UNIVERSIDADE DE BRASÍLIA
FACULDADE DE CEILÂNDIA
CURSO DE FARMÁCIA

LAÍS MANUELA BORGES RIBEIRO

PEPTÍDEOS ANTIMICROBIANOS DE ARTRÓPODES HEMATÓFAGOS

BRASÍLIA, 2021

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Monografia de Conclusão de Curso apresentada como requisito parcial para obtenção do grau de Farmacêutico, Faculdade de Ceilândia, Universidade de Brasília.

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Ficha catalográfica elaborada automaticamente,
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Bp

Borges Ribeiro, Lais Manuela
Peptídeos Antimicrobianos de Artrópodes Hematófagos / Lais Manuela Borges Ribeiro; orientador Carla Nunes de Araujo; co-orientador Paula Beatriz Santiago. -- Brasília, 2021.
56 p.

Monografia (Graduação - Farmácia - FCE) -- Universidade de Brasília, 2021.

1. Vetores de doenças. 2. Saliva. 3. Agentes antimicrobianos. 4. Cecropinas. 5. Defensinas. I. Nunes de Araujo, Carla , orient. II. Beatriz Santiago, Paula, co orient. III. Título.

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AGRADECIMENTOS

Agradeço à professora Carla Nunes de Araújo, orientadora deste trabalho, pessoa de personalidade afetuosa e que me deu a oportunidade de participar do Programa de Iniciação Científica da Universidade de Brasília.

À Paula Beatriz Santiago, pesquisadora do Programa de Pós-Graduação em Biologia Molecular e do Laboratório de Interação Patógeno-Hospedeiro (IB-UnB), co-orientadora deste trabalho, por todo o tempo dedicado e pela gentileza com que me recebeu rotineiramente no setor;

Aos professores Jaime Santana, Izabela Bastos, Flavia Motta do (LIPH), que proporcionaram a chance de vivenciar de forma prática o mundo científico na graduação;

Ao Kaio Luís da Silva Bentes, que com paciência e simpatia me auxiliou na realização dos meus primeiros experimentos no laboratório, e que me possibilitou compreender passo a passo do seu trabalho e dos demais com suas explicações;

Aos demais colegas do LIPH pela boa recepção e pelos ensinamentos disseminados ao longo dos diversos seminários apresentados;

Aos docentes do Colegiado de Bases Biológicas e da Saúde e do curso de Farmácia da Universidade de Brasília-FCE por compartilharem seus conhecimentos e experiências com tanta dedicação;

Ao Programa de Iniciação Científica da Universidade de Brasília;

À Fundação de Apoio à Pesquisa do Distrito Federal.

Agradeço ao meu pai, Wederson Soares Ribeiro, por sempre me estimular na busca do conhecimento, à minha mãe, Djane Borges Arcanjo, futura colega da profissão farmacêutica, pelo cuidado e pela motivação em trilhar os melhores caminhos;

À minha irmã Lívia Borges Ribeiro, por muitas vezes ter sido minha ouvinte enquanto eu estudava para os exames da faculdade;

Agradeço a Deus pela oportunidade de estar encerrando mais um ciclo.

BRASÍLIA, 2021

RESUMO:

Os peptídeos antimicrobianos (PAMs) são uma classe diversa de moléculas majoritariamente catiônicas de pequeno peso molecular presentes em organismos não metazoários e metazoários, incluindo artrópodes. Os PAMs atuam em mecanismos imunomoduladores para proteger os artrópodes hematófagos da infecção por microrganismos patogênicos que podem ser adquiridos durante sua alimentação de sangue. Foi realizada uma revisão da literatura para identificar os estudos de PAMs de artrópodes hematófagos. Três bases de dados eletrônicas (MEDLINE, SCIELO e BVS) foram sistematicamente pesquisadas sem restrição de ano de publicação. Apenas trabalhos experimentais foram incluídos. Os títulos e resumos dos estudos foram selecionados dentro dos critérios de elegibilidade e os artigos relevantes foram lidos na íntegra e incluídos na revisão. Trinta e cinco artigos foram para análise final. Os artigos incluídos foram publicados entre 1995 e 2019. Os primeiros estudos sobre PAMs de artrópodes hematófagos foram realizados em mosquitos. Os impactos das revistas variaram de 0,855 a 10,557. Os estudos foram realizados na América do Norte, América do Sul, Europa e Ásia. Defensina foi o principal PAM relatado em carrapatos (12 de 13 artigos; 92,31%), triatomíneos (100%) e mosquitos (100%), seguido por cecropina. O artigo exclusivo sobre PAMs de pulgas não relatou defensina. Os dados sobre PAMs expressos por artrópodes hematófagos ainda são escassos, mas estudos sugerem que eles podem ser promissores no desenvolvimento de medicamentos antimicrobianos e no controle de doenças transmitidas por vetores. Adicionalmente, os PAMs podem servir como marcadores biológicos comparativos, e portanto, ser utilizados para inferir as relações evolutivas entre diferentes espécies.

Palavras-chave: Vetores de doenças, Saliva, Agentes antimicrobianos, Cecropinas, Defensinas.

BRASÍLIA, 2021

ABSTRACT

Antimicrobial peptides (AMP) are a diverse class of mostly cationic molecules of small molecular weight present in non-metazoan and metazoan organisms, including arthropods. AMPs act in immunomodulatory mechanisms to protect hematophagous arthropods from infection by pathogenic microorganisms that can be acquired during their blood-feeding. Here, we reviewed the scientific literature on studies of AMPs from hematophagous arthropods. A literature review was conducted to identify all studies of AMP from hematophagous arthropods. Three electronic databases (MEDLINE, SCIELO and BVS) were systematically searched without restriction on year of publication. Only experimental works were included. Studies' titles and abstracts were screened for eligibility and relevant articles were read in full and included in the review. 35 articles were selected for the final analysis. The included articles were published between 1995 and 2019. The first reports on AMPs from hematophagous arthropods were realized on mosquitoes. The journal impact was ranged from 0.855 to 10.557. Studies were performed in North America, South America, Europe and Asia. Defensin was the main AMP reported in ticks (12 from 13 articles; 92.31%) triatomines (100%) and mosquitoes (100%), followed by cecropin. The single article on AMPs from fleas did not report defensin. Data on AMPs expressed by hematophagous arthropods are still scarce, but studies suggest they may be promising in antimicrobial medicine development and vector-borne diseases control. Additionally, AMPs may serve as comparative biological markers, and therefore, be used to infer the evolutionary relationships among different species.

Keywords: Disease vectors, Saliva, Antimicrobial agents, Cecropins, Defensins.

LISTA DE SIGLAS

OMS – Organização Mundial de Saúde

PAMs – Peptídeos antimicrobianos

MP – Membrana peritrófica

SJ- Junções septadas

ProPO - Profenoloxidase

MEDLINE - Medical Literature Analysis and Retrieval System Online

SCIELO - Scientific Electronic Library Online

BVS - Biblioteca Virtual em Saúde

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1. INTRODUÇÃO

Os artrópodes vetores de doenças pertencem às ordens Diptera, Hemiptera, Ixodida e Siphonaptera (RODHAIN, 2015; MARCONDES, 2016). Esses organismos são capazes de transmitir agentes etiológicos de muitas doenças infecciosas, tais como: tifo, febre recorrente transmitida por piolhos, chikungunya, dengue, filariose linfática, febre do Vale do Rift, febre amarela, zika, malária, encefalite japonesa, febre do Nilo Ocidental, oncocercose, leishmaniose, febre do mosquito-pólvora, doença do sono, doença de Chagas, febre hemorrágica da Crimeia-Congo, borreliose, rickettsioses, encefalite transmitida por carrapatos, tularemia, peste e tungíase (WHO, 2020).

Dentre as doenças citadas anteriormente, pertencem ao grupo de doenças negligenciadas definidas pela Organização Mundial da Saúde (OMS): dengue, doença de Chagas (tripanossomíase americana), doença do sono (tripanossomíase africana), leishmaniose, filariose linfática, oncocercose (cegueira dos rios) (DIAS et al., 2013). Esse grupo é caracterizado por doenças que possuem alta prevalência em espaços geográficos nos quais as condições sanitárias, de alimentação e de moradia são precárias, além de uma acentuada dificuldade no acesso ao sistema de saúde por parte da população (SILVEIRA VASCONCELOS et al., 2016). A negligência na prevenção e no tratamento de tais doenças se deve à falta de investimentos em pesquisas por instituições financeiras internacionais, ou mesmo da indústria farmacêutica nos locais em que o perfil de morbimortalidade de tais doenças é relevante e que oferecem um baixo nicho econômico rentável para esses mercados: notoriamente em países da África, Ásia e América Latina (SILVEIRA VASCONCELOS et al., 2016). Portanto, fica evidenciada a importância de pesquisas relacionadas aos vetores dessas doenças para melhor entender sua biologia e sua relação com agentes etiológicos, facilitando o desenvolvimento de estratégias no planejamento de políticas públicas de saúde efetivas que ajudem a diminuir os altos índices de morbidade ocasionados por estas doenças negligenciadas.

Sabe-se que o sistema de defesa dos artrópodes é muito eficiente. Tem a capacidade de enfrentar microrganismos patogênicos potenciais como bactérias, vírus, fungos, além de protozoários e helmintos, o que permite que esses organismos ocupem com sucesso quase todos os nichos ecológicos da Terra (LEMAITRE & HOFFMANN, 2007). Esses vetores não possuem imunidade adquirida. Apresentam

um sistema imunológico composto por barreiras anatômicas e fisiológicas hostis aos patógenos. Quando há o rompimento dessas barreiras, o patógeno entra na hemocele ativando mecanismos celulares e humorais inatos. Seus processos imunológicos envolvem fagocitose de patógenos e formação de cápsulas pelos hemócitos, reparo de feridas, melanização e síntese de peptídeos antimicrobianos (PAMs) (C. N. DE ARAÚJO et al., 2012).

