behavior. Citronellol, Citronellol acetate, Citronellal and Geranyl acetate showed similar interactions with OBP4 binding site as DEET. Thus, it is suggested that these compounds are able to bind to L. *longipalpis* OBP4 generating repellent behavior in sand f ies.

1. Introduction

Sand f ies are responsible for the transmission of protozoan of the species *Leishmania infantum*, etiologic agent of human and canine visceral leishmaniasis (VL) (McCall et al., 2013). In Brazil the lack of effectiveness in VL control is attributed to late human case diagnosis, dog culling delay after a positive diagnosis and low residual insecticide spray eff cacy on the most relevant sand f y species, *Lutzomyia longipalpis*

* Corresponding author.
E-mail address: tiagofmotavet@gmail.com (T.F. Mota).

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