Antibacterial activities of two potential peptides extracted from *Polistes wattii* Cameron, 1900 (Vespidae: Polistinae) wasp venom collected at Eastern Province, Saudi Arabia

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Abstract

Alternatives of conventional antibiotics have become an urgent need to control drug-resistant bacteria. Therefore, search for new antibacterial agents has become a trend in several microbiological and pharmaceutical scientific works. Insects, one of the most successful and evolved species on earth is known to be an effective natural source of several medically useful chemicals including antibacterial agents. There is considerable evidence of using wasp venom against medical ailments in several parts of the world. In this work venom from *Polistes wattii* Cameron, 1900 collected from Eastern Province, Saudi Arabia was evaluated for its antibacterial activities. Such activity was tested against four pathogenic bacteria: two-gram positive *Staphylococcus aureus* (ATCC 25923) and *Streptococcus mutans* (RCMB 017(1) ATCC 25175) and two gram-negative (*Salmonella typhimurium* NCTC 12023 ATCC 14028 and *Enterobacter cloacae* (RCMB 001(1) ATCC 23355). Also, chemical characterization of wasp venom was done using HPLC and two isolated peptides were sequenced. The result indicates the potent anti-microbial effect of the venom against the four tested bacteria. The most sensitive bacteria were *Staphylococcus aureus* (ATCC 25923) and *Streptococcus mutans*. The sequence of the two purified peptides indicates that they belong to mastoparan. The study results may pave way to use this wasp venom in future antibiotics especially in controlling skin infection by *Staphylococcus aureus*.

Introduction

Humans depended mostly on the natural resources for all their needs [1]. Diseases, being the most crucial limiting factor that negated the advancement of human race [2]. Plant derived phytochemicals were the primary resources exploited for human needs [3]. Several plant-derived phytochemicals were investigated for their activity against chronic debilitating diseases and have found to act through multiple pathways including their disease modifying effect and...
by general mechanisms like antioxidant defense [4, 5]. But, in the present scenario, where the disease-causing agents have attained resistance against the drug, these medicines failed drastically. In such situations, newer alternatives have been searched for in other life forms like animal derived drugs developed from their natural secretions. Animal derived glandular secretions like musk are being traditionally used for medicinal purposes [6]. Salivary secretions, specialized glandular secretions constituting venom, secretions for self-defense in the form of acrid irritant juices; all represents potent sources against human diseases [7]. From an evolutionary point of view, animal venom forms a very effective group of chemicals that was used to kill and digest prey [8, 9]. Many animals have evolved a wide range of chemical toxins to achieve this purpose [10]. The class Insecta, in particular, utilized a vast array of chemicals in their venom for the purpose of self-defense and predation [11, 12]. Among insects, the order Hymenoptera, which includes ants, bees and wasps are specifically equipped with effective venom and delivery systems which provided them an evolutionary advantage of becoming the most evolved life forms on earth [13, 14]. The chemical analysis of Hymenoptera venom showed the presence of an array of low molecular weight compounds like amino acids, biogenic amines, carbohydrates, small peptides and phospholipids with diverse biological activity [15].

The medicinal use of Hymenoptera venom dates back to ancient Egyptian civilizations, where the use of honeybee venom was common for alleviating arthralgia [16]. For years, immunotherapy was the main objective of medication by insect venom, as the venom enhances the immune defense and increases blood circulation on target sites [17, 18]. Recently, studies have shown that insect venom is active against viruses, fungi, and most importantly drug-resistant bacteria [19].

Drug-resistant bacteria poses a great threat in the form of escalating health expenditure and loss of precious human lives in different parts of the world [20]. Failure of conventional antibiotics against common bacterial infections is the nightmare faced by microbiologists and pharmacologists alike during the current century [21]. Search for newer and potent anti-microbial agents to combat infections is the urgent need of the hour to prevent wide spread infections without specific medications [22]. Very common pathogenic bacteria like *Staphylococcus aureus*, causing bacteremia and infective endocarditis along with soft tissue infections, and *Salmonella typhimurium*, the common cause for food poisoning, are resistant to a wide range of antibiotics available today [23, 24]. The above illustrated facts point to a grave crisis generated by drug-resistant microbes posing life-threatening conditions from common infections and minor injuries [25].