Os PAMs são os principais efetores da resposta imune humoral dos artrópodes. A maioria dos PAMs são peptídeos catiônicos anfipáticos de pequeno peso molecular. A presença de regiões hidrofílicas e hidrofóbicas, e também a sua carga elétrica, determinam suas propriedades antimicrobianas, pois facilitam a interação com as membranas fosfolipídicas microbianas de caráter elétrico negativo (SINHA & SHUKLA, 2018). Os PAMs são classificados nas estruturas folhas β, α-hélice e peptídeos com estrutura estendida (LEI et al., 2019). Apresentam ainda classificações funcionais, dividindo-se em peptídeos antivirais, antibacterianos, antifúngicos e antiparasitários (SINHA & SHUKLA, 2018).

Os PAMs de insetos mais explorados são as defensinas, cecropinas, drosocinas, attacinas, diptericinas, ponericinas, drosomicina e metchnikowin. No entanto, com o avançar de novas pesquisas relacionadas ao tema, mais PAMs podem ainda serem descobertos (WU et al., 2018).

As pesquisas relacionadas aos PAMs de artrópodes nos ajudam a compreender a evolução e a fisiopatologia desses animais, bem como a possível utilização dessas moléculas como medicamentos contra infecções de microrganismos em humanos e animais. As defensinas de insetos são PAMs que apresentam atividade contra bactérias Gram-positivas e Gram-negativas, principalmente contra bactérias Gram-positivas, como *Staphylococcus aureus* (WU et al., 2018). Em um experimento *in vivo*, animais infectados com protozoários do gênero *Babesia microti* demonstraram uma queda no grau de parasitemia quando expostos ao peptídeo parasiticida longicina, PAM encontrado em carrapatos da espécie *Haemaphysalis longicornis*. Foi observada uma redução de 60% da colonização de parasitas em ratos com parasitemia de 15% utilizando longicina na dose de 1,25 mg/kg, enquanto uma redução de 72% foi observada em ratos colonizados por *Babesia microti* (5% de parasitemia) utilizando-se a dose 3,0 mg/kg de longicina (TSUJI et al., 2007).

Dentre os mecanismos de resistência aos PAMs incluem-se: degradação proteolítica ou sequestro por proteínas secretadas, impedimento por exopolímeros e

moléculas de biofilme produzidos pelas bactérias, diminuição da atração da superfície celular/alteração da membrana e exportação por bombas de efluxo. As proteases são os primeiros mecanismos de defesa bacteriana que os PAMs encontram ao interagir com esses microorganismos (MEJÍA-ARGUETA ET AL., 2020).

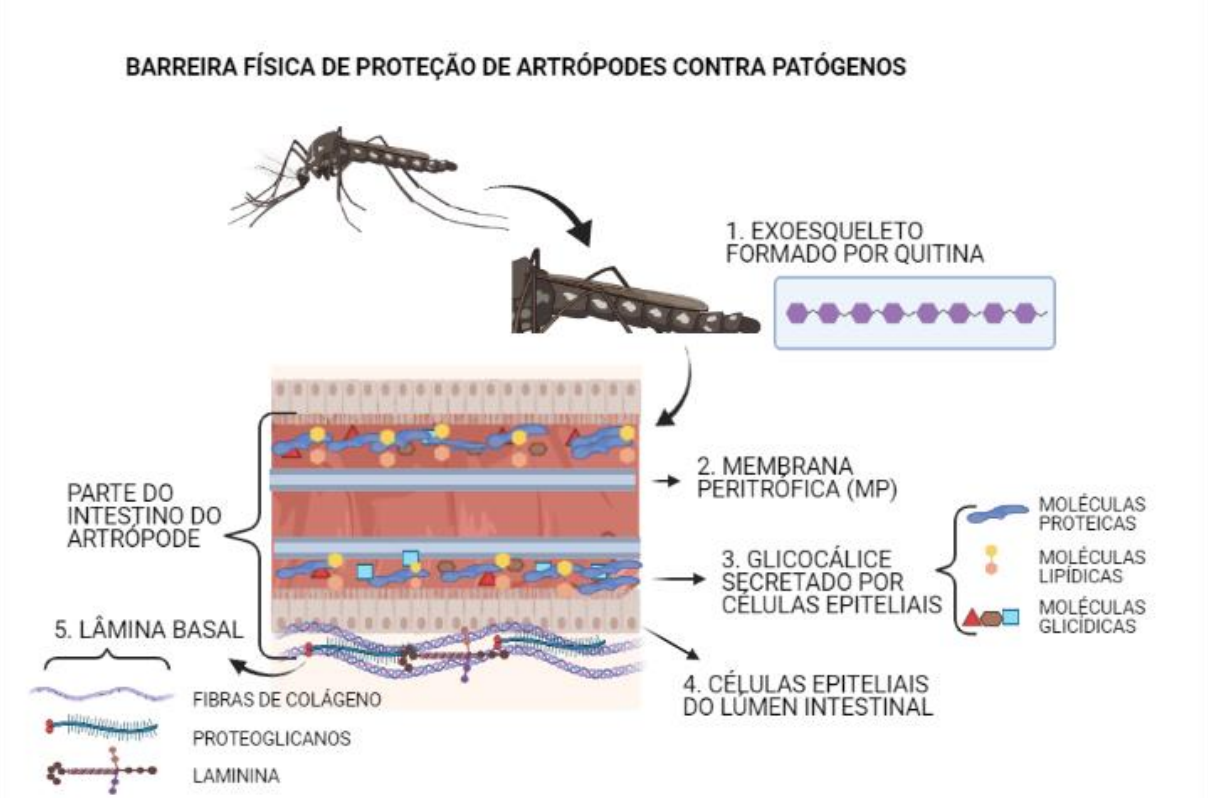
Os PAMs apresentam algumas limitações que dificultam seu desenvolvimento de sucesso para uso clínico, como por exemplo, a inibição da sua atividade em baixo pH e no ambiente pulmonar (MEJÍA-ARGUETA et al., 2020). Ainda sim, é esperado que com as características de estrutura tridimensional apresentadas pelos PAMs, sua tendência anfipática e seu caráter aniônico confiram a estas moléculas uma probabilidade muito menor de desenvolvimento de resistência bacteriana. Além do fato citado, sua intensa atividade espectral faz com que os PAMs se tornem potenciais candidatos para uso como agentes antimicrobianos (MEJÍA-ARGUETA et al., 2020).

2. REVISÃO BIBLIOGRÁFICA

2.1 Sistema de defesa de insetos

A primeira linha de defesa de um inseto é a defesa física (Figura 1), conferida pelo modo em que sua anatomia se organiza. Os insetos possuem um exoesqueleto formado de quitina, cuja cutícula é formada por lamelas duras e impermeáveis (BAXTER et al., 2017). A maior parte dos insetos também possui uma membrana quitinosa porosa não lamelar em forma de filme denominada membrana peritrófica. A membrana peritrófica é secretada pelo epitélio intestinal para separar fisicamente o alimento da camada de células do lúmen intestinal, protegendo o epitélio contra microrganismos e abrasão. Abaixo dessas barreiras estão as células epiteliais, que secretam proteínas e glicanos para formar uma matriz extracelular mucosa e uma camada de carboidrato conhecida como glicocálice para isolar a membrana plasmática do lúmen. As células epiteliais dos insetos formam junções conhecidas como junções septadas (SJ), a fim de criar uma barreira impermeável entre o exterior e a hemocele. Abaixo do epitélio, uma segunda barreira física - a lâmina basal - composta de colágeno, laminina e proteoglicanos separa o epitélio do fluido circulatório, ou hemolinfa (BAXTER et al., 2017).

Figura 1. Barreira física de proteção de artrópodes contra patógenos.



Fonte: Imagem criada pela autora através do site Biorender.com

Quando os microrganismos conseguem ultrapassar as barreiras físicas de defesa dos insetos, a resposta imune humoral e a celular são ativadas. Dentre as respostas geradas pela resposta imune humoral, destacam-se a produção de PAMs, ativação da profenoloxidase (proPO) e produção de espécies reativas de oxigênio. Dentre as respostas induzidas pelo sistema imune celular dos insetos, destacam-se os efeitos de nodulação, encapsulação e fagocitose (ROSALES, 2017).

Em um estímulo provocado por infecções microbianas, a produção de PAMs é induzida. A síntese ocorre principalmente no corpo gorduroso dos insetos, enquanto os hemócitos parecem ser sua segunda fonte de produção, liberando-os na hemolinfa após dano do tegumento ou invasão microbiana (YAKOVLEV et al., 2017). Nesta situação, a concentração dessas moléculas no sistema circulatório de insetos alcança concentrações micromolares na hemolinfa de insetos infectados (ROSALES, 2017).

2.2 Peptídeos antimicrobianos (PAMs)

Os PAMs são uma família de peptídeos que compõem o sistema imune mais primitivo de diversas espécies (TOMASINSIG et al., 2010). Esses peptídeos têm sido descritos em espécies de animais invertebrados, vertebrados e plantas (MEJÍA-ARGUETA et al., 2020). Ainda, os PAMs podem ser produzidos por microorganismos com a finalidade de limitar o crescimento de outros micróbios presentes na mesma área (ZHANG; GALLO, 2016). Eles são constituídos por 12 a 50 resíduos de aminoácidos, as diferentes composições destes compostos permitem dividí-los nos subgrupos: peptídeos ricos em cisteína (defensinas de insetos e drosomicina); peptídeos ricos em prolina (apidaecina, drosocina e lebocina); peptídeos ricos em glicina (atacina e gloverina), além de peptídeos alfa helicoidais (cecropina e moricina) (WU et al., 2018; YI et al., 2014). Originalmente, os PAMs de insetos foram identificados através da purificação de proteínas e peptídeos obtidos da hemolinfa de espécies imunologicamente ativas após contato com bactérias (YI et al., 2014).

O primeiro contato entre os PAMs e uma bactéria ocorre via interações eletrostáticas ou hidrofóbicas, que dependem diretamente da composição lipídica da membrana das bactérias (WU et al., 2018). Os mecanismos de ação dos PAMs são diversos, visto que essas moléculas podem ocasionar a ruptura da membrana de microrganismos, interferir com o metabolismo bacteriano e direcionar componentes citoplasmáticos à uma atividade danosa contra patógenos (WU et al., 2018).

