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ORIGINAL PAPER

Exploring the antibacterial potential of venoms from Argentinian animals

Micaela A. Gritti[1,2] · Karen Y. González[1,3] · Flavio L. Tavares[4] · Gladys P. Teibler[3] · María E. Peichoto[1,2,4]

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Abstract

The resistance to antimicrobials developed by several bacterial species has become one of the main health problems in recent decades. It has been widely reported that natural products are important sources of antimicrobial compounds. Considering that animal venoms are under-explored in this line of research, in this study, we screened the antibacterial activity of venoms of eight snake and five lepidopteran species from northeastern Argentina. Twofold serial dilutions of venoms were tested by the agar well-diffusion method and the minimum inhibitory concentration (MIC) determination against seven bacterial strains. We studied the comparative protein profile of the venoms showing antibacterial activity. Only the viperid and elapid venoms showed remarkable dose-dependent antibacterial activity towards most of the strains tested. *Bothrops diporus* venom showed the lowest MIC values against all the strains, and *S. aureus* ATCC 25923 was the most sensitive strain for all the active venoms. *Micrurus baliocoryphus* venom was unable to inhibit the growth of *Enterococcus faecalis*. Neither colubrid snake nor lepidopteran venoms exhibited activity on any bacterial strain tested. The snake venoms exhibiting antibacterial activity showed distinctive protein profiles by SDS–PAGE, highlighting that we could reveal for the first time the main protein families which may be thought to contribute to the antibacterial activity of *M. baliocoryphus* venom. This study paves the way to search for new antibacterial agents from Argentinian snake venoms, which may be a further opportunity to give an added value to the local biodiversity.

Keywords Antimicrobial · Bioprospection · Lepidoptera · Serpentes · Venom

Background

Antimicrobial resistance developed by several species of bacterial pathogens has become one of the major global health problems in the last decades. The emergence of newer resistant strains overcomes the challenge for developing novel, potent, and less toxic agents from natural sources against various infectious agents (Unemo et al. 2016; Upadhya et al. 2018). It has been extensively reported that natural products are important sources of medicinal compounds. Some of them have shown efficacy in killing bacteria or stopping/slowing down its growth. Animal venoms are produced by specialized organs called "venom gland" that are used for the production of toxic substances (mixture of proteins/peptides) deployed by injection into prey (Kularatne and Senanayake 2014; Utkin 2015). These animal venoms are remarkable natural sources for bioprospection of new molecules in drug research. Recent reports about molecules from animal venoms—bees, caterpillars, scorpions, spiders, snakes, lizards, among others—have postulated them as chemotherapeutic agents against infectious pathogens (Perumal Samy et al. 2017; Liu et al. 2018; Charvat et al. 2018). These therapeutic benefits justify their use for decades in traditional medicine (Yacoub et al. 2020). In addition, the understanding of venom microenvironments has

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✉ María E. Peichoto
mepeichoto@yahoo.com.ar; mepeichoto@conicet.gov.ar

1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

2 Instituto Nacional de Medicina Tropical (INMeT)-ANLIS "Dr. Carlos G Malbrán", Almafuerte y Ambar s/n, 3370 Puerto Iguazú, Argentina

3 Facultad de Ciencias Veterinarias (FCV), Universidad Nacional del Nordeste (UNNE), Sargento Cabral 2139, 3400 Corrientes, Argentina

4 Universidade Federal da Integração Latino-Americana (UNILA), Av. Tarquínio Joslin dos Santos, nº. 1.000, Jd. Universitário, Foz do Iguaçu, PR CEP 85870-901, Brazil

been purposed as a new and intertwined approach between venomics and venom-microbiomics that may open avenues to search new antimicrobial compounds (Adnani et al. 2017; Ul-Hasan et al. 2019).

Snake venoms are vast natural biological resources and each of them contains several components with potential therapeutic value across a broad range of diseases. However, snake venoms have not been thoroughly explored till date for their antimicrobial potency (Alam et al. 2019). In Argentina, research investigating the antimicrobial properties of snake venoms are scarce. Bustillo et al. (2008) verified the antibacterial activity of the venom of *Bothrops alternatus* against Gram-positive and -negative bacteria. However, there is an important number of non-studied venoms of snake species in Argentina, mainly in the subtropical northeastern region (Giraudo et al. 2012), opening a wide and unexplored field for the search of new biomolecules with antimicrobial action.

Lepidopterans belong to one of the most widely distributed and recognized group of insects around the world. They represent, together with hymenopterans, dipterans and coleopterans, one of the four most plentiful orders of the Insecta subphylum (Powell 2009). Lepidopterans are insects of complete metamorphosis, including four different stages: egg, larva (caterpillar), pupa (chrysalis) and adult moth (imago). Caterpillar bodies and some female moth abdomens are covered by bristles/spines/setae presenting in their cavities a mixture of macromolecules (venom and/or hemolymph) that play various biological roles. Although many species cause envenomation in humans, only few venoms have been thoroughly investigated, and they deserve attention in a bioprospecting context (Specht et al. 2008; Seldeslachts et al. 2020).

Aiming to go deeply into the research of animal venoms as a source of drug leads, and to provide an added value to the local biodiversity, in this study, we explore the rich and underexplored antibacterial potential of several snake and lepidopteran venoms from northeastern Argentina. We chose representatives—available in captivity or by authorized capture—of the main families of both group of venomous animals (Colubridade, Elapidae and Viperidae for snakes, and Megalopygidae and Saturniidae for lepidopterans).

Methods

Snake venoms

Pooled venom samples from adult specimens (≥ 1 m snout-vent length-SVL, except for *Leptodeira annula pulchriceps* with SVL ≥ 60 cm) of Colubridae (*Leptophis ahaetulla marginatus, Leptodeira annulata pulchriceps, Erythrolamprus aesculapii, Philodryas olfersii olfersii, Pseudablabes patagoniensis*), Elapidae *(Micrurus baliocoryphus*) and Viperidae (*Bothrops alternatus, Bothrops diporus)* snakes were used in this study. These animals were collected from the wild in northeastern Argentina by the local population, delivered at the serpentarium of the Centro Interactivo de Serpientes Venenosas de Argentina (CISVA, College of Veterinarian Sciences, University of Northeastern Argentina, Argentina) and kept in captivity; all snakes were provided water ad lib and fed with rodents. After the quarantine period, and being in good health, their venoms were collected sporadically. Venom samples were lyophilized and stored at − 20 °C. When required, venoms were dissolved in phosphate-buffered saline (PBS) pH 7.4, and filtered through a 0.22 mm Millipore membrane to remove insoluble material and to sterilize. The protein content of venom was determined by fluorometry using the Qubit 2.0 (Life Technologies, USA).

Lepidopteran venoms

Specimens of *Lonomia obliqua, Leucanella memusae, Podalia orsilochus, Megalopyge albicollis* and *Hylesia* aff. *nigridorsata* were collected in Misiones province, Argentina (authorized by the Ministry of Ecology and Natural Renewable Resources of this province, according to authorization numbers 050-072/17, 016-036/18, 012/19 and 003/20), transported, and maintained briefly in the Insectarium of the National Institute of Tropical Medicine (INMeT, Argentina) until use. Preparation of bristle extract (venom) was carried out by manually removing (with a scissor for caterpillars in last instar, and a soft brush for adult female *H*. aff. *nigridorsata* moth) the bristles (including spines and hairs/setae) from the whole caterpillar bodies or the whole moth abdomens, homogenizing them in cold PBS, and then the suspension was centrifuged and filtered to remove insoluble material (Quintana et al. 2017; Sánchez et al. 2019). The protein content of venom was determined by fluorometry using the Qubit 2.0 (Life Technologies, USA), and thereafter aliquots were stored at − 20 °C until use.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

The protein profile of venoms was analyzed by SDS–PAGE using 4% stacking and 12% resolving gels (Laemmli 1970), and Tricine–SDS–PAGE using 4% stacking, 10% spacer and 16.5% resolving gels (Schägger and von Jagow 1987). In both cases, samples were run under reducing (with 2-mercaptoethanol) and non-reducing (without 2-mercaptoethanol) conditions. Gels were stained with silver (Blum et al. 1987) or Coomassie G-250 (Candiano et al. 2004).