Although, advancements like passive immunization and phage therapy have substituted conventional antimicrobials to a greater extend, medical researchers are still behind exotic sources of novel antibiotics [26, 27]. Several natural sources were screened for antimicrobial activity and among them insects provided promising results in this regard [20, 28]. Recently, several publications highlighted the biological and chemical activities of hymenopteran insects including their antimicrobial activity [29].

Wasps, a hymenopteran insect, produces a venom which is a good source of alternative antibiotic agents [30]. Antimicrobial peptides (AMPs) isolated from wasp venom have shown strong bactericidal activity [31, 32]. Their mode of action depends on eliciting multiple pathways that include destructing the phospholipid bilayer membrane, perturbing cellular metabolism, or by interfering with cytoplasmic signaling. This makes them a safer alternative for human and animal consumption. Moreover, these compounds are highly conserved among the Vespidae family [33].

In Saudi Arabia, several species of Vespidae are reported [34], but their venoms were never characterized and analyzed before for their antimicrobial activity. This work aims at
characterizing two peptides isolated from *Polistes wattii* Cameron, 1900 collected from the Eastern Province of Saudi Arabia and to investigate their antimicrobial activity against four multi-drug resistant strains of *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhimurium* and *Enterobacter cloacae* for the development of a probable antimicrobial agents that can overcome drug resistance.

**Material and methods**

**Collected materials**

Live specimens of *Polistes wattii* wasps were collected using standard insect swiping net from Al-Ahsa Governorate (25°23′00″N 49°36′00″E) (Fig 1). The specimens were then transferred to the lab and identified according to the keys published by Temreshev, 2018. The venom was collected using an electric screen that was used for collecting honeybee venom with a 6 Volt electric charge [35]. The collected venom was harvested three time a week from electric screen and transferred to Eppendorf tubes containing mixture of 50:50 acetonitrile and water and preserved in refrigerator.

![Fig 1. The wasp *Polistes wattii* with its characteristic yellow coloration.](https://doi.org/10.1371/journal.pone.0264035.g001)
Antibacterial activity

Four multidrug-resistant bacteria were selected to evaluate the antibacterial activities: two-gram positive bacteria (Staphylococcus aureus (ATCC 25923) and Streptococcus mutans (RCMB 017(1) ATCC 25175) and two gram-negative (Salmonella typhimurium NCTC 12023 ATCC 14028 and Enterobacter cloacae (RCMB 001(1) ATCC 23355). Agar well diffusion method was used to demonstrate the antibacterial effect of the wasp venom against the selected microbes [36]. The selected strains of the bacteria were uniformly inoculated on to petri dishes containing nutrient agar media. Wells were made on the plates using a sterile 7 mm cork-borer and 100 ul of diluted wasp venom were poured into each well. The dilutions of 5, 2.5, 1.25, 0.75 mg/ml of wasp venom were selected for the present study and the plates were incubated for 24 h at 37˚C [37]. The zone of inhibition of bacterial growth was measured using calipers at the end of the incubation [20]. Each experiment was carried out in triplicates for each concentration and organism.

Chemical analysis

Wasp venom compounds were isolated using HPLC under a specific column (Vydac® 218TP C18 HPLC Columns, Avantor) with unique selectivity for small peptides. The peptides were collected after a period of 30 min runtime at every minute [38]. Certain pure peptide fractions were then transferred to Porton LF3000G protein sequencing machine to get the amino acid sequence [39]. Chemoffice (chem draw) and Discovery Studio software were used to visualize the selected peptide chemical orientation and 3D shape.

Statistical analysis

One way ANOVA was done between the different venom concentrations for each bacterial pathogen and the mean zone of inhibition was done using IBM SPSS ver.22 followed by post Hoc Tukey’s test were done to evaluate the differences between the different concentrations. Matrix cluster analyses using two-way single linkage Euclidian distance was made using SYSTAT version 13, from Systat Software, Inc., San Jose, CA, USA, www.sigmaplot.com to show the degree of antimicrobial activity of wasp venom for each pathogenic species [20].