2.3 Resistência bacteriana

A resistência bacteriana aos antibióticos é um fenômeno natural que vem sofrendo expansão devido ao uso irracional desses medicamentos. Sua ocorrência acarreta graves consequências clínicas e econômicas na sociedade, provocando um maior tempo de permanência dos indivíduos no ambiente hospitalar e o uso de antibióticos diferentes dos de primeira geração, que seriam administrados inicialmente (LOUREIRO et al., 2016). Estima-se que anualmente 700 mil mortes ocorram devido à resistência aos antimicrobianos (ESTRELA, 2018).

Dentre as formas de resistência bacteriana aos antibióticos, são 4 os principais mecanismos: modificação ou destruição enzimática da estrutura molecular do antibiótico; redução da permeabilidade celular ao antibiótico ou presença de bombas de efluxo de antibiótico nas células bacterianas; alterações do sítio de ligação dos antibióticos no microrganismo; e produção de sítios alvo alternativos de ligação, não

inibidas pelo antibiótico (LOUREIRO et al., 2016). Medicamentos antivirais, antiparasitários e antifúngicos também possuem certas características de resistência, assim como os antibióticos (ESTRELA, 2018).

Além disso, o baixo investimento em novas tecnologias de saúde por parte de laboratórios e empresas farmacêuticas ao enfrentamento de infecções ocasionadas por bactérias tem sido um dos empecilhos na redução dos índices de resistência antimicrobiana, já que para estas o investimento em pesquisas para desenvolvimento de novos fármacos não é considerado um bom investimento devido a uma baixa lucratividade na venda desses medicamentos (ESTRELA, 2018). Como exemplo do fato citado, a baixa de penicilina enfrentada pelo Brasil tem relação à falta de interesse na fabricação desse medicamento por indústrias do país, como resultado, entre outros fatores, doenças como a sífilis tiveram um reaparecimento, aumentando os registros de casos no Brasil nos últimos anos (ESTRELA, 2018).

3. OBJETIVOS

3.1 Objetivo geral

O objetivo geral deste trabalho foi realizar uma revisão sistemática dos dados literários sobre os PAMs dos artrópodes vetores de doenças infecciosas. Essa revisão sistemática concentrou-se em artrópodes hematófagos, a fim de listar os PAMs identificados nesses vetores e seus diferentes campos de aplicação, relacionando-os com a biologia do vetor e a doença transmitida pelo mesmo.

3.2 Objetivos específicos

- Identificar os PAMs de artrópodes hematófagos já descritos na literatura;
- Identificar para quais grupos de artrópodes hematófagos já foram descritos PAMs;
- Apresentar o estado da arte da pesquisa sobre os PAMs de artrópodes hematófagos em forma de revisão que possa servir como incentivo a pesquisadores e empresas do setor biotecnológico e farmacêutico a se aprofundarem no tema e avaliarem a utilização dessas moléculas promissoras em diversos campos da ciência, o que poderia trazer benefícios clínicos e epidemiológicos para a sociedade.

4. JUSTIFICATIVA

Os artrópodes hematófagos são vetores de várias doenças para os humanos. A maioria dessas doenças ocasiona problemas de saúde pública importantes, principalmente nos países em desenvolvimento. O conhecimento sobre as interações entre os vetores, os patógenos que os mesmos albergam e os hospedeiros vertebrados nos quais se alimentam é de suma importância para os campos da biologia e da saúde. Uma revisão sistemática sobre os PAMs apresentando o estado da arte da pesquisa sobre esses peptídeos traz informações importantes sobre a biologia dos vetores, mas também pode auxiliar na identificação de moléculas naturais farmacologicamente ativas e de seus possíveis alvos terapêuticos, podendo virem a ser empregadas na prática clínica. Há trabalhos que demonstraram a atividade dessas moléculas contra patógenos humanos e de animais.

Destacam-se entre estratégias potenciais para o uso dessas moléculas: 1) seu uso como agentes anti-infecciosos únicos, (2) agentes sinérgicos ao uso de antimicrobianos, (3) agentes imunomoduladores, (4) agentes neutralizantes de endotoxinas para prevenir complicações associadas à virulência de bactérias capazes de causarem choque séptico (GALLO et al., 2002).

Atualmente, os PAMs em teste para o desenvolvimento de medicamentos para utilização clínica têm sido originados a partir de humanos e animais vertebrados. Alguns desses medicamentos encontram-se em fase pré-clínica, em estudos de fases I/II ou em fase III. Ainda, poucos estudos baseados no mapeamento do desenvolvimento de resistência aos PAMs e a exploração de como esses mecanismos ocorrem foram realizados (MEJÍA-ARGUETA et al., 2020). Portanto, as investigações de peptídeos naturais são uma nova área de foco de pesquisa para o futuro (WU et al., 2018).

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6. MANUSCRITO

Antimicrobial peptides from hematophagous arthropods: A systematic review

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Abstract

Background: Antimicrobial peptides (AMP) are a diverse small molecular weight class of molecules (mostly cationic) present in non-metazoan and metazoan organisms, including arthropods. AMPs act in immunomodulatory mechanisms to protect hematophagous arthropods from infection by pathogenic microorganisms that can be acquired during their blood-feeding. Here, we reviewed the scientific literature on studies of AMPs from hematophagous arthropods.

Methods: A literature review was conducted to identify all studies of AMP from hematophagous arthropods. Three available electronic databases (MEDLINE, SCIELO and BVS) were systematically searched without restriction on year of publication. Only experimental works were included. Studies' titles and abstracts were screened for eligibility and relevant articles were read in full and included in the review.

Results: A total of 35 articles were selected for the final analysis. The included articles were published between 1995 and 2019. The first reports on hematophagous arthropods AMPs were realized on mosquitoes. The journal impact was ranged from 0.855 to 10.557. Studies were performed in North America, South America, Europe and Asia. Defensin was the main AMP reported in ticks (12 from 13 articles; 92.31%) triatomines (100%) and mosquitoes (100%), followed by cecropin. The single article on AMPs from fleas in our search did not report defensin.

Conclusions: Data on AMP expressed by hematophagous arthropods are still scarce, but studies suggest they may be promising in antimicrobial medicine development and vector-borne diseases control. Still, AMPs may serve as comparative biological markers among different species belonging to the same insect group, clarifying idea about evolutive mechanisms between these animals.

Keywords: Disease vectors, Saliva, Antimicrobial agents, Cecropins, Defensins

Background

Arthropods, the most diverse group of animals, occupy almost all ecological niches on earth [1]. These facts reveal their success in evolution. Hematophagous arthropods are present in the orders Diptera, Heteroptera, Siphonaptera and Ixodida [2, 3]. They can transmit several infectious diseases, collectively known as vector-borne diseases, such as typhus, louse-borne relapsing fever, chikungunya, dengue, lymphatic filariasis, Rift Valley fever, yellow fever, zika, malaria, Japanese encephalitis, West Nile fever, onchocerciasis, leishmaniasis, sand-fly fever, sleeping sickness, Chagas disease, Crimean-Congo hemorrhagic fever, Lyme disease, borreliosis, rickettsial diseases, tick-borne encephalitis, tularemia, plague, and tungiasis [4]. Vector-borne diseases cause more than 700,000 deaths annually [4].

While feeding on blood, hematophagous arthropods can become infected by potential pathogenic microorganisms such as bacteria, viruses, fungi, protozoan, and helminth parasites. Their innate immune defense system comprises physical and chemical barriers, and cells, that together allow cellular and humoral responses [5]. Cellular immunologic processes include phagocytosis, nodulation, capsule formation by hemocytes and antiviral response. Humoral responses include coagulation, wound repair, melanization and the synthesis of antimicrobial peptides (AMPs), for which hematophagous arthropods are a potential source [6, 7]. Hematophagous arthropods' ability to evade microbial infections is a result of their innate immune response including the production of several AMPs.

AMPs are mostly small sized cationic peptides [8]. Their major activity is to kill pathogens directly. This activity is initiated due to their electric charge that facilitates interaction with microbial membranes, negatively charged, followed by cell membrane disruption [9]. AMPs are classified into α-helical, β-sheet, and peptides with extended/random-coil structure based in their secondary structure. The most common categories of AMPs are the first two categories cited [10]. Anionic AMP has also been reported [8]. In general, these peptides exhibit broad spectrum of functions (antiviral, antibacterial, antifungal and antiparasitic activities) [8], which belong to two major subfamilies: Defensin, with approximately 4 kDa in size and usually characterized by disulfide bridges formed by 6 cysteine residues [11] and another subfamily of linear molecules, with no cysteine, such as cecropin, magainin, or rich in proline, glycine, arginine, histidine or tryptophan [12, 13].

Microorganisms drug resistance is a serious health problem worldwide and leads to the risk that several infections, mainly in hospitals, become untreatable. Need for new antimicrobial molecules is an urgent demand [14]. Natural arthropod AMPs could be used to develop potential new drugs against microorganism infections in humans. Differences between cytoplasmatic membrane of mammalian cells and bacteria, for instance, can provide AMP selectivity against microorganisms. The mammalian cell membrane characteristically presents a neutral charge, avoiding AMP attraction, and a high content of cholesterol, which confers stabilization to the phospholipid bilayer [10]. Moreover, AMP research help to understand the evolution and physiopathology of hematophagous arthropods. In this review, we synthesize the studies and provide a comprehensive overview of hematophagous arthropod AMPs.

Methods

Search Strategies and Selection Criteria

A systematic literature review was conducted by two independent authors to synthesize the studies' results related to AMPs in hematophagous vectors of diseases. The research was performed on 13 May 2020 by searching the following electronic databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Scientific Electronic Library Online (SCIELO) and Virtual Health Library (BVS, Biblioteca Virtual em Saúde). The research terms used were 'Arthropod Vectors' and 'Antimicrobial Cationic Peptides'. According to the search algorithm of each database, these keywords were detected in the title, abstract and/or text. There were no restrictions on language or on time of publication. Inclusion criteria were experimental scientific works involving the study of AMPs from hematophagous arthropods. Review articles, Master's and Doctoral theses were excluded, and duplicated studies were removed.