Mass spectrometry

For protein identification of the main components in *M. baliocoryphus* venom, target bands were excised from Tricine–SDS–PAGE gels stained with Coomassie Brilliant Blue G 250. Protein digestion and Mass Spectrometry analysis were performed at the Proteomics Core Facility CEQUIBIEM, at the University of Buenos Aires/CONICET as follows: excised protein bands were sequentially washed with 50 mM ammonium bicarbonate (AB), 25 mM AB, 50% ACN, and 100% ACN; reduced and alkylated with 20 mM dithiothreitol (DTT) and 60 mM iodoacetamide (IAA), and in-gel digested with 100 ng Trypsin (Promega V5111) in 25 mM AB overnight at 37 °C. Peptides were recovered by elution with 50% ACN/0.5% TFA, including brief sonication, and then further concentrated by speed-vacuum drying. Samples were resuspended in 15 µL of water containing 0.1% Formic Acid (FA), desalted using C18 zip tips (Merck Millipore), and eluted in 10 µL of H_2O:ACN:FA 40:60:0.1%. Samples were dried and resuspended in 15 µL of water containing 0.1% Formic Acid (FA). Digests were analyzed by nanoLC–MS/MS in a nanoHPLC EASY-nLC 1000 (Thermo Scientific) coupled to a QExactive Mass Spectrometer. A 75-min gradient of H_2O:ACN at a flow of 33 nL/min was used with a C18 2 mm Easy Spray column × 150 mm. Data-dependent MS2 method was used to fragment the top 15 peaks in each cycle. The raw data from mass spectrometry analysis were processed using the Proteome Discoverer, version 2.2.0388 (Thermo Scientific) software for database searching with the SEQUEST search algorithm. The search was performed against a Uniprot database generated using 'snake' and 'venom' as keywords. In the search parameters trypsin was selected as the enzyme used. Precursor mass tolerance was set to 10 ppm and product ion tolerance to 0.05 Da. Static modification was set to carbamidomethylation of Cys, and dynamic modifications were set to oxidation of Met and N-terminal acetylation. Protein hits were filtered for high confidence peptide matches with a maximum protein and peptide false discovery rate of 1% calculated by employing a reverse database strategy.

Antimicrobial activity

The antibacterial activity of venoms was tested against the following strains: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* ATCC 17666, *Enterococcus faecalis* ATCC 29212. Their bacterial features are summarized in Table 1. These bacterial strains were obtained as a generous gift from a collaborator's laboratory, and preserved in 20% glycerol at – 20 °C until use.

Agar diffusion assay

This was used to screen the antibacterial activity of venoms and was carried out according to the general guidelines of the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) (CLSI 2022), with minor modifications as described by Nair et al. (2007). Bacterial suspension corresponding to the 0.5 Mac Farland scale was distributed in a sterile Petri dish in culture medium (Müller–Hinton agar). Wells of 3-mm diameter were made in the agar using a sterile punch, and 10 µL of twofold serial dilutions of each venom sample were placed in wells. One of the wells was used as a negative control, seeding 10 µL of PBS, while a commercial antibiotic was used as a positive control. The plates were examined after incubation at 37 ºC for 24 h.

Table 1 Bacterial strains used in this study and their features according to the criteria defined by the CLSI (2022)

Bacterial strains	Features
Staphylococcus aureus ATCC 25923	β-Lactamase negative, mecA negative
ATCC 29213	Weak β-lactamase, mecA negative
Enterococcus faecalis ATCC 29,212	Resistance to Cephalosporins, Aminoglycosides, Clindamycin, Quinupristin–dalfopristin, Trimethoprim, Trimethoprim–sulfamethoxazole, Fusidic Acid
Pseudomonas aeruginosa ATCC 27853	Resistance to Ampicillin, Amoxicillin, Ampicillin–sulbactam, Amoxicillin-clavulanate, Cefotaxime, Ceftriaxone, Ertapenem, Tetracyclines/Tigecycline, Trimethoprim, Trimethoprimsulfamethoxazole, Chloramphenicol
Stenotrophomonas maltophilia ATCC 17666	Resistance to Ampicillin, Amoxicillin, Piperacillin, Ticarcillin, Ampicillin–sulbactam, Amoxicillin-clavulanate, Piperacillin–tazobactam, Cefotaxime, Ceftriaxone, Aztreonam, Imipenem, Meropenem, Ertapenem, Aminoglycosides, Tetracyclines but not to doxycycline, minocycline, or tigecycline, Trimethoprim, Fosfomycin
Escherichia coli ATCC 25922	β-Lactamase negative
ATCC 35218	β-Lactamase positive (TEM-1)

Diameters of the growth-inhibition halos were measured in mm. For those venoms that did not exhibit any activity in solid agar medium, an assay in liquid growth medium was carried out to confirm the negative result. Assays were performed at least three times in triplicate.

Determination of the minimum inhibitory concentration (MIC)

Based on the preliminary screening, venoms that revealed potent antibacterial activity were further tested to determine the MIC for each bacterial strain. In the case of strains of the same species, we evaluated only that showing the highest inhibitory effect. The MIC of these venoms was determined by the broth dilution method in accordance with the CLSI guidelines (CLSI 2022), with minor modifications as described by Piaru et al. (2012) and Shi et al. (2007). Suspension of the microorganism was prepared to contain approximately 10^5 colony-forming units per milliliter (CFU/mL) in Mueller–Hinton–Broth medium (MHB medium). The MIC was determined by the microdilution method at concentrations from 60 to 0.1 mg/mL. Ten µL of twofold serial dilutions of each venom was poured in 96-well microplates, and then 100 µL of bacterial suspension was added to each well. The microplate was incubated at 37 °C for 24 h; thereafter, the bacterial growth was first determined by measuring turbidity (absorbance at 650 nm). Sequentially, a colorimetric assay based on the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was carried out according to the protocols of Piaru et al. (2012) and Shi et al. (2007) with our own modifications. Briefly, 10 µL of MTT solution (5 mg/mL of MTT in PBS) was added to each well and incubated for 30 min, then 100 µL of a 10% SDS solution was added and incubated for an additional 60 min. The MICs were defined as the lowest venom concentrations showing no color change and exhibited complete inhibition of bacterial growth. Experimental samples were evaluated in triplicate and experiments were repeated at least twice.

Results and discussion

Bacterial infections are one of the major causes of human diseases and death in both developing and developed countries (Willyard 2017; Jones et al. 2008). This is mainly attributed to the emergence of multi-drug resistant (MDR) bacteria stimulated by antibiotic overuse in people, animals, and the environment, as well as a dearth of new antibiotic development (Alekshun and Levy 2007; Piddock 2016). The exploration of animal venoms—rich and complex natural sources of biologically active molecules—constitutes a very good strategy for finding new antibacterial drugs. In the present work, antibacterial effect of venoms of eight snake and five lepidopteran species from northeastern Argentina was studied on seven bacterial strains (three Gram-positive cocci, two non-fermenting Gram-negative bacilli, and two fermenting Gram-negative bacilli).