Results

The present work illustrates the antagonistic activity of different Polistes wattii wasp venom (PWWV) concentrations to the spectrum of gram-positive and gram-negative human pathogenic bacteria. The result of the agar well diffusion method showed a concentration dependent inhibition of the pathogenic agents (Table 1; Fig 2). All tested bacteria were inhibited by wasp venom with different degrees: the highest inhibition is shown by Staphylococcus aureus under the highest concentration 29.3±1.5 while the lowest concentration shows no effect to

Table 1. Antimicrobial activity indicated as inhibition zone in (mm) of different wasp venom concentrations against selected pathogens.

| Venom concentration (mg/ml) | Gram-positive bacteria | Gram-negative bacteria |
|-----------------------------|------------------------|------------------------|
|                             | Zone of inhibition (mm)|                        |
|                             | Staphylococcus aureus  | Streptococcus mutans   | Salmonella typhimurium | Enterobacter cloacae   |
| 0.75                        | 4.3±0.9                | Na                     | Na                     | Na                     |
| 1.25                        | 17.0±1.4               | 5.3±1.3                | Na                     | 2.7±0.7                |
| 2.5                         | 24.3±1.3               | 9.7±1.9                | 3.3±0.7                | 6.7±1.2                |
| 5                           | 29.3±1.5               | 16.7±1.8               | 10.7±1.9               | 14.0±1.7               |

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**Streptococcus mutans**, *Salmonella typhimurium*, and *Enterobacter cloacae* (Fig 2). Matrix cluster analyses produce a heat map that represents each concentration effect on the target bacterial species (Fig 2).

The statistical analysis indicates that there is a significant difference between mean inhibition zone of the different venom concentrations with a P = 0.03. The post hoc Tukey’s test showed a significant difference between each venom concentration (Table 2).

The results of HPLC analysis of the venom with the separated venom components are shown in Fig 3. From the literature, the effective small peptides were targeted as the main bioactive compounds that can produce antimicrobial activity so two pure peptides that isolated at retention time 34.778 and 39.693 were sequenced to identify their identity. The sequence result shows that the two peptides have belonged to Mastoparan (a group of toxic peptides that are common in wasp venoms) (Table 3). The two new peptides were given the Acronym of MP-PW1 and MP-PW2 where the MP represents the peptide group mastoparan and the PW represents the wasp species *Polistes wattii*. The chemical drawing software indicated the spiral shape 3D dimension of the isolated peptide that has great ability to disintegrate the phospholipid bilayer of bacterial cells (Fig 4).

**Discussion**

The drug-resistant microbial infection poses the most perilous issue in health sector due to the escalating health costs and loss of human resources [40]. World is now facing pandemic attacks from ‘superbugs’ that are resistant to almost all the known antibiotics in use today [41]. Researches ramified in varied related streams like phytomedicine, ethnomedicine and nanomedicine, which searched for effective antimicrobials that could replace the existing antibiotics or could potentiate their action so as to curb the menace created by multidrug resistant varieties of microbes [42–45]. Though succeeded to a certain extent, these remedies largely failed to provide a leap in the medical armamentarium of antibiotic agents [46]. The search has now

![Fig 2. a. Minimum inhibitory concentrations of wasp venom towards certain strains of gram-positive and gram-negative bacteria; b. the heat map that represents the effect of venom on each bacterial species where the darker color indicates the highest effect.](https://doi.org/10.1371/journal.pone.0264035.g002)

| Venom concentration (mg/ml) | Mean   | Standard error | Non-significant ranges |
|----------------------------|--------|----------------|-----------------------|
| 0.75                       | 1.07   | 0.3            | a                     |
| 1.25                       | 6.25   | 0.27           | b                     |
| 2.5                        | 11     | 0.8            | c                     |
| 5                          | 17.6   | 1.1            | d                     |

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extended to other natural sources like the venom derived from reptiles and insects. Among these the members of the class insecta evolved with much unique venom profile and diverse biogenic activities. In insects, wasps, belonging to the family of Vespidae distributed all around the world and with more than 5000 species has been the center of attraction owing to its diverse pharmacological activities. Wasp venom is a complex mixture of chemicals containing proteins, peptides, enzymes and small molecules. The common peptides isolated from the wasp venom are mastoparan, eumenitin, eumenitin-R, rumenitin-F, EpVP, decoralin and anoplpin [47]. The enzymes, on the other hand included hyaluronidase, α-glucosidase, phosphatase, phospholipase A2 and phospholipase B [48]. In this regard, the variety of antimicrobial, anticancer, neuroprotective anti-oxidant and anti-inflammatory activities exhibited by wasp-derived peptides are well established. The bioactive peptides derived from PWWV was investigated for its potential to target drug-resistant microbes in the present study. Present study investigated the venom at doses of 5, 2.5, 1.25 and 0.75 mg/ml against the multidrug-resistant bacterial species of Staphylococcus aureus, Streptococcus mutans, Salmonella typhimurium and Enterobacter cloacae. The results showed a statistically significant growth inhibition of Wasp venom on the selected microorganisms. Thus, the four selected organisms were inhibited by PWWV with a very predominant and dose dependent activity against Staphylococcus aureus. In earlier studies, the mastoparan-c peptide isolated from Vespa cabro venom also showed activity against drug resistant gram-positive and gram-negative microorganisms [49]. PWWV also showed the presence of two mastoparan peptides in HPLC analysis which were sequenced in the present study. Thus, PWWV forms a promising substitute for old and conventional sources of antimicrobial drugs [50]. The presence of wide ranges of antimicrobial peptides in wasp venom encourages the study of more wasp species from around the world to isolate these