Based on inclusion/exclusion criteria, all abstracts were independently screened by each examiner to determine their eligibility. In case of doubt about the inclusion after reading the abstract, the article was completely read. Next, the included full-text articles were reviewed in duplicate. Disagreements were solved by discussion between the examiners to reach a consensus. The bibliographic reference lists of the selected studies were screened for potentially relevant articles.

Using the same algorithm, a second search was performed on 19 April 2021. Four new articles were found, although none of them was included in this review.

Data Extraction

The extracted data from each full-text article included in the systematic review were (when available): (i) publication characteristics (study title, PMID or DOI, year of publication, periodic name, journal impact factor, country where the study was performed); (ii) arthropod vector species and pathogens; and (iii) antimicrobial peptide families and comments. Data extraction process was performed by one member of the study who inserted the relevant data into a table summarizing all the aspects investigated.

Results

Initially, 80 studies were identified. After reading the title and abstract, 45 articles were rejected. The remaining 35 studies were completely read, and all studies met the inclusion criteria. In a second search, although 4 studies had been added to the result, none of them were included in this review after reading the title and abstract. A total of 35 articles were admitted in this systematic review. Articles (48) not included in this review had non-arthropod vectors and vertebrate animals as target of studies, or did not aim at studying PAMs. The study flow can be observed in Figure 1.

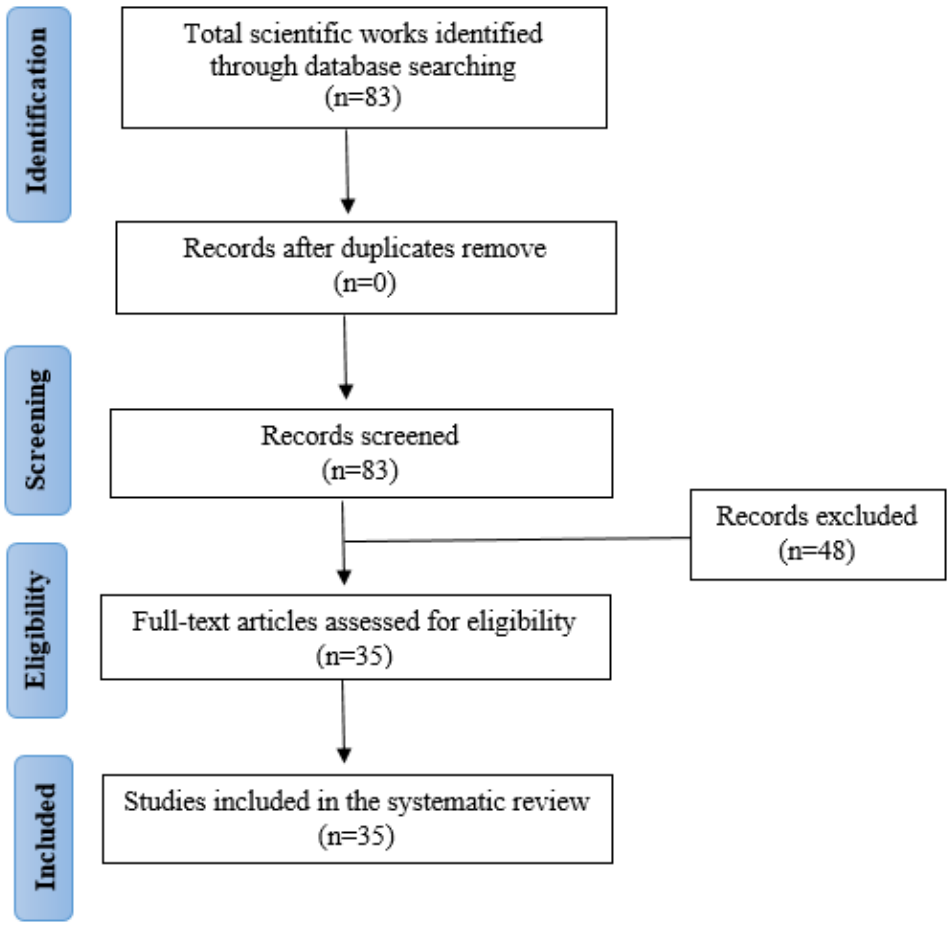

Figure 1. Flow diagram for selected studies.

The 35 articles assessed for eligibility were published between 1995 and 2019. The first reports on hematophagous arthropod AMPs concerned to mosquitoes. Ten (28%) articles were published in the last five years (2015 to 2019). The journal impact was ranged from 0.855 to 10.557. All publications were written in English. Studies were performed in the following continents: North America, South America, Europe and Asia, (Table 1).

Thirteen articles (37.14%) reported tick AMPs, 6 (17,14%) reported triatomine AMPs, 15 (42.85%) reported mosquitoes AMPs and 1 (2.85%) reported AMPs from fleas. To examine the overall panorama of AMPs in the studies, a column of AMP was included in Table 1. Defensin was the main AMP reported in ticks (12 from 13 articles; 92.31%) triatomines (100%) and mosquitoes (100%), followed by cecropin. However, the unique article on AMPs from fleas returned in our search did not report defensin,

but polymyxin B and cecropin. Other AMPs reported in the articles were ixosin, prolixicin and attacin (Table 1).

Discussion

Main Findings of the Research on AMPs from Hematophagous Arthropods

In Ticks

Ticks are obligatory blood-feeding ecto-parasitic arthropods. The maintenance of blood feeding in these arthropods is dependent on some bioactive molecules able to counteract host's hemostasis, to evade host immune surveillance and/or to suppress their immune response. Pharmacological effects of tick saliva and its components have been studied for almost half a century [15], AMPs are among them. Some of the relevant pathogens transmitted during tick feeding are *Borrelia* (spirochetes; borreliosis), *Anaplasma* (Gram-negative bacteria; rickettsial diseases) and *Babesia* (protozoan; babesiosis) [16]. Crimean-Congo hemorrhagic fever, Lyme disease, tick-borne encephalitis, and tularemia are among tick-borne diseases [4].

Defensins are major components of innate immunity in ticks [17], and were the main AMP reported on the studies analyzed. AMPs as ixosin are other components of ticks' innate immunity mentioned on the studies. Defensin may be stored in granulocytes of *Dermacentor variabilis* hemolymph and secreted when this arthropod seems to be upon bacterial insult [18]. *Ornithodoros moubata* showed an increased expression of defensin after *Escherichia coli* ingestion. By 24 days after infection, no *E. coli* were identified in the tick midgut [19]. *Haemaphysalis longicornis* produces a novel defensin-like parasiticidal peptide, named longicin, which seems to have evolved from a common ancestral peptide resembling spider and scorpion toxins. Its activity was shown through an *in vivo* experiment, where longicin induced significant reduction of parasitemia in animals infected with the zoonotic and murine *Babesia microti*. In a group of infected mice there was parasitemia reduction of 72% using a dose of 3.0 mg/kg of longicin. In a second group, mice treated with 1.25 mg/kg of longicin had showed a parasitemia reduction of 60% [20]. *Dermacentor silvarum* defensin showed bactericidal activity against various Gram-positive, Gram-negative bacteria and *Candida albicans* [21]. *Haemaphysalis longicornis* HlDFS1 and HlDFS2 defensins significantly protected mice against *Staphylococcus aureus* and *Micrococcus luteus* lethal bacterial infection, the survival time had increased from 1.5 days to more than 4 days in *S. aureus*-infected mice. Mice survival had increased from 4 days to more than

6 days in *M. luteus* infection [17]. Moreover, in this species, the synthetic HEdefensin peptide showed effectiveness in killing Gram-positive bacteria, such as *Micrococcus luteus,* by an increase in membrane permeability [22]. *Dermacentor marginatus* defensin (defDM) expression was analyzed in its hemolymph, midgut, and salivary glands, and showed activity against *Borrelia afzelii* [23].

Transcription of *Amblyomma americanum* defensin, termed amercin (amn), revealed this gene has low similarity to *Amblyomma hebraeum* defensins, the only other *Amblyomma* species for which a defensin has been described. Similarity with other tick defensins ranges from 42% to 71% [24]. Mature defensin from *I. scapularis* shows 78.9% similarity with *D. variabilis* [11]. *I. scapularis* has two multigene families of defensin-like peptides (DLPs), one named scapularisin, similar to defensins from primitive insects, bivalves, arachnids, and fungi; and another named scasin, which corresponds to a novel family, unusual to any known defensins [25]. Six novel defensins (DefMT2, DefMT3, DefMT4, DefMT5, DefMT6 and DefMT7) were reported in *Ixodes ricinus* [26], for which only two defensins were initially identified [27, 28]. Only DefMT7 was intronless and non-cationic, while the others contained two introns and were cationic. Some of them were tissue specific. There was phylogenetic difference among these novel defensins [26]. DefMT3, DefMT5 and DefMT6 showed in vitro antimicrobial activity. These defensins were active against distantly related bacteria and fungi. Authors suggested that differences in electrostatic potential, and amino acid substitutions may affect the antimicrobial activity of DefMT2 and DefMT7. DefMT3, DefMT6, and DefMT7 antimicrobial activities, considering only the g-core motif (functional region) were not comparable to those of the whole peptides, unless the antifungal activity, which was higher for the g-core in comparison to full peptides [29].

Finally, ixosin, an AMP that presents an Amino Terminal Cu(II)- and Ni(II) (ATCUN) motif indispensable to its oxidative mode of action, and ixosin B, both isolated from the salivary glands of the hard tick *Ixodes sinensis,* act synergistically to promote bacterial death. Ixosin with ATCUN motif utilizes metal ions in its activity, and presents how ticks can employ a variety of effectors in their immune response [30]

In Triatomines

Triatomines are the vectors of Chagas disease, a chronic health problem that occurs mainly in Latin America. Epidemiologic data provide an estimated number of 6 to 7 million infected people worldwide with the causative agent of the disease,

Trypanosoma cruzi [4]. Transmission occurs mainly by infected triatomine excreta after blood feeding on vertebrate hosts. Congenital, blood transfusion and organ transplantation are also forms of transmission [31]. Outbreaks of oral transmission are due to contaminated food/beverage in different countries as Brazil, Argentina, Bolivia, Colombia, Ecuador, French Guyana, and Venezuela [32]. In the last decades, Chagas disease has been detected in non-endemic continents such as Asia, Europe, North America and Oceania as a result of migration of *T. cruzi* infected individuals from endemic countries [31].