Of all venoms studied, only three (of snakes from the Viperidae and Elapidae families) exhibited antibacterial activity towards most of the strains tested. This is in agreement with other studies showing that venoms from both snake families display more antibacterial potency than other venoms (Perumal Samy et al. 2006). The venoms of *B. alternatus* and *B. diporus* (Viperidae family) are known by their hemotoxic effects during snake envenomation, with LD_{50} of 4.6 (Lanari et al. 2010) and 3.7 µg/g mouse (de Oliveira et al. 2011), respectively, while the venom of *M. baliocoryphus* (Elapidae family) is characterized by neurotoxic effects, exhibiting a LD_{50} of 0.5 µg/g mouse (Da Silva and D. Aird 2001). Despite these undesirable effects, they may also have desirable pharmacological effects, such as the antibacterial activity unveiled here. The molecules responsible for this effect may be used to develop chemically synthesized molecules devoid of toxicity, such was the case of Captopril®, an antihypertensive drug derived from an angiotensin converting enzyme inhibitor from *Bothrops jararaca* venom (Opie and Kowolik 1995).

Figure 1 shows representative images of the halos of inhibition obtained for *B. alternatus*, *B. diporus* and *M. baliocoryphus* venoms against the five most susceptible bacterial strains. For all cases, the inhibitory effect was dose-dependent with larger zones of inhibition observed at higher concentration, and they exhibited different efficacy levels against the bacterial strains (Fig. 2). In accordance with previous studies (Abtahi et al. 2014), and looking at both previous figures, it is apparent that both bothropic venoms displayed a broader spectrum of antibacterial activity than the *Micrurus* venom. The latter was unable to inhibit the growth of *E. faecalis* which is known to exhibit intrinsic resistance to many antibiotics and to physical and chemical stresses (Baureder et al. 2012). Noticeably, and contrary to our results, Ciscotto et al. (2009) reported no activity of *Bothrops jararacussu* and *Bothrops jararaca* venoms on *E. faecalis* and *E. coli*. In addition, Bustillo et al. (2008), which is the only previous investigation into the antibacterial screening of a venom from northeastern Argentina, showed that *B. alternatus* venom exhibits no activity against *E. faecalis*, and lower activity against *S. aureus*, *E. coli* and *P. aeruginosa* than obtained in this study. Some reasons for these differences may be attributed to the concentrations tested or even the storage conditions of venom samples used in each study, since antibacterial activity depends mainly on enzymatic activity and the stability of enzymes is dependent on the temperature of storage, particularly after dilution (Munekiyo and Mackessy 1998; Kurth and Aurich 1976).

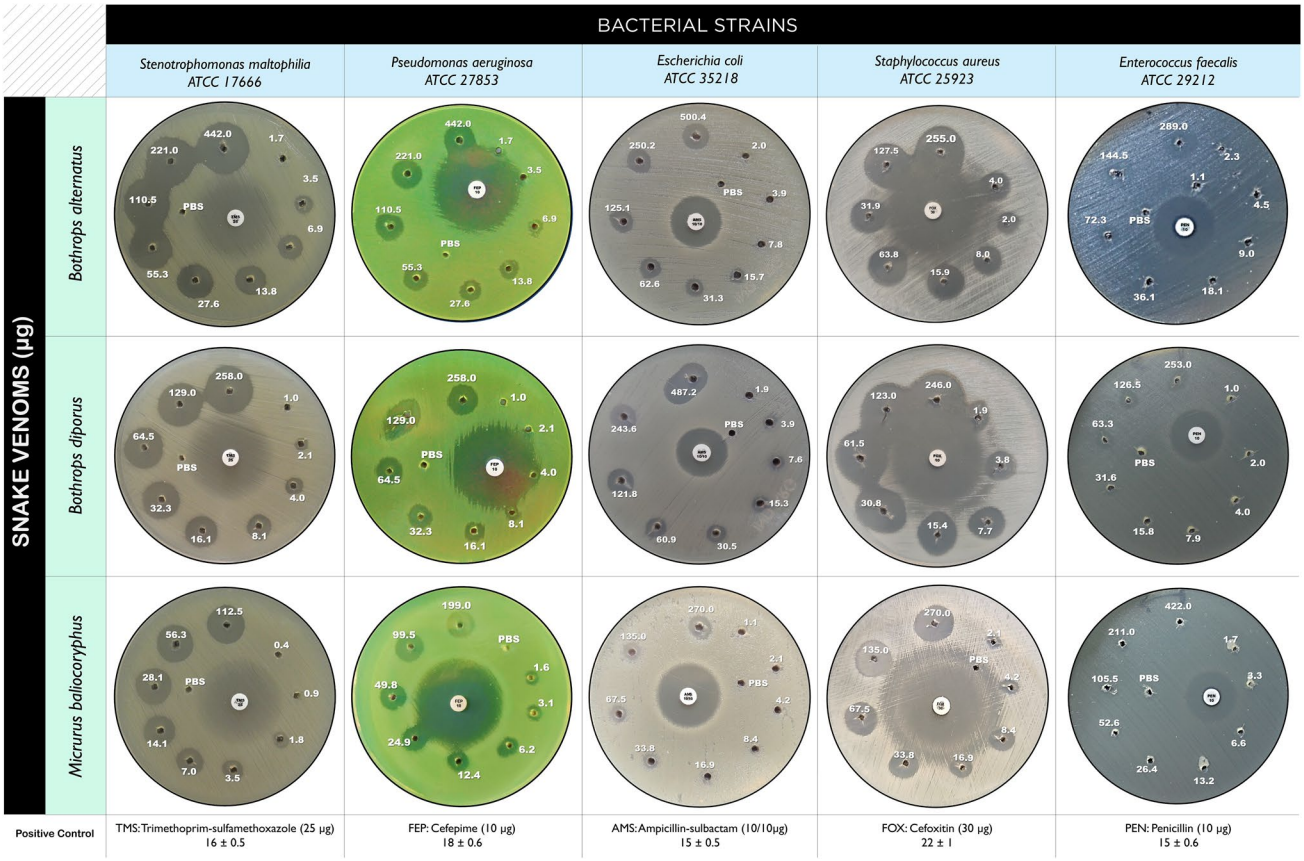

Fig. 1 Representative images of the halos of inhibition obtained for *B. alternatus*, *B. diporus* and *M. baliocoryphus* venoms against the five bacterial species tested. Data are representative of at least three experiments done in at least triplicates with freshly dissolved venom samples

Table 2 presents the comparative MIC values (in µg/mL) of both bothropic and *M. baliocoryphus* venoms against the four bacterial strains tested in this assay. We point out that our MIC values for antibiotics (controls) are in the range reported previously (CLSI 2022). As can be seen from the table, *B. diporus* venom showed the lowest MIC values against all the strains, and the *S. aureus* strain from the American Type Culture Collection (ATCC) 25923—a fully antibiotic-sensitive strain—was the most sensitive strain for all the three venoms. These results agree with previous reports documenting that venoms from the Elapidae and Viperidae are more effective against Gram-positive bacteria, such as *S. aureus* (Ciscotto et al. 2009; Rangsipanuratn et al. 2019). In addition, our MIC values for both bothropic venoms against *S. aureus* ATCC 25923 were at least three times lower than those reported previously for several viperid venoms against the same strain (Moridikia et al. 2018; Canhas et al. 2017).

It is necessary to draw attention to the relatively potent action of venoms on *Stenotrophomonas maltophilia*—with an efficacy statistically comparable to that of the fully antibiotic-sensitive strain of *S. aureus* (Fig. 2), which is a Gram-negative bacterium considered an opportunistic human pathogen that has the ability to grow in most moist environments and cause serious nosocomial infections. Treatment of *S. maltophilia* infection is very challenging due to its high level of resistance to multiple classes of antibiotics, since its genome harbors a large number of antibiotic resistance determinants, including antibiotic inactivating enzymes and efflux pumps (Tanimoto 2013; Güvenir et al. 2018). Although there are no studies testing the antimicrobial action of any venom against *S. maltophilia*, our MIC value for the venom of *B. diporus* was only fourfold greater than that for a L-amino acid oxidase (LAAO) from *Bothriechis schlegelii* venom against another non-fermenting bacillus with similar resistance determinants, *Acinetobacter baumannii* (Vargas Muñoz et al. 2014).