![HPLC profile of Polistes wattii venom](https://doi.org/10.1371/journal.pone.0264035.g003)

Table 3. Amino acid sequences of tested mastoparan family of peptides.

| Acronym | Retention time (min.) | Sequence                      |
|---------|-----------------------|-------------------------------|
| MP-PW1  | 34.778                | Ile-Asn-Leu-Lys-Ala-Leu-Ala-Leu-Ala-Met-Lys-Ile-Leu-NH2 |
| MP-PW2  | 39.693                | Ile-Asn-Arg-Lys-Ala-Leu-Ala-Leu-Met-Met-Lys-Leu-Leu-NH2 |

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chemicals and evaluate their antagonistic activity against common multi-drug-resistant bacterial species [51].

Present work showed highest activity against *Staphylococcus aureus*, which is a known notorious pathogen causing skin and respiratory tract infections and other life-threatening conditions like infective endocarditis, toxic shock syndrome, scalded skin syndrome, osteomyelitis, necrotizing fasciitis and necrotizing pneumonia [52, 53]. The versatility and virulence of *Staphylococcal* infections are attributed to a variety of virulence factors encoded in its genes. For its pathogenicity for human skin [54], *Streptococcus mutans* is a member of the natural flora of human oral cavity mostly dwelling on dental plaques and on biofilms over dental surfaces and is considered one of the common etiological agents for dental caries [55]. *Salmonella typhimurium*, is considered the principal cause for food poisoning and accounts for 3 million deaths in endemic zones annually. It poses a huge impact on the health expenditure of several nations owing to its endemicity and its capability to cause gastroenteritis which is considered a major factor responsible for under 5 years’ mortality among children [56, 57]. *Enterobacter cloacae* is a common Gram-negative facultative, anaerobic, non-sporing bacterium of human gut which gained clinical significance recently owing to its capability to cause opportunistic and nosocomial infections in patients under mechanical ventilation [58, 59]. The four species show quite different responses to the range of diluted concentrations of PWWV. Such outcomes came compatible with other works concerning antipathogenic activity of different wasp venoms throughout the world. Various pathogens show great variation in their response to the venoms [33, 37, 57, 60, 61]. *Staphylococcus aureus* shows highest sensitivity to PWWV even at a very low concentration. This result makes this a potent candidate to be developed as a future drug resistant anti-streptococcal agent. As a common skin pathogen and a predominant species causing soft tissue infections, use of PWWV as a topical agent also could be considered. The incorporation of PWWV into oral toiletries may also be beneficial to contain *Streptococcus mutans* and its propensity to cause oral infections. In contrast, the *Enterobacter cloacae* show less sensitivity to PWWV; this could be due to the exposure of these agents to “antibiotic-pollution” leading to attainment of resistance to multiple drugs [62]. The venom of the very common wasp species *Vespa orientalis* also shows very few effects on *Enterobacter cloacae* [37].