Defensin was the main AMP expressed by triatomines and the subject of 6 (100%) articles. Concerning *R. prolixus*, the Immune-deficiency pathway (IMD) from the triatomine innate immune system was revealed to be incomplete after the genome publication and thought to be non-functional. However, RNAi strategies were used to demonstrate the IMD pathway is functional and plays a role in regulating the expression of specific AMPs in the fat body of the insect [33].

According to some authors, the phylogenetic setting of triatomines among the Reduviidae is not clear due to controversial results. Phylogenetic analyses among triatomines are based on molecular markers such as Cytochrome b (Cytb) encoding gene and internal transcribed spacer (ITS). Defensin genes were used as putative new molecular markers in order to clarify the origin of triatomines, since various defensin gene isoforms have been identified in this subfamily, for instance in the species *R. prolixus, Triatoma brasiliensis, Triatoma infestans, Triatoma sordida, Rhodnius nasutus and Panstrongylus megistus*. Genetic variability was also investigated in members of the *T. brasiliensis* species complex [34].

In *R. prolixus,* defensin A transcription was shown to increase significantly in the fat body of immune activated insects [35]. Also, *R. prolixus* has different AMP transcription pattern in the midgut compartments and in the fat body depending on the *T. cruzi* genotype it hosts. Defensin (defC) has been induced by *T. cruzi* Dm 28c infection and reduced *Serratia marcescens* and *R. rhodnii* colonies in the midgut. Interestingly, *T. cruzi* Y strain did not induce AMP gene expression, what did not decrease bacteria colonization in the triatomine anterior midgut. *T. cruzi* Dm 28c strain caused a stronger activity in colony-forming unit reduction than *T. cruzi* Y strain. The first one showed ($p < 0.001$) 2.5×10^8 CFU/mL−26-fold less, while the second had ($p< 0.05$) 1.64×10^9 CFU/mL−4-fold less in comparison with uninfected insects (6.57 × 109 CFU/mL) [36].

Defensin variability was investigated in *T. pallidipennis*. Two defensins type 1 and one type 4 were identified. Their pro-peptide domain was highly variable, but not the mature peptide [37]. In *T. brasiliensis,* Def3 and Def4 localization and temporal expression were studied. These AMPs showed difference in both parameters cited. While Def3 was constitutively expressed in the fat body and more expressed in the salivary glands 5 days after blood ingestion, Def4 had an up-regulation in stomach after the same period. Three days after blood ingestion, Def3 had increased in *T. brasiliensis* small intestine and Def4 expression had increased in its fat body tissues. These defensins showed structural similarity to *Anopheles gambiae* defensin (62–74% identity). Comparisons of electrostatic potential revealed that Def4 have a more cationic structure than Def3 [38].

In Mosquitoes

Mosquitoes are important vectors of known diseases as chikungunya, dengue, lymphatic filariasis, Rift Valley fever, yellow fever, zika, malaria, Japanese encephalitis, West Nile fever [4]. Chikungunya disease is transmitted by *Aedes*, and over the past 13 years this infection has rushed more than 4 million human cases [39]. In tropical and subtropical countries, dengue is one of the most serious public health problems. Transmission to humans occurs through *Aedes aegypti* and *Aedes albopictus* bite [40]. *Aedes* is also the vector of lymphatic filariasis, Rift Valley fever, yellow fever and zika. *Anopheles* is the vector of malaria. According to WHO (World Health Organization), malaria results in more than 400,000 deaths every year [4]. *Culex* is the vector of lymphatic filariasis, Japanese encephalitis and West Nile fever [4]. Due to the complexity of mosquitoes-host interactions, these insects have been the target of researchers aiming to improve prevention to the diseases transmitted by them.

Defensin was also the most common AMP studied among mosquitoes. As discussed before, this AMP family has a wide distribution in different species of arthropods. Cecropin was the second AMP more reported (5 articles), followed by attacin (1 article).

Members of the defensin family were induced following bacterial infection in *An. gambiae* larvae. Thus, defensin is induced in larval and adult stages when exposed to bacterial infection. In *An. gambiae* pupae, defensin mRNA is expressed constitutively [41]. Increased levels of mRNAs encoding defensin were found in *An. gambiae* upon an infection by *Plasmodium berghei,* demonstrating response against the parasite

invasion in the insect midgut epithelium. Though it was initiated in the midgut, expression extended to fat body and/or hemocytes, indicating a systemic response [42]. *An. gambiae* recombinant defensin showed activity against Gram-positive bacteria and growth inhibitory activity against filamentous fungi species, but no activity against yeast. Authors also showed defensin was induced in the hemolymph of bacteria infected adult female [43]. In *An. stephensi* salivary glands, several novel immune-related transcripts, including defensin and cecropins, were identified upon infection with *Plasmodium* [44]. Investigation of temperature effects on DEF1 (defensin) and CEC1 (cecropin) gene expression in response to injury, heat-killed *E. coli* challenge or no manipulation revealed that temperature influences the expression of the AMPs in *An. stephensi*. Results showed defensin expression peaked around 18°C [45].

In *Ae. aegypti* infected by *Plasmodium gallinaceum*, different AMPs (metalnikowin from *Palomena prasine*, defensins from *Aeschna cyanea* and *Phormia terranovae,* thanatin from *Podisus maculiventris*, drosocin from *Drosophila* and metchnikowin from *Drosophila*) were tested in order to examine parasite development in the mosquito. *Aeshna cyanea* and *Phormia terraenovae* defensins had a potent effect against *P. gallinaceum* oocyst in *Ae. aegypti* (in a time-dependent manner) and against *P. gallinaceum* isolated sporozoites. In vitro experiments showed no effect of AMPs on the zygotes and ookynetes stages of the parasite. In vitro, both defensins had toxicity against isolated sporozoites. It seems this toxicity occurs by damage in parasite membrane and loss of motility [46].

The transcription of defensin genes occurs in the midguts of naive *Ae. aegypti* and *An. gambiae* mosquitoes and from those ingesting a bacterial infected or non-infected bloodmeal. However, bacteria-challenged mosquito shows high levels of mature defensin in the hemolymph and this correlates with a lower prevalence and mean intensity of infection with *P. gallinaceum* oocysts [47].

E. coli and *M. luteus* injection into the hemocoel from *Ae. aegypti* induces potent antibacterial activity in the hemolymph. Three defensin found in the insect hemolymph after infection were not reported in naive mosquitoes. These isoforms differentiated only by one or two amino acid residues [48]. Transgenic *Ae. aegypti* were shown to be extremely susceptible to the infection by Gram-negative bacteria because of reduced post infection levels of defensin and cecropin [49]. Comparative modeling prediction of the defensin three-dimensional structures isoforms (Defensin A, defensin B and

defensin C) of *Ae. aegypti* defensins showed that Defensin A and Defensin C primary structures are identical presenting 5 glycine residues. Defensin B presents 6 glycine residues [40]. *Ae. aegypti* defensin A (DefA) and C (DefC) were examined following ingestion of chikungunya (CHIKV) or Zika virus (ZIKV). DefA and DefC relative activity changed depending on whether the insect was infected with CHIKV or ZIKV. The results suggested differences in antiviral defense responses. Moreover, adult males had higher expression than different aged adult female [39].

Ae. albopictus defensin is highly homologous to that of *Ae. aegypti*, and presents six cysteine residues potentially capable of forming three disulfide bridges, common characteristic to insect defensin family. *Ae. albopictus* infection with *Wolbachia* did not induce or suppress the transcription of any of the three AMPs (diptericin, cecropin and defensin) [50].

In *Culex quinquefasciatus* infected with *Wuchereria bancrofti,* defensin and attacin showed increased peptide levels [51]. Infection of *Ae. aegypti* with *W. bancrofti* also resulted in AMPs increase as soon as 2 h post-infection and peaked before 48 hours. Worm development inside the insect was abnormal since the beginning of infection [52].

In Fleas

Fleas transmit several human pathogens, for instance *Bartonella henselae, Rickettsia felis, R. typhi*, *Yersinia pestis* [53], *Dipylidium caninum* and *Hymenolepis diminuta* [54], among others. Flea-borne infections are emerging or re-emerging worldwide, and their incidence is greater than is commonly recognized by health authorities [55]. One article in our search analyzed by signature-tagged mutagenesis the genes required for *Y. pestis* maintenance in *Xenopsylla cheopis*. *Y. pestis* mutants with insertions in genes encoding glucose-1-phosphate uridylyltransferase (galU) and UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase (arnB) displayed reduced fitness in fleas. Both enzymes are involved in resistance to AMPs. The mutants had sensibility to cecropin A, a linear α-helical AMP produced by insects, including *X. cheopis* [56] and to polymyxin B [57].

Conclusions

The findings presented in this review show the main AMP produced by hematophagous arthropods are defensins. Understanding features and roles of these

molecules in insects against different pathogen seems to be a way to determine vector competency and their biology. It was also proposed AMPs can be a new target/ biological marker to phylogenetic analysis. In addition, suggestion of AMPs as potential molecules to block pathogens development may be considered since it has been demonstrated in several studies their effects against microorganisms as bacteria, virus, fungi and protozoa. AMPs could be potential candidates against multidrug-resistant bacteria, a major health threat worldwide.

Supplementary information

Additional file 1: Table S1. PRISMA checklist.

Abbreviations

AMP: Antimicrobial peptide

RT–PCR: reverse transcription–polymerase chain reaction

MEDLINE - Medical Literature Analysis and Retrieval System Online

SCIELO - Scientific Electronic Library Online

BVS - Biblioteca Virtual em Saúde

IMD **-** Immune-deficiency pathway

CYTB - Cytochrome b

ITS - Internal transcribed spacer

WHO – World Health Organization

CHIKV - Chikungunya

ZIKV – Zika Virus

galU - Glucose-1-phosphate uridylyltransferase

arnB - UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase

Acknowledgments

The authors would like to acknowledge the Institutional Program for Scientific Initiation Grants (PIBIC) from University of Brasília.