To determine the relatedness among the three snake species venoms showing antibacterial activity, their comparative venom composition was evaluated on SDS–PAGE. In both bothropic venoms, higher mass toxins were prevalent, whereas in *M. baliocoryphus* venom, smaller toxins predominated (Fig. 3). It is important to note that the venom proteomes of *B. alternatus* and *B. diporus* are already reported in the literature (Öhler et al. 2010; Gay et al. 2015). Whereas *B. alternatus* venom is mainly constituted

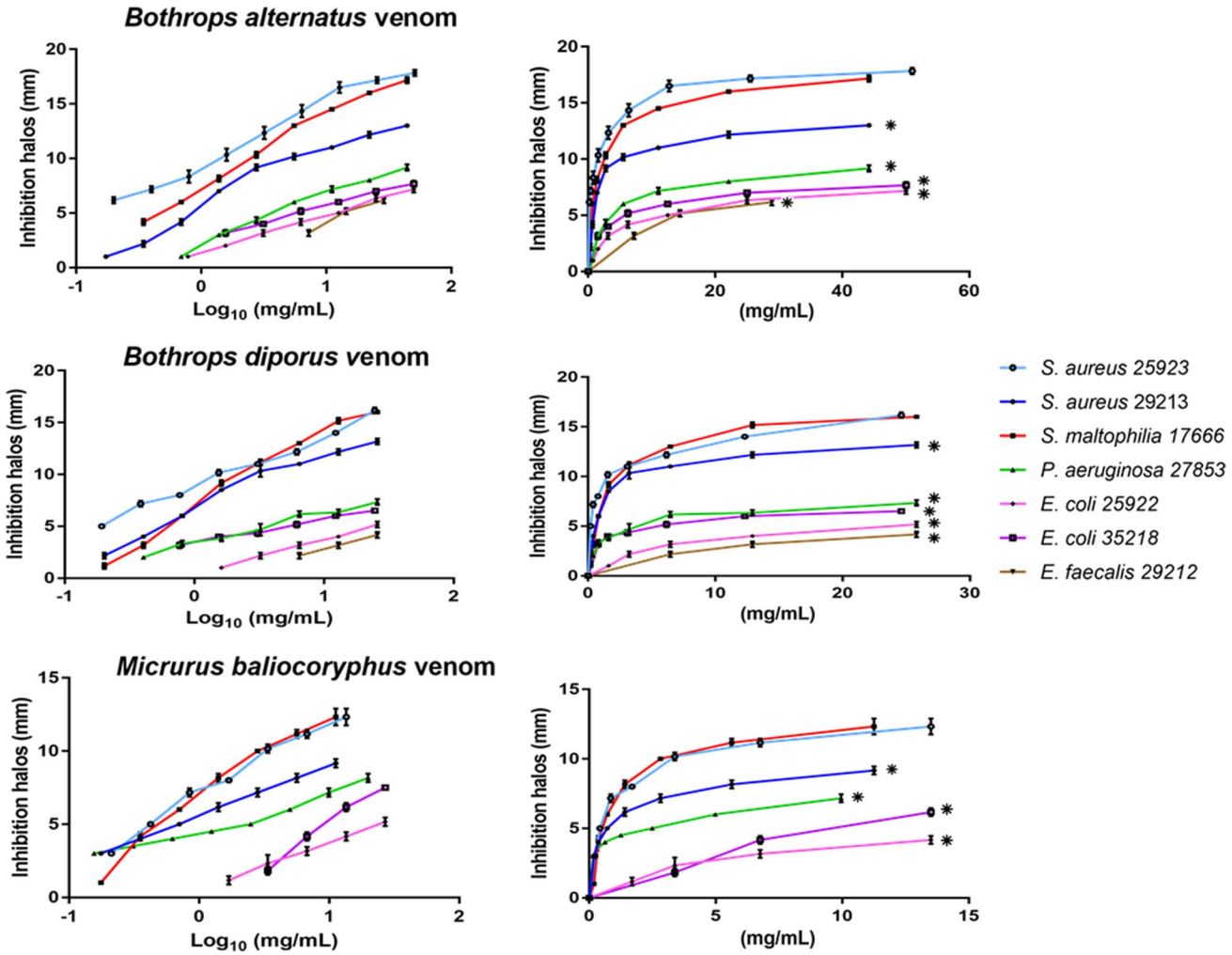

Fig. 2 Growth inhibition of bacterial strains by different concentrations of *B. alternatus*, *B. diporus* and *M. baliocoryphus* venoms. Left: log concentration–response curves showing the concentration-dependent effect. Right: linear concentration–response curves showing the maximum efficacy levels (plateau) against each bacterial strain tested. Data points show the mean and standard deviation of triplicates. An * marks statistically significant differences ($p < 0.05$) in maximum efficacy level in comparison with the fully antibiotic-sensitive strain of *S. aureus* (ATCC 25923)

Table 2 Minimum inhibitory concentration (MIC) values (in µg/mL) for *B. alternatus*, *B. diporus* and *M. baliocoryphus* venoms against the four bacterial strains tested

	Bacterial strains			
Snake venoms (µg/mL, final concentration[a])	*Stenotrophomonas maltophilia* ATCC 17666	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 35218	*Staphylococcus aureus* ATCC 25923
Bothrops alternatus	39.8 ± 3.7	144.6 ± 13.4	268.1 ± 23.3	8.4 ± 0.5
Bothrops diporus	8.4 ± 0.7	34.9 ± 2.7	64.6 ± 4.5	1.6 ± 0.2
Micrurus baliocoryphus	48.6 ± 3.1	76.7 ± 3.6	306.8 ± 15.2	11.2 ± 0.8
Antibiotic (control)	TMS[b] 1.1 ± 0.4/5.2 ± 1.8	ME[c] 1.3 ± 0.5	TMS[b] 2.1 ± 0.7/10.4 ± 3.6	TMS[b] 0.83 ± 0.4/4.2 ± 1.4

[a]Values within columns are means (left) and standard deviation (right)