![Fig 4. a. Discovery Studio software diagram represents the 3D orientation of mastoparan showing the spiral shape; b. Chem draw diagram which represents the molecular configuration of mastoparan: gray atoms represent Carbon, blue atoms represent Nitrogen, red atoms represent Oxygen and white atoms represent Hydrogen.](https://doi.org/10.1371/journal.pone.0264035.g004)
The chemical analysis of PWWV showed similarity to other wasp species of the family Vespidae [63]. The sequenced peptides through this study are very similar to those identified from family Vespidae with an aspartate residue in the second position and very few amino acids substitutions [64]. As all identified mastoparan the molecular orientation of the two identified antimicrobial peptides MP-PW1 and MP-PW2 have a $\alpha$-helical shape with 14 amino acid residues and an amide group at the C-terminus. Being a small molecule, it has an ability to penetrate the bacterial cell wall easily. AMPs is a versatile molecule that typically acts through a variety of mechanisms of action, which can range from direct interactions and membrane destabilization to intracellular targets [65, 66]. The chemical mode of action of antagonistic activity of these peptides needs to be reviewed and studied for help in developing very effective pharmaceutical final products that will be the antibiotics of the future.

Further works are needed to compare the sequences of different AMPs collected from other wasp species around the world. This will help in synthesizing very effective artificial peptides that could be more effective as antimicrobial agents. The way is still far from getting a complete understanding of such group of new antibiotics till found them on the market, but no doubt they will form a part of our future medicines.

**Supporting information**

**S1 File.** Inhibition zone induced by wasp venom using well diffusion method on the left side the control using solvent only and on the right side the venom application (all photos represent the high concentration of the venom): a. *Staphylococcus aureus*; b. *Streptococcus mutans*; c. *Salmonella typhimurium*; d. *Enterobacter cloacae*.

**Author Contributions**

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**References**

1. Wang H, Hashimoto S, Moriguichi Y, Yue Q, Lu Z. Resource use in growing China: Past trends, influence factors, and future demand. Journal of Industrial Ecology. 2012 Aug; 16(4):481–92.

2. Dobson AP, Carper ER. Infectious diseases and human population history. Bioscience. 1996 Feb 1; 46(2):115–26.

3. Rafieian-Kopaei M. Medicinal plants and the human needs. Journal of HerbMed Pharmacology. 2012;1.

4. Mukhopadhuy MK, Banerjee P, Nath D. Phytochemicals–biomolecules for prevention and treatment of human diseases-a review. IJER. 2012; 9(7):1–32.
5. Benil PB, Nimisha P, Arokiyaraj S, Rajakrishnan R, AlFarhan A, AlAnsary A. (2020). Antitumour and anti-haematotoxic activity of Asparagus racemosus L total dissolved solids in co-administration with cyclophosphamide in mice. Journal of King Saud University-Science, 32(5), 2582–9.

6. Liu K, Xie L, Deng M, Zhang X, Luo J, Li X. Zoology, chemical composition, pharmacology, quality control and future perspective of Musk (Moschus): a review. Chinese Medicine. 2021 Dec; 16(1):1–21. https://doi.org/10.1186/s13020-020-00418-7 PMID: 33407732

7. Mahawar MM, Jaroli DP. Animals and their products utilized as medicines by the inhabitants surrounding the Ranthambhore National Park, India. Journal of Ethnobiology and Ethnomedicine. 2006 Dec; 2(1):1–5. https://doi.org/10.1186/1746-4269-2-46 PMID: 17081314

8. Mebs D. (2001). Toxicity in animals. Trends in evolution?. Toxicon, 39(1), 87–96. https://doi.org/10.1016/s0041-0101(00)00155-0 PMID: 10936625

9. Arbuckle K. (2017). Evolutionary context of venom in animals. Evolution of venomous animals and their toxins, 24, 3–31.

10. Pucca M.B., Fry B.G., Sartim M.A., Peigneur S., & Monteiro W.M. (2021). Venoms and Toxins: At the Crossroads of Basic, Applied and Clinical Immunology. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.716508 PMID: 34249021

11. Golden D. B., Marsh D. G., Kagey-Sobotka A., Freidhoff L., Szklo M., Valentine M. D., et al. (1989). Epidemiology of insect venom sensitivity. Jama, 262(2), 240–244. PMID: 2739018

12. Libersat F. (2003). Wasp uses venom cocktail to manipulate the behavior of its cockroach prey. Journal of Comparative Physiology A, 189(7), 497–508. https://doi.org/10.1007/s00359-003-0432-0 PMID: 12898169

13. De Lima P. R., & Brochetto-Braga M. R. (2003). Hymenoptera venom review focusing on Apis mellifera. Journal of Venomous Animals and Toxins including Tropical Diseases, 9(2), 149–162.