Author’s contributions

CNA and PBS conceived the study. LMBR and KLSB analyzed the articles. LMBR wrote the manuscript. CNA, PBS and JMS critically revised the manuscript. All authors edited and approved the final manuscript.

Funding

This work was supported by grants and fellowships awarded by Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF, grants 0193.001802/2017), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, INCT-MCTI/CNPq/FAPs 16/2014), Financiadora de Estudos e Projetos (Finep, CT-Infra grants 0439/11).

Availability of data and materials

All data generated during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All the authors of this manuscript declare that they do not have any conflict of interest.

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573

574 **Table 1.** General characteristics of the 35 studies included in the systematic review.

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
Ticks										
1. An arthropod defensin expressed by the hemocytes of the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae)	10.1016/S0965-1748(03)00122-X	2003	Insect Biochemistry and Molecular Biology	3.562	USA	*Dermacentor variabilis*	*Borrelia burgdorferi*	Defensin	Injection of *Borrelia burgdorfer*i results in secretion of defensin into the *Dermacentor variabilis* hemolymph	[18]
2. Fate of GFP-expressing *Escherichia coli* in the midgut and response to ingestion in a tick, *Ornithodoros moubata* (Acari: Argasidae)	10.1016/j.exppara.2004.07.014	2004	Experimental Parasitology	1.821	Japan	*Ornithodoros moubata*	*Escherichia coli*	Defensin	Expression of defensin increases after *Escherichia coli* ingestion	[19]
3. A defensin-like gene expressed in the black-legged tick, *Ixodes scapularis*	10.1111/j.1365-2915.2005.00579.x	2005	Medical and Veterinary Entomology	1.688	USA	*Ixodes scapularis*	-	Defensin	A defensin gene (slnA) was obtained RT–PCR using mRNA extracted from tissues of female ticks	[11]
4. Babesial Vector Tick Defensin against *Babesia sp.* Parasites	10.1128/IAI.00256-07	2007	Infection and Immunity	3.256	Georgia and Japan	*Haemaphysalis longicornis*	*Babesia microti*	Longicin (defensin-like peptide)	In an *in vivo* experiment, longicin induced significant reduction of parasitemia in animals infected with the zoonotic and murine *B. microti.*	[20]
5. Tissue and life-stage distribution of a defensin gene in the Lone Star tick, *Amblyomma americanum*	10.1111/j.1365-2915.2007.00682.x	2007	Medical and Veterinary Entomology	2.178	USA	*Amblyomma americanum*	-	Amercin (defensin)	Amercin gene shows little similarity to either of the defensins from *Amblyomma hebraeum* Koch	[24]
6. The defensin gene family expansion in the tick *Ixodes scapularis*	10.1016/j.dci.2011.03.030	2011	Developmental and Comparative Immunology	2.913	China	*Ixodes scapularis*	Gram-positive and Gram-negative bacteria	Defensin	Two multigene families of defensin-like peptides (DLPS) were described: Scapularisins and Scasins	[25]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
7. Defensin from the ornate sheep tick *Dermacentor marginatus* and its effect on Lyme borreliosis spirochetes	10.1016/j.dci.2014.04.005	2014	Developmental and Comparative Immunology	2.913	Czech Republic	*Dermacentor marginatus*	*Borrelia afzelii*	DefDM	Anti-gram-positive bacterial role for the defensin was shown; There was a very clear defensin borrelicidal activity	[23]
8. Identification and partial characterisation of new members of the *Ixodes ricinus* defensin family	10.1016/j.gene.2014.03.002	2014	Gene	2.498	Czech Republic, UK, USA, and Spain	*Ixodes ricinus*	-	DefMT2, defMT3, defMT4, defMT5, defMT6, defMT7	Identification of six novel putative defensins; Their expression pattern was reported (location, phylogeny and structure), Mainly activity was against Gram-positive bacteria	[26]
9. Central Role of the Copper-Binding Motif in the Complex Mechanism of Action of Ixosin: Enhancing Oxidative Damage and Promoting Synergy with Ixosin B	10.1021/acsinfecdis.5b00140	2015	ACS Infectious Diseases	4.325	USA	*Ixodes sinensis*	-	Ixosin B	Tick AMP depend on metal ions for activity	[30]
10. *Ixodes ricinus* defensins attack distantly-related pathogens	10.1016/j.dci.2015.08.001	2015	Developmental and Comparative Immunology	2.913	Czech Republic, Germany, France, Spain	*Ixodes ricinus*	Gram-negative and Gram-positive bacteria; Fungi *Fusarium culmorum* and *Fusarium graminearum*	DefMT3, DefMT5, DefMT6, DefMT2, DefMT7	Evolution of tick defensins was investigated; DefMT3, DefMT5, DefMT6 showed in vitro activity; These peptides showed activity against distantly related bacteria and fungi	[29]
11. Molecular characterization of a defensin gene from a hard tick, *Dermacentor silvarum*	10.1186/s13071-014-0625-0	2015	Parasites and Vectors	3.163	China	*Dermacentor silvarum*	Gram-positive and Gram-negative	Ds-Defensin	Ds-Defensin showed bactericidal activity against Gram-positive and Gram-negative bacteria; and against	[21]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
							bacteria, and *Candida albicans* fungus		the fungus *Candida albicans*	
12. Functional characterization of two defensins, HlDFS1 and HlDFS2, from the hard tick *Haemaphysalis longicornis*	10.1186/s13071-017-2397-9	2017	Parasites and Vectors	3.163	China	*Haemaphysalis longicornis*	Gram-negative, Gram-positive bacteria, and *C. albicans* fungus	Defensins (HlDFS1 and HlDFS)	Bactericidal activity against Gram-positive and Gram-negative bacteria; Significantly protect mice against lethal bacterial infection	[17]
13. Hemolymph defensin from the hard tick *Haemaphysalis longicornis* attacks Gram-positive bacteria	10.1016/j.jip.2018.07.005	2018	Journal of Invertebrate Pathology	2.511	Philippines and Japan	*Haemaphysalis longicornis*	Gram-positive bacteria *Micrococcus luteus*	HEdefensin	Antibacterial activity against *Micrococcus luteus;* HEdefensin increased the membrane permeability of *M. luteus*.	[22]
Triatomines										
1. Isolation and characterization of a novel insect defensin from *Rhodnius prolixus*, a vector of Chagas disease	10.1016/S0965-1748(03)00008-0	2003	Insect Biochemistry and Molecular Biology	3.562	Colombia and USA	*R. prolixus*	*E. coli* and *M. luteus*	Defensin A	Exceptionally low baseline transcription of defensin A in naive insects; Transcription increases significantly in the fat body of immune activated insects	[35]
2. Two novel defensin-encoding genes of the Chagas disease vector *Triatomabrasiliensis* (Reduviidae, Triatominae): gene expression and peptide-structure modeling	10.1016/j.jinsphys.2009.05.015	2009	Journal of Insect Physiology	2.733	Brazil	*Triatoma brasiliensis*	-	Def3 and def4	Two novel defensin-encoding cDNAs and the respective genomic DNAs (*def3* and *def4*) were identified and their tissue-specific and temporal expression were characterized	[38]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
3. Genes encoding defensins of important Chagas disease vectors used for phylogenetic studies	10.1007/s00436-015-4694-6	2015	Parasitology Research	2.558	Brazil	*Triatoma sordida, R. prolixus, Rhodnius nasutus, Panstrongylus megistus,* and members of the *T. brasiliensis* complex (*Triatoma sherlock, Triatoma juazeirensis, Triatoma brasiliensis macromelasoma, Triatoma melanica*)	-	Triatoma Def3/4 Rhodnius DefA/B	Genes were sequenced and used as a molecular markers for phylogenetic analysis; First time defensin genes were reported in *Triatoma sordida, Rhodnius nasutus,* and *Panstrongylus megistus*; The deduced defensin amino acid sequences were highly conserved mainly in their mature peptide	[34]
4. Impact of *Trypanosoma cruzi* on antimicrobial peptide gene expression and activity in the fat body and midgut of *Rhodnius prolixus*	10.1186/s13071-016-1398-4	2016	Parasites and Vectors	3.163	Brazil	*R. prolixus*	-	Defensin (defA, defB, defC) and prolixicin (prol)	*R. prolixus* AMP gene expression and cultivable midgut bacterial microbiota were modulated in distinct patterns, which depended on the *T. cruzi* genotype used for infection	[36]
5. Variability of defensin genes from a Mexican endemic Triatominae: *Triatoma 1* (Meccus) *pallidipennis* (Hemiptera: Reduviidae)	10.1042/BSR20180988	2018	Bioscience Reports	2.899	Mexico	*Triatoma (Meccus) pallidipennis*	-	Two Defensin type 1 and one type 4	Two defensins type 1 and one type 4 were identified; The pro-peptide domain was highly variable, but not the mature peptide	[37]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
6. *Rhodnius prolixus*: Identification of missing components of the IMD immune signaling pathway and functional characterization of its role in eliminating bacteria	10.1371/journal.pone.0214794	2019	PLoS ONE	2.766	Brazil	*R. prolixus*	*Enterobacter cloacae* and *Staphylococcus aureus*	Defensin-A, Defensin-C	RNAi strategies were used to demonstrate the role of the IMD pathway in regulating the expression of specific AMPs in the fat body	[33]
Mosquitoes										
1. Insect immunity: Isolation of three novel inducible antibacterial defensins from the vector mosquito, *Aedes aegypti*	10.1016/0965-1748(95)00043-u	1995	Insect Biochemistry and Molecular Biology	3.562	USA and France	*Aedes aegypti*	*E. coli* and *M. luteus*	Defensin	The injection of *Escherichia coli* and *Micrococcus luteus* into the hemocoel of *Aedes aegypti* induces a potent antibacterial activity in the hemolymph	[39]
2. Inducible immune factors of the vector mosquito *Anopheles gambiae*: biochemical purification of a defensin antibacterial peptide and molecular cloning of preprodefensin cDN	10.1111/j.1365-2583.1996.tb00055.x	1996	Insect molecular biology	2.492	Germany and France	*Anopheles gambiae*	*E. coli* and *M. luteus*	Defensin	Antimicrobial activities were observed following bacterial infection; Defensin expression was induced in response to bacterial infection, in both adult and larval stages	[48]
3. *Plasmodium* activates the innate immune response of *Anopheles gambiae* mosquitoe	10.1093/emboj/16.20.6114	1997	EMBO Journal	10.557	Germany	*An. gambiae*	*Plasmodium* sp	Defensin	Increased levels of mRNAs encoding defensin were found in *Anopheles gambiae* upon an infection by *Plasmodium berghei*	[42]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
4. *Plasmodium gallinaceum*: Differential Killing of Some Mosquito Stages of the Parasite by Insect Defensin	10.1006/expr.1998.4212	1998	Experimental Parasitology	1.821	France and USA	*A. aegypti*	*Plasmodium gallinaceum*	Defensin	Two defensins had toxic effect in *Plasmodium gallinaceum* oocyst in *A. aegypy* and isolated sporozoites; The peptides had no detectable effect on the zygotes and ookinetes *in vitro*	[46]
5. Mosquito-*Plasmodium* interactions in response to immune activation of the vector	10.1006/expr.1999.4350	1999	Experimental Parasitology	1.821	USA and France	*Ae. aegypti*	Bacteria	Defensin	Defensins transcription occurs in the midguts of naive mosquitoes and those ingesting a bloodmeal, either infectious or noninfectious; Bacteria-challenged mosquito showed high levels of mature defensin in the hemolymph; this correlated with a lower prevalence and mean intensity of infection with *Plasmodium* oocysts	[47]
6. *Wolbachia* neither induces nor suppresses transcripts encoding antimicrobial peptides	10.1046/j.1365-2583.2000.00224.x	2000	Insect Molecular Biology	2.492	USA and Greece	*Aedes albopictus*	*Wolbachia* sp.	Defensin,	The *Ae. albopictus* defensin sequence is highly homologous to that of *Ae. aegypti,* and presented six cysteine residues potentially capable of forming three disulphide bridges, characteristic common to insect defensins;	[50]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
									Wolbachia did not induce or suppress the transcription of any of the three AMPs	
7. The defensin peptide of the malaria vector mosquito *Anopheles gambiae*: antimicrobial activities and expression in adult mosquitoes	10.1016/S0965-1748(00)00143-0	2000	Insect Biochemistry and Molecular Biology	3.562	France, USA, Germany	*An. gambiae*	Gram-positive and Gram-negative bacteria, filamentous fungi and yeast	Defensin	Activity was detected against Gram-positive bacteria; Growth inhibitory activity against filamentous fungi species, but no activity against yeast; Defensin was induced in the hemolymph of bacteria infect adult female	[43]
8. Relish-mediated immune deficiency in the transgenic mosquito *Aedes aegypti*	10.1073/pnas.0537347100	2003	Proceedings of the National Academy of Sciences of the United States of America	9.504	USA	*Ae. aegypti.*	Gram-positive and gram-negative bacteria.	Defensin and cecropin	Transgenic mosquitoes were extremely susceptible to the infection by Gram-negative bacteria because of reduced post infection levels of antimicrobial peptide genes, defensin and cecropin	[49]
9. The malaria vector mosquito *Anopheles gambiae* expresses a suite of larval-specific defensin gene	10.1111/j.1365-2583.2008.00786.x.	2008	Insect Molecular Biology	2.492	UK	*An. gambiae*	-	Defensin 2 (AgDef2), Defensin 3 (AgDef3) and Defensin 4 (AgDef4)	cDNAs of *Anopheles gambiae* Defensin 2 (AgDef2), Defensin 3 (AgDef3) and Defensin 4 (AgDef4), identified in the genome sequence, were characterized and their expression profiles investigated	[58]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
10. Actin protein up-regulated upon infection and development of the filarial parasite, *Wuchereria bancrofti* (Spirurida: Onchocercidae), in the vector mosquito, *Culex quinquefasciatus* (Diptera: Culicidae)	10.1016/j.exppara.2007.08.012	2008	Experimental Parasitology	1.821	India	*Culex quinque fasciatus*	*Wuchereria bancrofti*	Attacin, Defensin	Analysis of the hemolymph of infected *C. quinquefasciatus* by *W. bancrofti* showed up-regulation of five proteins; Attacin and defensin are among them	[51]
11. In-silico homology modeling of three isoforms of insect defensins from the dengue vector mosquito, *Aedes aegypti* (Linn., 1762)	10.1007/s00894-008-0408-7	2008	Journal of Molecular Modeling	1.507	India	*Ae. aegypti*	-	Defensin A, Defensin B and Defensin C	Comparative modeling prediction of three-dimensional structures from three isoforms of *Ae. aegypti* defensins	[40]
12. Expression of defensin, cecropin, and transferrin in *Aedes aegypti* (Diptera: Culicidae) infected with *Wuchereria bancrofti* (Spirurida: Onchocercidae), and the abnormal development of nematodes in the mosquito	10.1016/j.exppara.2008.09.003	2008	Experimental Parasitology	1.821	Brazil	*Ae. aegypti*	*W. bancrofti*	Defensin, Cecropin	Higher transcription of both AMPs in infected *Ae. aegypti* as soon as 2 h post-infection and peak before 48 hours	[52]
13. Salivary gland transcriptome analysis during *Plasmodium* infection in malaria vector *Anopheles stephensi*	10.1016/j.ijid.2008.07.027	2009	International Journal of Infectious Diseases	3.202	India	*An. stephensi*	*Plasmodium*	Defensin and Cecropin	Identification of several novel immune-related transcripts, including defensin and cecropins	[44]