[b]TMS: trimethoprim–sulfamethoxazole; [c]ME: meropenem

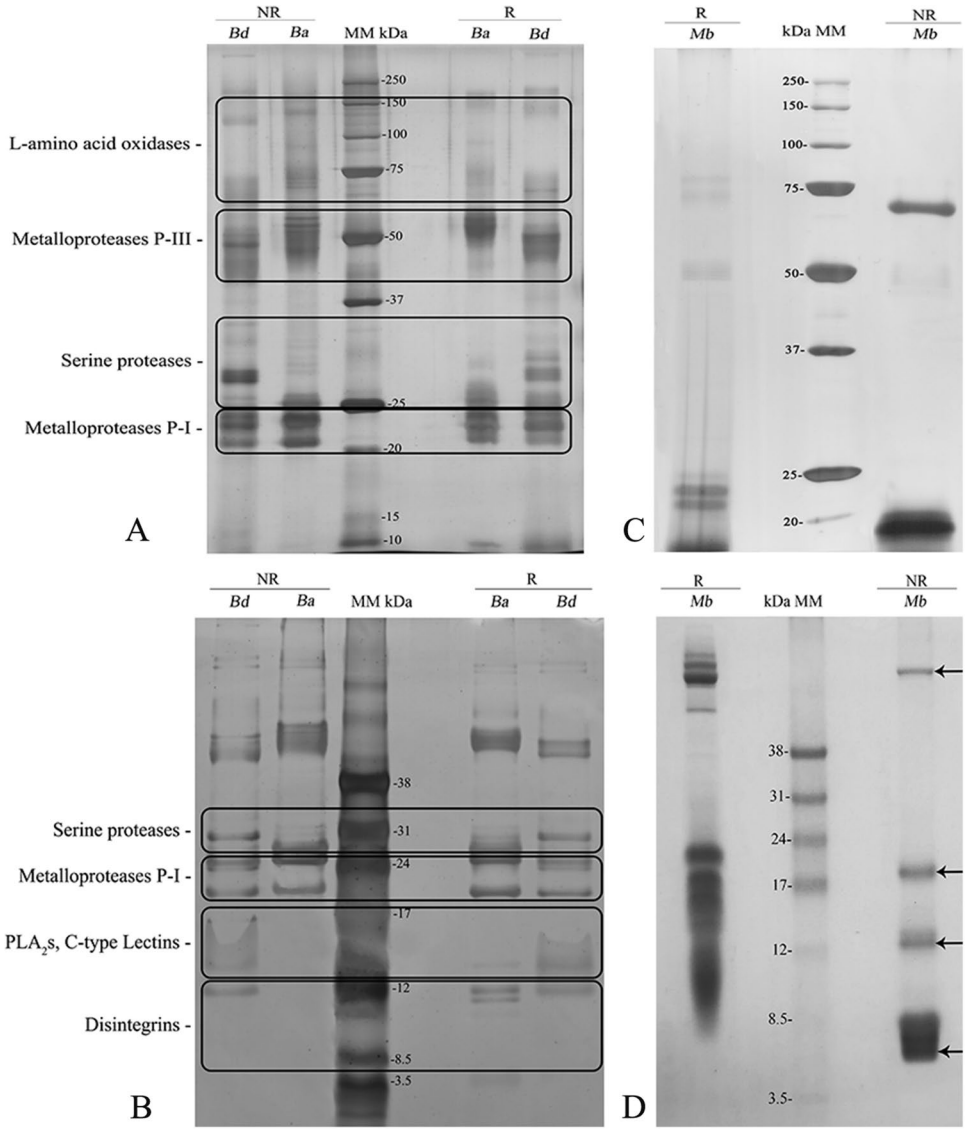

Fig. 3 **A** Electrophoretic profiles of *B. alternatus* (Ba) and *B. diporus* (Bd) venoms in 12% SDS–PAGE. Gel was silver stained. **B** Electrophoretic profiles of *B. alternatus* (Ba) and *B. diporus* (Bd) venoms in 16.5% Tricine–SDS–PAGE. Gel was silver stained. Based on the corresponding venom proteomes and published masses (Öhler et al. 2010; Gay et al. 2015), major protein families are given on the left of (**A**) and (**B**), highlighting that only protein families with known antibacterial activity are indicated. **C** Electrophoretic profile of *M. baliocoryphus* (Mb) venom in 12% SDS–PAGE. Gel was silver stained. **D** Electrophoretic profile of *M. baliocoryphus* (Mb) venom in 16.5% Tricine–SDS–PAGE, pointing out (with arrows) the protein bands identified by MS/MS analysis. Gel was stained with Coomassie Brilliant Blue G-250. In all cases, samples were run under reducing (R) and non-reducing (NR) conditions. Each lane was loaded with 10 µg of venom sample. *MM* molecular mass markers

(> 60%) by proteinases (metalloproteinases, and serine/thrombin-like proteinases) (Öhler et al. 2010), *B. diporus* venom is mainly comprised (~ 60%) of metalloproteinases, and phospholipase A_2 molecules (Gay et al. 2015). However, in agreement with de Roodt et al. (2021), we could neither find data on the proteome composition of *M. baliocoryphus* venom. To gain insights into its main components, we identified by mass spectrometry (with high hit scores) the main SDS–PAGE-separated protein bands that were stained with Coomassie Brilliant Blue G-250 (Fig. 3D). Tryptic peptides of protein bands at ~ 17 and ~ 12 kDa were matched by SEQUEST to internal sequences from a phospholipase A_2 (PLA_2) of *Micrurus altirostris* (EFVCNCDLEAANCFAK and APYIEENYNINLNR; Uniprot accession N° F5CPE9) and *Micrurus lemniscatus* (AFVCNCDR and GGSGTPVDELDR; Uniprot accession N° A0A2D4J1R3), respectively. Tryptic peptides of a protein band at ~ 6 kDa were matched to three internal sequences (ITSAFIIER, GCGCPETSR, and TCISPICYEK) from a three-finger toxin (3FTx) of *Micrurus altirostris* (Uniprot accession N° F5CPE3). In fact, based on published masses, all bands in the range of 6–9 kDa (Fig. 3D) seem to be representatives of this superfamily of proteins. The most intense protein band at ~ 70 kDa yield tryptic peptides that matched to seven internal sequences (RFDEIVGGFDR, RPLGECFR, STTDLPSR, EADYEEFLEIAR, FDEIVGGFDR, FWEADGIHGGK, YPVKPSEEGK) from a LAAO of *Micrurus tener* (Uniprot accession N° A0A194ARE6). These results are in agreement with the available composition data of other *Micrurus* venoms (Aird et al. 2017), highlighting that all of them are mainly constituted by 3FTxs and PLA_2s.

Though venom composition varies, often significantly, among species (Mackessy 2009), more closely related species of reptiles generally tend to have venoms that are

more similar in composition than do more distantly related venoms. The varying compositions may relate to their distinct effects on the various bacterial classes/strains, such as revealed here with *Bothrops* (Viperidae) and *Micrurus* (Elapidae) venoms. It is interesting to note that the differential inhibitory effect of both bothropic venoms compared to *M. baliocoryphus* venom on *E. faecalis* may be related to the presence of lectins in the former, such as demonstrated for *Bothrops leucurus* venom (Nunes et al. 2011). It is known that lectins are potent antimicrobials through binding to carbohydrates on microbial surfaces (Breitenbach Barroso Coelho et al. 2018).

Considering that antimicrobial activity of snake venoms has been widely associated with their LAAO and/or PLA_2 activities (Ciscotto et al. 2009), and that both viperid venoms show similar content of LAAO according to Ohler et al. (2010) and Gay et al. (2015), the fact that *B. diporus* venom displayed greater potency against all strains than *B. alternatus* venom may be due to their differential PLA_2 content (Fig. 3B), since the former has twice the amount of this enzyme than the latter (Öhler et al. 2010; Gay et al. 2015), and this is in agreement with the ideas of Perumal Samy et al. (2006). It is believed that PLA_2 enzymes exert their bactericidal effect by permeabilizing the bacterial membrane by forming pores (Brogden 2005).

Taking into consideration the lowest potency of the venom of *M. baliocoryphus* to all strains tested, we can infer that its distinguishing content of 3FTxs seems to have little influence on its antibacterial activity. In accordance with this, Rheubert et al. (2020) have demonstrated that 3FTX are a poor predictor of antibacterial effectiveness of venoms. On the other hand, the PLA_2 and LAAO content may be of great importance in the antibacterial action of this venom. Regarding the latter, the hydrogen peroxide (H_2O_2) generated during its enzymatic reaction is highly toxic, capable of acting on nucleic acids, proteins and plasma membranes of cells, altering the permeability of the targeted area, and can also participate in necrosis or apoptosis (Alam et al. 2019).