14. Pennacchio F., & Strand M. R. (2006). Evolution of developmental strategies in parasitic Hymenoptera. Annu. Rev. Entomol., 51, 233–258. https://doi.org/10.1146/annurev.ento.51.110104.151029 PMID: 16332211

15. Moffitt J. E. (2003). Allergic reactions to insect stings and bites. Southern Medical Journal, 96(11), 1073–1080. https://doi.org/10.1097/01.SMJ.0000097885.28467.21 PMID: 14633254

16. Gupta RK, Stangaciu S. Apitherapy : holistic healing through the honeybee and bee products in countries with poor healthcare system. In Beekeeping for poverty alleviation and livelihood security 2014 (pp. 413–446). Springer, Dordrecht.

17. Alqutub A. N., Masoodi I., Alsayari K., & Alomair A. (2011). Bee sting therapy-induced hepatotoxicity: A case report. World Journal of Hepatology, 3(10), 268. https://doi.org/10.4254/wjh.v3.i10.268 PMID: 22059110

18. King G. F. (2011). Venoms as a platform for human drugs: translating toxins into therapeutics. Expert opinion on biological therapy, 11(11), 1469–1484. https://doi.org/10.1517/14712598.2011.621940 PMID: 21939428

19. Yacoub T., Rima M., Karam M., Sabatier J. M., & Fajloun Z. (2020). Antimicrobials from venomous animals: An overview. Molecules, 25(10), 2402. https://doi.org/10.3390/molecules25102402 PMID: 32455792

20. Amer A., Hamdy B., Mahmoud D., Elanany M., Rady M., Alahmadi T., et al. (2021). Antagonistic Activity of Bacteria Isolated from the Periplaneta americana L. Gut against Some Multidrug-Resistant Human Pathogens. Antibiotics, 10(3), 294. https://doi.org/10.3390/antibiotics10030294 PMID: 33799712

21. Willyard C. (2017). The drug-resistant bacteria that pose the greatest health threats. Nature News, 543 (7643), 15. https://doi.org/10.1038/nature.2017.21550 PMID: 28252092

22. Parish T. (2019). Steps to address anti-microbial drug resistance in today’s drug discovery. Expert opinion on drug discovery, 14(2), 91–94. https://doi.org/10.1080/17460441.2019.1550481 PMID: 30466326

23. Helms M., Vastrup P., Germer-Smidt P., & Melbak K. (2002). Excess mortality associated with antimicrobial drug-resistant Salmonella Typhimurium. Emerging infectious diseases, 8(6), 490. https://doi.org/10.3201/eid0806.010267 PMID: 11996664

24. Hiramatsu K., Katayama Y., Matsuo M., Sasaki T., Morimoto Y., Sekiguchi A., & Baba T. (2014). Multidrug-resistant Staphylococcus aureus and future chemotherapy. Journal of Infection and Chemotherapy, 20(10), 593–601. https://doi.org/10.1016/j.jiac.2014.08.001 PMID: 25172776

25. Tang Q., Song P., Li J., Kong F., Sun L., & Xu L. (2016). Control of antibiotic resistance in China must not be delayed: the current state of resistance and policy suggestions for the government, medical facilities, and patients. Bioscience trends, 10(1), 1–6. https://doi.org/10.5582/bst.2016.01034 PMID: 26961210

26. Slifka M. K., & Amanna I. J. (2018). Passive immunization. Plotkin's Vaccines, 84.
27. Gordillo Altamirano F. L., & Barr J. J. (2019). Phage therapy in the postantibiotic era. Clinical microbiology reviews, 32(2), e00066–18. https://doi.org/10.1128/CMR.00066-18 PMID: 30651225

28. Bhagavathy S., Sumathi P., & Bell I. J. S. (2011). Green algae Chlorococcum humicola: a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine, 1(1), S1–S7.

29. Konno K., Hisada M., Fontana R., Lorenzi C. C., Naoki H., Itagaki Y., et al. (2001). Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp Anoplius samariensis. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1550(1), 70–80. https://doi.org/10.1016/S0167-4888(01)00271-0 PMID: 11738089

30. Fratini F., Cilia G., Turchi B., & Felicioli A. (2017). Insects, arachnids and centipedes venom: A powerful weapon against bacteria. A literature review. Toxicon, 130, 91–103. https://doi.org/10.1016/j.toxicon.2017.02.020 PMID: 28242227

31. Palma M. S. (2006). Insect venom peptides. In Handbook of biologically active peptides (pp. 389–396). Academic Press.