Study title	PMID or DOI	Year	Journal	J IF*	Country	Vector species	Pathogens	AMP	Comments	Ref
14. Complex effects of temperature on mosquito immune function	10.1098/rspb.2012.0638	2012	Proceedings of the Royal Society B: Biological Sciences	4.847	USA	*An. stepensi*	*E. coli*	Defensin 1 (DEF1), cecropin 1 (CEC1)	Investigation of the effects of temperature on DEF1 and CEC1 gene expression in response to injury, heat-killed *E. coli* challenge or no manipulation; Temperature influenced the expression of the AMPs	[45]
15. Transcription Profiling for Defensins of *Aedes aegypti* (Diptera: Culicidae) During Development and in Response to Infection With Chikungunya and Zika Viruses	10.1093/jme/tjx174	2018	Journal of Medical Entomology	1.968	USA	*Ae. aegypti*	Chikungunya and Zika Viruses	DefA and defC	DefA and DefC relative activity changed depending on whether the insect was infected with CHIKV or ZIKV; The results suggested differences in antiviral defense responses; Adult males had higher expression than different aged adult females.	[39]
Fleas										
1. LPS modification promotes maintenance of *Yersinia pestis* in fleas	10.1099/mic.0.000018	2014	Microbiology (United Kingdom)	0.855	USA	*Xenopsylla cheopis*	*Yersinia pestis*	Polymyxin B and cecropin A	Cecropin A and polymyxin B showed higher activity against *Y. pestis* mutants	[57]

575 *J IF – Journal Impact Factor

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ANEXO I

INFORMAÇÕES SUPLEMENTARES

Supplementary information

Additional file 1: Table S1. PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Antimicrobial Peptides from Hematophagous Arthropods: A systematic review	
ABSTRACT			
Structured summary	2	A systematic literature review was conducted by two independent authors in order to synthesize the studies' results related to antimicrobial peptides in hematophagous vectors of diseases. The research was performed on 13 May 2020 by searching the following electronic databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Scientific Electronic Library Online (SCIELO) and Virtual Health Library (BVS, Biblioteca Virtual em Saúde). The research terms used were 'Arthropod Vectors' and 'Antimicrobial Cationic 'Peptides'. According to the search algorithm of each database, these keywords were detected in the title, abstract and/or text. There were no restrictions on language or on time period of publication. Inclusion criteria were experimental scientific works involving the study of proteins from hematophagous arthropods. Review articles, Master's and Doctoral theses were excluded, and duplicated studies were removed.	
INTRODUCTION			
Rationale	3	Antimicrobial peptides (AMPs) studies bring information on arthropods' immune response, expression variability, distribution pattern in the arthropod body and toxicity upon different microorganisms. In addition, some studies suggest as perspectives AMPs investigation to better understanding of arthropod evolution, and to improve medicines.	
Objectives	4	Summarize relationship between AMPs and four arthropods groups (ticks, triatomines, mosquitoes, and fleas), facilitating the comprehension about how these potential molecules can be used in science different broads.	
METHODS			
Protocol and registration	5	It was not used a review protocol.	
Eligibility criteria	6	The included articles were published between 1995 and 2019. Most of these articles (12) have been published in the last five years (2015 to 2019). The journal impact was ranged from 0.945 to 41.845. All publications included were written in English. Studies were performed in the following continents North America, South America, Europe and Asia.	
Information sources	7	The research was performed on 13 May 2020 by searching the following electronic Databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Scientific Electronic Library Online (SCIELO) and Virtual Health Library (BVS, Biblioteca Virtual em Saúde).	
Search	8	The research terms used were 'Arthropod Vectors' and 'Antimicrobial Cationic 'Peptides' by searching the following electronic databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Scientific Electronic Library	