Despite having several and varied components with recognized antibacterial activity, venoms from the Elapidae are less effective in this regard than venoms from the Viperidae, as shown here and in other works (Abtahi et al. 2014). This may be aligned with their less complexity, but it is also worth to consider the possible presence of specific components that may function synergistically (or antagonistically) to impart (or potentially impair) the antibacterial capabilities of snake venoms (Charvat et al. 2018). For instance, it is likely that PLA_2 from elapid venoms may protect the bacterial membrane from toxic H_2O_2 generated by LAAO, with a mode of action comparable to that of a PLA_2 from Malaysian *Naja sumatrana* venom against H_2O_2-induced cell damage and apoptosis (Abdullah et al. 2022).

No activity was exhibited by any colubrid snake and lepidopteran venoms tested in this study (data not shown). Similarly, snail venoms have shown little antibiotic potency against human pathogens (Perumal Samy et al. 2017), which may be related to their microenvironments, where the composition of the microbiome play a key role. According with this point of view, Torres et al. (2017) demonstrated that the venom ducts of cone snails contain symbiotic bacteria similar to those of the genus *Stenotrophomonas*, which may suppose that similar microorganisms will be resistant to snail venoms.

Previous studies suggested that venom from the less toxic (LD50 ≥ 10 µg/g mouse) Colubridae species may contain antimicrobial properties without the adverse effects on human health (Mackessy 2002; Mackessy and Saviola 2016). However, up to now, very few studies have been conducted on the antibacterial effects of venoms from this family (Jansen 1983; Perumal Samy et al. 2007). Herein, five colubrid venoms—four rich in enzymatic components (Sánchez et al. 2019, 2021; Zelanis et al. 2010), and one, *L. a. marginatus*, rich in 3FTXs (Sánchez et al. 2018)—were tested but found to be ineffective against all bacterial strains, even at concentrations higher than 10 mg/mL. This is in line with the findings of Charvat et al. (2018) that showed, although without statistical significance, that Colubridae crude venoms were not effective against any bacterial class tested.

What is surprising is that Badari et al. (2021) have recently identified a protein with antimicrobial activity from the venom of the colubrid snake *Philodryas patagoniensis* (currently named *Pseudablabes patagoniensis*), but the whole venom of this species showed here to be devoid of antibacterial activity (in liquid and solid growth media; Fig. 4), even when tested against the same strain of *P. aeruginosa* used by them. This difference may be explained, because those authors used venom from only one adult female specimen and we used pooled venom samples from adult specimens, and/or because specimens of both studies were collected from different geographic areas (northeastern Argentina and southeastern Brazil) which is known to be related to different venom compositions. Moreover, it is important to recognize that some active proteins may be antagonized by other components present in the whole venom (Chippaux et al. 1991).

Regarding lepidopteran venoms, there are also very few studies exploring their antimicrobial properties. A recently published article by Walker et al. (2021) has showed strong antibacterial activity for *Doratifera vulnerans* limacodid caterpillar venom, which is dominated by peptide toxins (< 10 kDa). However, all venoms (from the Megalopygidae and Saturniidae families) tested here showed to be devoid of this type of activity, and they are mainly constituted by proteins > 10 kDa (Sánchez et al. 2019; Quintana et al. 2017;

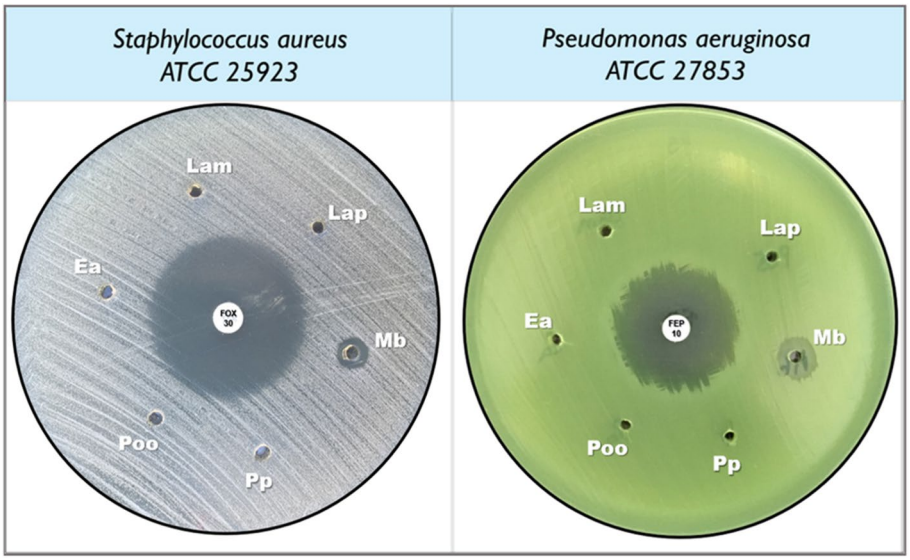

Fig. 4 Representative images showing no inhibition of bacterial growth by the five colubrid snake venoms (concentration ≥ 10 mg/mL) against a Gram-positive (left) and a Gram-negative (right) bacterium as examples, since all of them were ineffective against all bacterial strains tested here. *Lam Leptophis ahaetulla marginatus, Lap Leptodeira annulata pulchriceps, Ea Erythrolamprus aesculapii, Poo Philodryas olfersii olfersii, Pp Pseudablabes patagoniensis*. As positive controls we used: *Micrurus baliocoryphus* (Mb) venom, and the antibiotics Cefoxitin (FOX) and Cefepime (FEP)

Casafús et al. 2021). Considering that proteins/peptides from hemolymph are commonly found in any lepidopteran venom (Sánchez et al. 2019), our result is somewhat counterintuitive, since defense peptides and proteins constitute key factors in immune hemolymph against invading microorganisms (Bulet et al. 1999). However, it is interesting to note that these components are commonly associated with an inducible antibacterial immunity, i.e., expressed after immune challenge with bacteria (Jarosz 1995; Cytryńska et al. 2007; Zdybicka-Barabas and Cytryńska 2011), and we used, at least apparently, healthy specimens in this study.

Conclusion

Although animal venoms exhibit toxicological effects, still several isolated snake venom proteins, enzymes, and peptides have found practical application as pharmaceutical agents (Koh et al. 2006). In addition to providing insights for the main protein families which may be thought (at least some of them) to contribute to the antibacterial activity of *M. baliocoryphus* venom, the results presented here will contribute to the search for new antimicrobial agents exhibiting strong activity against resistant bacterial strains, such as the MDR *S. maltophilia*. Since the spectrum and potential of Argentinian snake venoms for antibacterial activity have been established, further study is in progress to purify their active components.

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Author contributions Conceived and designed the analysis: MAG, MEP; collected the data: MAG; contributed data or analysis tools: FLT, GPT, KYG; performed the analysis: MAG, MEP; wrote the original draft of the manuscript: MAG, FLT, MEP; contributed to discussion and reviewed and edited the manuscript: MAG, FLT, GPT, KYG, MEP. All authors have read and agreed to the published version of the manuscript.

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Data availability The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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Consent to participate Not applicable.