32. Bahar A. A., & Ren D. (2013). Antimicrobial peptides. Pharmaceuticals, 6(12), 1543–1575. https://doi.org/10.3390/ph6121543 PMID: 24287494

33. Yang X., Wang Y., Lee W. H., & Zhang Y. (2013). Antimicrobial peptides from the venom gland of the social wasp Vespa tropica. Toxicon, 74, 151–157. https://doi.org/10.1016/j.toxicon.2013.08.056 PMID: 24012601

34. Carpenter J. M., & Gadallah N. S. (2013). Biodiversity of the aculeate wasps (Hymenoptera: Aculeata) of the Arabian Peninsula: Vespoidea, Vespidae. Zootaxa, 4754(1), 191–216. https://doi.org/10.11646/zootaxa.4754.1.20 PMID: 32230225

35. Besson T., Debayle D., Diochot S., Salinas M., & Lingueglia E. (2016). Low cost venom extractor based on Arduino® board for electrical venom extraction from arthropods and other small animals. Toxicon, 118, 156–161. https://doi.org/10.1016/j.toxicon.2016.05.001 PMID: 27158113

36. Nalawade T. M., Bhat K. G., & Sogi S. (2016). Antimicrobial activity of endodontic medicaments and vehicles using agar well diffusion method on facultative and obligate anaerobes. International journal of clinical pediatric dentistry, 9(4), 335. https://doi.org/10.5005/jp-journals-10005-1388 PMID: 28127166

37. Farag R., & Swaby S. (2018). Antimicrobial effects of wasp (Vespa orientalis) venom. Egyptian Pharmaceutical Journal, 17(3), 218.

38. Kele M., & Guiochon G. (2001). Repeatability and reproducibility of retention data and band profiles on reversed-phase liquid chromatography columns: V. Results obtained with Vydc 218TP C18 columns. Journal of Chromatography A, 913(1–2), 89–112. https://doi.org/10.1016/s0021-9673(00)01042-6 PMID: 11355847

39. Henzel W., Admon A., Carr S., Davis G., De Jongh K., Lane W., et al. (2000). ABRF-98SEQ: Evaluation of peptide sequencing at high sensitivity. Journal of biomolecular techniques: JBT, 11(2), 92. PMID: 19499042

40. Norby S. R., Nord C. E., & Finch R. (2005). Lack of development of new antimicrobial drugs: a potential serious threat to public health. The Lancet infectious diseases, 5(2), 115–119. https://doi.org/10.1016/S1473-3099(05)01283-1 PMID: 15680781

41. Gootz T. D. (2010). The global problem of antibiotic resistance. Critical Reviews™ in Immunology, 30(1). https://doi.org/10.1615/critrevimmunol.v30.i1.60 PMID: 20370622

42. Mundy L, Pendry B, Rahman M. Antimicrobial resistance and synergy in herbal medicine. Journal of Herbal Medicine. 2016 Jun 1; 6(2):53–8.

43. Anand U, Jacobo-Hererra N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019 Nov 9(11):258. https://doi.org/10.3390/metabo9110258 PMID: 31683833

44. Roy A, Saral S. Ethnomedicinal Approach in Biological and Chemical Investigation of Phytochemicals as Antimicrobials with Special Emphasis to Antibacterials. 10(3):206. https://doi.org/10.3390/toxins10030206 PMID: 33809401
48. Piek T, editor. Venoms of the Hymenoptera: biochemical, pharmacological and behavioural aspects. Elsevier; 2013 Oct 22.

49. Chen X.; Zhang L.; Wu Y.; Wang L.; Ma C.; Xi X.; et al. Evaluation of the bioactivity of a mastoparan peptide from wasp venom and of its analogues designed through targeted engineering. Int. J. Biol. Sci. 2018, 14, 599–607. https://doi.org/10.7150/ijbs.23419 PMID: 29904274

50. Monteiro M. C., Romao P. R., & Soares A. M. (2009). Pharmacological perspectives of wasp venom. Protein and peptide letters, 16(8), 944–952. https://doi.org/10.2174/092986609789823275 PMID: 19689421

51. Freire D. O., da Cunha N. B., Leite M. L., Kostopoulos A. G., da Silva S. N., de Souza A. C., et al. (2020). Wasp venom peptide, synoeca-MP, from Synoeca surinama shows antimicrobial activity against human and animal pathogenic microorganisms. Peptide Science, 112(3), e2414. https://doi.org/10.1002/psc.4063

52. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, et al. Severe community-onset pneumonia in healthy adults caused by methicillin resistant Staphylococcus aureus carrying the Panton-Valentine leukocidin genes. Clin Infect Dis. 2005; 40:100–107. https://doi.org/10.1086/427148 PMID: 15614698

53. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles. N Engl J Med. 2005; 352:1445–1453. https://doi.org/10.1056/NEJMoa042683 PMID: 15814880

54. Boldock E., Surewaard B. G., Shamarina D., Na M., Fei Y., Ali A., et al. (2018). Human skin commensals augment Staphylococcus aureus pathogenesis. Nature microbiology, 3(8), 881–890. https://doi.org/10.1038/s41564-018-0198-3 PMID: 30013237

55. Lembo F. L., Longo P. L., Ota-Tsuzuki C., Rodrigues C. R. M. D., & Mayer M. P. A. (2007). Genotypic and phenotypic analysis of Streptococcus mutans from different oral cavity sites of caries-free and caries-active children. Oral microbiology and immunology, 22(5), 313–319. https://doi.org/10.1111/j.1399-302X.2007.00361.x PMID: 17803628

56. Pui CF, Wong WC, Chai LC, Tunung R, Jayalechthum P, Noor Hidayah MS, et al. (2011) Review article Salmonella: a foodborne pathogen. Int Food Res J 18:465–473.

57. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles. N Engl J Med. 2005; 352:1445–1453. https://doi.org/10.1056/NEJMoa042683 PMID: 15814880

58. Boldock E., Surewaard B. G., Shamarina D., Na M., Fei Y., Ali A., et al. (2018). Human skin commensals augment Staphylococcus aureus pathogenesis. Nature microbiology, 3(8), 881–890. https://doi.org/10.1038/s41564-018-0198-3 PMID: 30013237

59. Lembo F. L., Longo P. L., Ota-Tsuzuki C., Rodrigues C. R. M. D., & Mayer M. P. A. (2007). Genotypic and phenotypic analysis of Streptococcus mutans from different oral cavity sites of caries-free and caries-active children. Oral microbiology and immunology, 22(5), 313–319. https://doi.org/10.1111/j.1399-302X.2007.00361.x PMID: 17803628

60. Pui CF, Wong WC, Chai LC, Tunung R, Jayalechthum P, Noor Hidayah MS, et al. (2011) Review article Salmonella: a foodborne pathogen. Int Food Res J 18:465–473.

61. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles. N Engl J Med. 2005; 352:1445–1453. https://doi.org/10.1056/NEJMoa042683 PMID: 15814880

62. Boldock E., Surewaard B. G., Shamarina D., Na M., Fei Y., Ali A., et al. (2018). Human skin commensals augment Staphylococcus aureus pathogenesis. Nature microbiology, 3(8), 881–890. https://doi.org/10.1038/s41564-018-0198-3 PMID: 30013237

63. Čeřovsky V., Slaninová J., Fučíková V., Hulačová H., Borovičkova L., Ježek R., et al. (2008). New potent antimicrobial peptides from the venom of Polistinae wasps and their analogs. Peptides, 29(6), 992–1003. https://doi.org/10.1016/j.peptides.2008.02.007 PMID: 18375018

64. Torres M. D., Andrade G. P., Sato R. H., Pedron C. N., Manieri T. M., Cerchiaro G., et al. (2018). Natural and redesigned wasp venom peptides with selective antitumoral activity. Beilstein Journal of Organic Chemistry, 14(1), 1693–1703. https://doi.org/10.3762/bjc.14.144 PMID: 30013694

65. de la Fuente-Nunez C., Torres M. D., Mojica F. J., & Lu T. K. (2017). Next-generation precision antimicrobials: towards personalized treatment of infectious diseases. Current opinion in microbiology, 37, 95–102. https://doi.org/10.1016/j.mib.2017.05.014 PMID: 28623720

66. Torres M. D., Sothiselvam S., Lu T. K., & de la Fuente-Nunez C. (2019). Peptide design principles for antimicrobial applications. Journal of molecular biology, 431(18), 3547–3567. https://doi.org/10.1016/j.jmb.2018.12.015 PMID: 30611750