		Online (SCIELO) and Virtual Health Library (BVS, Biblioteca Virtual em Saúde). According to the search algorithm of each database, these keywords were detected in the title, abstract and/or text.	
Study selection	9	Based on inclusion/exclusion criteria, all abstracts were independently screened by each examiner to determine their eligibility. In case of doubt about the inclusion after reading the abstract, the article was completely read. Next, included full-text articles were reviewed in duplicate. Disagreements were solved by discussion between the examiners to reach a consensus. The reference lists of the selected studies were screened for potentially relevant articles.	
Data collection process	10	The systematic literature review was conducted by two independent authors in order to synthesize the studies' results related to antimicrobial peptides in hematophagous vectors of diseases.	
Data items	11	The keywords 'Arthropod Vectors' and 'Antimicrobial Cationic Peptides' were detected in the title, abstract and/or text. Data as Vector species, pathogens and AMPs were collected.	
Risk of bias in individual studies	12	Not applicable.	
Summary measures	13	Not applicable.	
Synthesis of results	14	Based on inclusion/exclusion criteria, all abstracts were independently screened by each examiner to determine their eligibility. In case of doubt about the inclusion after reading the abstract, the article was completely read. Results were presented in discussion for each arthropod group and arranged in Table 1: General characteristics of the studies included in the systematic review.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Not applicable.	
Additional analyses	16	It was not necessary to do additional analyses in this review.	
RESULTS			
Study selection	17	Initially, 80 studies were identified. After reading the title and abstract, 45 articles were rejected. The remaining 35 studies were completely read, and all studies met the inclusion criteria. In a second search, although 4 studies had been added to the result, none of them were included in this review after reading the title and abstract. A total of 35 articles were admitted in this systematic review.	
Study characteristics	18	The data extracted from each full-text article included in the systematic review were (when available): (i) publication characteristics (study title, year of publication, periodic name, journal impact factor, country where the study was performed); (ii) arthropod vector	

		species and transmitted pathogens; and (iii) antimicrobial peptide families and comments. Data extraction process was performed by one member of the study who inserted the relevant data into a table summarizing all the aspects investigated.	
Risk of bias within studies	19	All the authors of this manuscript declare that they do not have any conflict of interest.	
Results of individual studies	20	Not applicable.	
Synthesis of results	21	Not applicable.	
Risk of bias across studies	22	Not applicable.	
Additional analysis	23	Additional analyses were not included.	
DISCUSSION			
Summary of evidence	24	The main findings of this study is related to interactions and relations between vector species, pathogens and antimicrobial peptides (AMPs). The review relevance is aimed to biologists, pharmacists and scientists that needs a comprehensive study on antimicrobial peptides, and may target these molecules in different areas of application. In this review we will see potential activity of different AMPs in pathogenic microorganisms, which can be a source of new medicine; phylogenetic analysis between species; etc. All studies included are experimental and are arranged in journals ranged from 0.945 to 41.845.	
Limitations	25	Using the keywords 'Arthropod Vectors' and 'Antimicrobial Cationic Peptides' it was possible to find just one article which had fleas antimicrobial peptides as target of discussion. Information related to this molecules and fleas seems to be not common.	
Conclusions	26	Understanding features of antimicrobial peptides in insects against different pathogens infections seems to be a way in determining vector competency and its biology. On the other hand, antimicrobial peptides studies can be a target to phylogenetic analysis between species which has genes to transcript these molecules. In addition, suggestion of antimicrobial peptides as potential molecules to block pathogens development may be considered since it has been demonstrated in several studies the capacity of antimicrobial peptides in damaging microorganisms as bacteria, virus, fungus and yeasts.This potential action may be an alternative to control the occurrence of diseases transmitted by insects.	
FUNDING			
Funding	27	This work was supported by grants and fellowships awarded by Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF, grants 0193.001802/2017), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, INCT-MCTI/CNPq/FAPs 16/2014), Financiadora de Estudos e Projetos (Finep, CT-Infra grants 0439/11).	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

ANEXO II
NORMAS DO PERIÓDICO

Parasites & Vectors

Objetivos e Escopo

Parasites & Vectors é um jornal online revisado por pares, de acesso aberto, que trata da biologia de parasitas, doenças parasitárias, hospedeiros intermediários, vetores e patógenos transmitidos por vetores. Os manuscritos publicados nesta revista estarão disponíveis para todo o mundo, sem barreiras de acesso, imediatamente após a aceitação. No entanto, os autores detêm os direitos autorais de seu material e podem usá-lo ou distribuí-lo como desejarem.

Além das áreas tradicionais e bem estabelecidas da ciência nesses campos, também pretendemos fornecer um veículo para a publicação de recursos e tecnologia em rápido desenvolvimento em genômica de parasitas, hospedeiros intermediários e vetores e seus impactos na pesquisa biológica. Somos capazes de publicar filmes e também grandes conjuntos de dados e resultados extensos, frequentemente associados a tecnologias genômicas e pós-genômicas, que não são prontamente acomodadas em periódicos tradicionais. Manuscritos abordando questões mais amplas, por exemplo, economia, ciências sociais e mudanças climáticas globais em relação a parasitas, vetores e controle de doenças, também são bem-vindos.

PREPARO DO MANUSCRITO

PÁGINA DE TÍTULO

A página de título deve:

• Apresentar um título que inclua, se for o caso, o desenho do estudo, por exemplo:

- "A versus B no tratamento de C: um ensaio clínico randomizado", "X é um fator de risco para Y: um estudo de caso-controle", "Qual é o impacto do fator X no sujeito Y: uma revisão sistemática"

- Para estudos não clínicos ou não de pesquisa: uma descrição do que o artigo relata

• Liste os nomes completos, endereços institucionais e endereços de e-mail de todos os autores.

- Se um grupo de colaboração deve ser listado como um autor, liste o nome do grupo como um autor. Se você quiser que os nomes dos membros individuais do Grupo possam ser pesquisados em seus registros individuais do PubMed, inclua essas informações na seção "Agradecimentos" de acordo com as instruções abaixo

• Indicar o autor para correspondência.

RESUMO

O resumo não deve exceder 350 palavras. Minimize o uso de abreviações e não cite referências no resumo.

PALAVRAS-CHAVE

Três a dez palavras-chave que representam o conteúdo principal do artigo.

INTRODUÇÃO

Deve explicar os antecedentes do artigo, seus objetivos, um resumo de uma pesquisa da literatura existente e o assunto em discussão.

TEXTO PRINCIPAL

Deve conter o corpo do artigo e também pode ser dividido em subseções com títulos curtos e informativos.

CONCLUSÕES

Deve indicar claramente as principais conclusões e explicar a importância e relevância do caso, dados, opinião, base de dados ou software relatado.

LISTA DE ABREVIAÇÕES

Se abreviações forem usadas no texto, elas devem ser definidas no texto na primeira utilização, e uma lista de abreviações deve ser fornecida.

DECLARAÇÕES

Todos os manuscritos devem conter as seguintes seções sob o título 'Declarações':

• Aprovação ética e consentimento para participar, se aplicável;
• Consentimento para publicação, se aplicável;
• Disponibilidade de dados e materiais;
• Conflito de interesses;
• Financiamento;
• Contribuições dos autores;
• Reconhecimentos;
• Informações dos autores (opcional).

Veja os detalhes sobre as informações a serem incluídas nessas seções. Se alguma das seções não for relevante para o seu manuscrito, inclua o título e escreva 'Não aplicável' para essa seção.

REFERÊNCIAS

Todas as referências, inclusive URLs, devem ser numeradas consecutivamente, entre colchetes, na ordem em que são citadas no texto, seguidas de quaisquer tabelas ou legendas. Os números de referência devem ser finalizados e a lista de referências totalmente formatada antes do envio.

Exemplos do estilo de referência: BioMed Central.

TABELAS

• As tabelas devem ser numeradas e citadas no texto em sequência em algarismos arábicos (ou seja, Tabela 1, Tabela 2 etc.);

• Tabelas com menos de uma página A4 ou Carta podem ser colocadas no local apropriado dentro do manuscrito.

• Tabelas maiores que uma página A4 ou Carta podem ser colocadas no final do arquivo de texto do documento. Cite e indique onde a tabela deve aparecer no local relevante no arquivo de texto para que a tabela possa ser adicionada no local correto durante a produção;

• Conjuntos de dados maiores ou tabelas muito largas para página paisagem A4 ou Carta podem ser carregados como arquivos adicionais. Por favor, veja abaixo para mais informações;

• Os dados tabulares fornecidos como arquivos adicionais podem ser carregados como uma planilha do Excel (.xls) ou valores separados por vírgula (.csv). Use as extensões de arquivo padrão;

• Os títulos das tabelas (máximo de 15 palavras) devem ser incluídos acima da tabela, e as legendas (máx. 300 palavras) devem ser incluídas abaixo da tabela;

• As tabelas não devem ser incorporadas como figuras ou arquivos de planilha, mas devem ser formatadas usando a função 'Objeto de tabela' em seu programa de processamento de texto;

• Cor e sombreamento não podem ser usados. Partes da tabela podem ser destacadas usando sobrescrito, numeração, letras, símbolos ou texto em negrito, cujo significado deve ser explicado na legenda da tabela;

• As vírgulas não devem ser usadas para indicar valores numéricos.

ARQUIVOS ADICIONAIS

Como o comprimento e a quantidade de dados não são restritos a muitos tipos de artigos, os autores podem fornecer conjuntos de dados, tabelas, filmes ou outras informações como arquivos adicionais.

Todos os arquivos adicionais serão publicados junto com o artigo aceito. Não inclua arquivos como formulários de consentimento do paciente, certificados de edição de idioma ou versões revisadas do documento manuscrito principal com alterações rastreadas. Tais arquivos, se solicitados, devem ser enviados por e-mail para o e-mail editorial da revista, citando o número de referência do manuscrito. Não envie formulários de consentimento do paciente preenchidos, a menos que solicitado.

Os resultados que seriam indicados como "dados não mostrados" devem ser incluídos como arquivos adicionais. Como muitos links da web e URLs quebram rapidamente, o BioMed Central requer que os dados de suporte sejam incluídos como arquivos adicionais ou depositados em um repositório reconhecido. Não crie links para dados em um site pessoal / departamental. Não inclua nenhum detalhe individual do participante. O tamanho máximo de arquivo para arquivos adicionais é de 20 MB cada,

e os arquivos serão verificados quanto a vírus no envio. Cada arquivo adicional deve ser citado em seqüência no corpo principal do texto.

• Nome do arquivo (por exemplo, arquivo adicional 1);

• Formato de arquivo incluindo a extensão de arquivo correta, por exemplo .pdf, .xls, .txt, .pptx (incluindo o nome e o URL de um visualizador apropriado se o formato for incomum);

• Título dos dados;

• Descrição dos dados;

Os arquivos adicionais devem ser nomeados "Arquivo adicional 1" e assim por diante e devem ser referenciados explicitamente pelo nome do arquivo no corpo do artigo, por exemplo, 'Um arquivo de filme adicional mostra isso com mais detalhes [consulte o arquivo adicional 1]'.