References

Abdullah NAH, Sainik NQAV, Esa E et al (2022) Neuroprotective effect of phospholipase A_2 from Malaysian *Naja sumatrana* venom against H_2O_2-induced cell damage and apoptosis. Front Pharmacol 13:1–14. https://doi.org/10.3389/fphar.2022.935418

Abtahi B, Khorjestan M, Saeedeh Ghezellou P, Aliahmadi A et al (2014) Effects of Iranian snakes venom; true sea and terrestrial snakes on some bacterial cultures. J Persian Gulf 5:27–36

Adnani N, Rajski SR, Bugni TS (2017) Symbiosis-inspired approaches to antibiotic discovery. Nat Prod Rep 34:784–814. https://doi.org/10.1039/c7np00009j

Aird SD, da Silva NJ, Qiu L et al (2017) Coralsnake Venomics: analyses of venom gland Transcriptomes and proteomes of six Brazilian taxa. Toxins (basel) 9:1–64. https://doi.org/10.3390/toxins9060187

Alam MI, O R, A MA et al (2019) Therapeutic potential of snake venoms as antimicrobial agents. Front Drug Chem Clin Res. https://doi.org/10.15761/FDCCR.1000136

Alekshun MN, Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Badari JC, Díaz-Roa A, Teixeira Rocha MM et al (2021) Patagonin-CRISP: antimicrobial activity and source of antimicrobial molecules in Duvernoy's gland secretion (*Philodryas patagoniensis* Snake). Front Pharmacol 11:1–11. https://doi.org/10.3389/fphar.2020.586705

Baureder M, Reimann R, Hederstedt L (2012) Contribution of catalase to hydrogen peroxide resistance in *Enterococcus faecalis*. FEMS Microbiol Lett 331:160–164. https://doi.org/10.1111/j.1574-6968.2012.02567.x

Blum H, Beier H, Gross HJ (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis 8:93–99. https://doi.org/10.1002/elps.1150080203

Breitenbach Barroso Coelho LC, dos Santos M, Silva P, Felix de Oliveira W et al (2018) Lectins as antimicrobial agents. J Appl Microbiol 125:1238–1252. https://doi.org/10.1111/jam.14055

Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 3:238–250. https://doi.org/10.1038/nrmicro1098

Bulet P, Hetru C, Dimarcq JL, Hoffmann D (1999) Antibacterial peptides in insects; structure and function. Dev Comp Immunol 23:329–344

Bustillo S, Leiva LC, Merino L et al (2008) Antimicrobial activity of *Bothrops alternatus* venom from the Northeast of Argentine. Rev Latinoam Microbiol 50:79–82

Candiano G, Bruschi M, Musante L et al (2004) Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25:1327–1333. https://doi.org/10.1002/elps.200305844

Canhas IN, Heneine LGD, Fraga T et al (2017) Antibacterial activity of different types of snake venom from the Viperidae family against *Staphylococcus aureus*. Acta Sci Biol Sci 39:309. https://doi.org/10.4025/actascibiolsci.v39i3.33826

Casafús MG, Favalesso MM, Gritti MA et al (2021) A hidden deadly venomous insect: first eco-epidemiological assessment and risk mapping of lonomism in Argentina. PLoS Negl Trop Dis 15:1–21. https://doi.org/10.1371/journal.pntd.0009542

Charvat RA, Strobel RM, Pasternak MA et al (2018) Analysis of snake venom composition and antimicrobial activity. Toxicon 150:151–167. https://doi.org/10.1016/j.toxicon.2018.05.016

Chippaux JP, Williams V, White J (1991) Review article snake venom variability: methods of study, results and interpretation. Toxicon 29:1279–1303

Ciscotto P, Machado de Avila RA, Coelho EAF et al (2009) Antigenic, microbicidal and antiparasitic properties of an l-amino acid oxidase isolated from *Bothrops jararaca* snake venom. Toxicon 53:330–341. https://doi.org/10.1016/j.toxicon.2008.12.004

CLSI (2022) Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically. CLSI standard M07 (11th ed), Clinical and Laboratory Standards Institute, Wayne

Cytryńska M, Mak P, Zdybicka-Barabas A et al (2007) Purification and characterization of eight peptides from *Galleria mellonella* immune hemolymph. Peptides 28:533–546. https://doi.org/10.1016/j.peptides.2006.11.010

Da Silva JN, Aird SD (2001) Prey specificity, comparative lethality and compositional differences of coral snake venoms. Comp Biochem Physiol Part C Toxicol Pharmacol 128:425–456. https://doi.org/10.1016/S1532-0456(00)00215-5

de Oliveira VC, Lanari LC, Hajos SE, de Roodt AR (2011) Toxicity of Bothrops neuwiedi complex ("yarará chica") venom from different regions of Argentina (Serpentes, Viperidae). Toxicon 57:680–685. https://doi.org/10.1016/j.toxicon.2011.01.012

de Rafael RA, Lanari LC, Ramírez JE et al (2021) Cross-reactivity of some *Micrurus* venoms against experimental and therapeutic anti-*Micrurus* antivenoms. Toxicon 200:153–164. https://doi.org/10.1016/j.toxicon.2021.07.011

dos Nunes ES, de Souza MAA, de Vaz AFM et al (2011) Purification of a lectin with antibacterial activity from *Bothrops leucurus* snake venom. Comp Biochem Physiol Part B Biochem Mol Biol 159:57–63. https://doi.org/10.1016/j.cbpb.2011.02.001

Gay C, Sanz L, Calvete JJ, Pla D (2015) Snake venomics and antivenomics of *Bothrops diporus*, a medically important pitviper in Northeastern Argentina. Toxins (basel) 8:1–13. https://doi.org/10.3390/toxins8010009

Giraudo AR, Arzamendia V, Bellini GP et al (2012) Categorización del estado de conservación de las Serpientes de la República Argentina. Cuad Herpetol 26:303–326

Güvenir M, Otlu B, Tunc E et al (2018) High Genetic diversity among *Stenotrophomonas maltophilia* isolates from single hospital: nosocomial outbreaks or genotypic profile changes during subcultures. Malays J Med Sci 25:40–49. https://doi.org/10.21315/mjms2018.25.2.5

Jansen DW (1983) A possible function of the secretion of Duvernoy's gland. Copeia 1983:262. https://doi.org/10.2307/1444728

Jarosz J (1995) Haemolymph immune proteins protect the insect body cavity from invading bacteria. Comp Biochem Physiol Part C Comp 111:213–220. https://doi.org/10.1016/0742-8413(95)00038-P

Jones KE, Patel NG, Levy MA et al (2008) Global trends in emerging infectious diseases. Nature 451:990–993. https://doi.org/10.1038/nature06536

Koh DCI, Armugam A, Jeyaseelan K (2006) Snake venom components and their applications in biomedicine. Cell Mol Life Sci 63:3030–3041. https://doi.org/10.1007/s00018-006-6315-0

Kularatne SAM, Senanayake N (2014) Venomous snake bites, scorpions, and spiders. Handb Clin Neurol 120:987–1001. https://doi.org/10.1016/B978-0-7020-4087-0.00066-8

Kurth J, Aurich H (1976) The effect of pH value and temperature on the stability of L-aminoacidoxidase from the venom of the sand viper. Acta Biol Med Ger 35:175–182

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685. https://doi.org/10.1038/227680a0

Lanari LC, Rosset S, González ME et al (2010) A study on the venom of *Bothrops alternatus* Duméril, Bibron and Duméril, from different regions of Argentina. Toxicon 55:1415–1424. https://doi.org/10.1016/j.toxicon.2010.01.015

Liu G, Yang F, Li F et al (2018) Therapeutic potential of a scorpion venom-derived antimicrobial peptide and its homologs against antibiotic-resistant Gram-positive bacteria. Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.01159

Mackessy SP (2002) Biochemistry and pharmacology of colubrid snake venoMS. J Toxicol Toxin Rev 21:43–83. https://doi.org/10.1081/TXR-120004741

Mackessy SP (2009) Handbook of venoms and toxins of reptiles. CRC Press

Mackessy SP, Saviola AJ (2016) Understanding biological roles of venoms among the Caenophidia: the importance of rear-fanged snakes. Integr Comp Biol 56:1004–1021. https://doi.org/10.1093/icb/icw110

Moridikia A, Zargan J, Sobati H et al (2018) Anticancer and antibacterial effects of Iranian viper (*Vipera latifii*) venom; an in-vitro

study. J Cell Physiol 233:6790–6797. https://doi.org/10.1002/jcp.26428
Munekiyo SM, Mackessy SP (1998) Effects of temperature and storage conditions on the electrophoretic, toxic and enzymatic stability of venom components. Comp Biochem Physiol Part B Biochem Mol Biol 119:119–127. https://doi.org/10.1016/S0305-0491(97)00294-0
Nair DG, Fry BG, Alewood P et al (2007) Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom proteins. Biochem J 402:93–104. https://doi.org/10.1042/BJ20060318
Öhler M, Georgieva D, Seifert J et al (2010) The venomics of *Bothrops alternatus* is a pool of acidic proteins with predominant hemorrhagic and coagulopathic activities. J Proteome Res 9:2422–2437. https://doi.org/10.1021/pr901128x
Opie LH, Kowolik H (1995) The discovery of captopril: from large animals to small molecules. Cardiovasc Res 30:18–25. https://doi.org/10.1016/S0008-6363(95)00006-2
Perumal Samy R, Pachiappan A, Gopalakrishnakone P et al (2006) In vitro antimicrobial activity of natural toxins and animal venoms tested against *Burkholderia pseudomallei*. BMC Infect Dis 6:100. https://doi.org/10.1186/1471-2334-6-100
Perumal Samy R, Gopalakrishnakone P, Thwin MM et al (2007) Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A_2 enzymes. J Appl Microbiol 102:650–659. https://doi.org/10.1111/j.1365-2672.2006.03161.x
Perumal Samy R, Stiles BG, Franco OL et al (2017) Animal venoms as antimicrobial agents. Biochem Pharmacol 134:127–138. https://doi.org/10.1016/j.bcp.2017.03.005
Piaru SP, Mahmud R, Perumal S (2012) Determination of antibacterial activity of essential oil of *Myristica fragrans* Houtt. using Tetrazolium microplate assay and its cytotoxic activity against vero cell line. Int J Pharmacol 8:572–576. https://doi.org/10.3923/ijp.2012.572.576
Piddock LJV (2016) Reflecting on the final report of the O'Neill review on antimicrobial resistance. Lancet Infect Dis 16:767–768. https://doi.org/10.1016/S1473-3099(16)30127-X
Powell JA (2009) Lepidoptera (moths and butterfflies). In: Resh VH, Cardé AT (eds) Encyclopedia of insects, 2a edn. Academic Press, San Diego, pp 557–587
Quintana MA, Sciani JM, Auada AVV et al (2017) Stinging caterpillars from the genera *Podalia, Leucanella* and *Lonomia* in Misiones, Argentina: a preliminary comparative approach to understand their toxicity. Comp Biochem Physiol Part C Toxicol Pharmacol 202:55–62. https://doi.org/10.1016/j.cbpc.2017.07.007
Rangsipanuratn W, Sandee A, Daduang J, Janwithayanuchit I (2019) Antibacterial activity of snake venoms against bacterial clinical isolates. Pharm Sci Asia 46:80–87. https://doi.org/10.29090/psa.2019.01.018.0003
Rheubert JL, Meyer MF, Strobel RM et al (2020) Predicting antibacterial activity from snake venom proteomes. PLoS One 15:e0226807. https://doi.org/10.1371/journal.pone.0226807
Sánchez MN, Teibler GP, López CA et al (2018) Assessment of the potential toxicological hazard of the Green Parrot Snake (*Leptophis ahaetulla marginatus*): characterization of its venom and venom-delivery system. Toxicon 148:202–212. https://doi.org/10.1016/j.toxicon.2018.04.027
Sánchez MN, Teibler GP, Sciani JM et al (2019) Unveiling toxicological aspects of venom from the Aesculapian false coral snake *Erythrolamprus aesculapii*. Toxicon 164:71–81. https://doi.org/10.1016/j.toxicon.2019.04.007
Sánchez MN, Gonzalez KY, Sciani JM et al (2021) First insights into the biochemical and toxicological characterization of venom from the banded cat-eyed snake *Leptodeira annulata pulchriceps*. Comp Biochem Physiol Part C Toxicol Pharmacol 239:108897. https://doi.org/10.1016/j.cbpc.2020.108897
Schägger H, von Jagow G (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166:368–379. https://doi.org/10.1016/0003-2697(87)90587-2
Seldeslachts A, Peigneur S, Tytgat J (2020) Caterpillar venom: a health hazard of the 21st century. Biomedicines 8:143. https://doi.org/10.3390/biomedicines8060143
Shi L, Ge H-M, Tan S-H et al (2007) Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. Eur J Med Chem 42:558–564. https://doi.org/10.1016/j.ejmech.2006.11.010
Specht A, Corseuil E, Abella HB(2008) Lepidópteros de Importância Médica: principais espécies no Rio Grande do Sul. USEB, Pelotas
Tanimoto K (2013) *Stenotrophomonas maltophilia* strains isolated from a university hospital in Japan: genomic variability and antibiotic resistance. J Med Microbiol 62:565–570. https://doi.org/10.1099/jmm.0.051151-0
Torres JP, Tianero MD, Robes JMD et al (2017) *Stenotrophomonas*-like bacteria are widespread symbionts in cone snail venom ducts. Appl Environ Microbiol. https://doi.org/10.1128/AEM.01418-17
Ul-Hasan S, Rodríguez-Román E, Reitzel AM et al (2019) The emerging field of venom-microbiomics for exploring venom as a microenvironment, and the corresponding Initiative for Venom Associated Microbes and Parasites (iVAMP). Toxicon X. 4:100016. https://doi.org/10.1016/j.toxcx.2019.100016
Unemo M, del Rio C, Shafer WM (2016) Antimicrobial resistance expressed by *Neisseria gonorrhoeae*: a major global public health problem in the 21st century. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.ei10-0009-2015
Upadhya RK, Shenoy L, Venkateswaran R (2018) Effect of intravenous dexmedetomidine administered as bolus or as bolus-plus-infusion on subarachnoid anesthesia with hyperbaric bupivacaine. J Anaesthesiol Clin Pharmacol 34:46–50. https://doi.org/10.4103/joacp.JOACP
Utkin YN (2015) Animal venom studies: current benefits and future developments. World J Biol Chem 6:28. https://doi.org/10.4331/wjbc.v6.i2.28
Vargas Muñoz LJ, Estrada-Gomez S, Núñez V et al (2014) Characterization and cDNA sequence of *Bothriechis schlegelii* l-amino acid oxidase with antibacterial activity. Int J Biol Macromol 69:200–207. https://doi.org/10.1016/j.ijbiomac.2014.05.039
Walker AA, Robinson SD, Paluzzi J-PV et al (2021) Production, composition, and mode of action of the painful defensive venom produced by a limacodid caterpillar, *Doratifera vulnerans*. Proc Natl Acad Sci 118:1–12. https://doi.org/10.1073/pnas.2023815118
Willyard C (2017) The drug-resistant bacteria that pose the greatest health threats. Nature 543:15–15. https://doi.org/10.1038/nature.2017.21550
Yacoub MF, Ferwiz HF, Said F (2020) Effect of interleukin and hepcidin in anemia of chronic diseases. Anemia 2020:1–5. https://doi.org/10.1155/2020/3041738
Zdybicka-Barabas A, Cytryńska M (2011) Involvement of apolipophorin III in antibacterial defense of *Galleria mellonella* larvae. Comp Biochem Physiol - B Biochem Mol Biol 158:90–98. https://doi.org/10.1016/j.cbpb.2010.10.001
Zelanis A, da Rocha Teixeira MM, de Fátima Furtado MD (2010) Preliminary biochemical characterization of the venoms of five Colubridae species from Brazil. Toxicon 55:666–669. https://doi.org/10.1016/j.toxicon.2009.09.015