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Systematic Review

Bactericidal Effects of Snake Venom Phospholipases A2: A Systematic Review and Analysis of Minimum Inhibitory Concentration

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Abstract: Background: Infections caused by multi-drug resistance (MDR) strains are potentially fatal public health issues worldwide that need pressing attention. Previous reports suggested using snake venom fractions as an effective alternative mechanism to the already available antibacterial drugs. In this study, we conducted a systematic review to analyze the bactericidal effects of snake venom phospholipases (PLA_2s). Methods: From the beginning through 30 March 2022, we searched the PubMed and Embase databases in accordance with the most recent PRISMA recommendations. We also conducted a manual search to identify relevant reports to improve literature coverage. Results: A total of 24 studies were included based on the selection criteria to compile this review. Of them, 16 studies were obtained from the abovementioned databases and eight through manual searches. The other 8 studies were obtained through the references of the included studies. According to the review, we reported that some PLA_2s showed more vigorous bactericidal activity on some Gram-negative and a moderate effect on Gram-negative and Gram-positive. Furthermore, we reported that the presence of p-bromophenacyl bromide (p-BPP) showed a significant decrease in enzymatic and associated antibacterial activities. Moreover, we observed that about 80% of the PLA_2s reported in our systematic review study were those from the Viperidae family, whereas 20% came from the Elapidae family. Moreover, some variations were revealed in the current study regarding the mechanism of actions of the snake venom PLA_2s ($svPLA_2$s). Conclusion: This systematic review provides a comprehensive overview of the bactericidal effect of snake venom PLA_2s and the analysis of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PLA_2s for bacterial strains. Varying bactericidal effects from various snake species and South American rattlesnakes were reported, presenting compelling concepts to the alternative search for therapies against bacterial resistance. Thus, further analysis of the bactericidal effects of other snake venoms PLA_2s considering different strains is needed. Moreover, more data are needed to investigate other bacteria of public health priority using peptides and other purified snake toxins.

Keywords: antimicrobial resistance; bactericidal effect; snake venoms; multi-drug-resistant; systematic review

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1. Introduction

Antimicrobial resistance is one of the most substantial risks to public health worldwide, including in developed countries in Europe [1]. According to the World Health Organization (WHO), infections caused by the multi-drug-resistant (MDR) strains are among the top ten reasons for mortality globally [2]. It is noteworthy that evidence about the development and spread of antibiotic-resistant bacteria is increasingly growing [3]. Furthermore, previous investigation has demonstrated that the improper use of antibiotics, such as vancomycin, can result in the development of vancomycin-intermediate (VISA) and vancomycin-resistant strains of bacteria such *Enterococci* [4]. Similarly, various bacteria, including *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Acinetobacter*, and *Salmonella*, can resist several antibiotics [5]. Thus, laboratory- and clinical-based research geared toward discovering new and potent bactericidal candidates with unique mechanisms of action that could overcome antimicrobial resistance is warranted.

Studies on crude snake venoms and/or their fractions often result in potential therapeutic molecules against bacteria and other parasites [6]. Snake venoms are composed of a spectrum of protein-based constituents. These components could be categorized into four broad groups [6], namely: (1) The dominant group, which consists of the three-finger toxins (3FTx), phospholipases A2 (PLA_2), snake venom metalloproteases (SVMP), and snake venom serine proteases (SVSP), (2) The second group consists of a small number of proteins, which includes Kunitz peptides (KUN), cysteine-rich secretory proteins (CRiSP), L-amino acid oxidases (LAAO), C-type lectins (CTL), disintegrins (DIS), and natriuretic peptides (NP), (3) The third group contains the rarely observed snake venom proteins, including venom nerve growth factor (VNGF), vascular endothelial growth factor (VEGF), acetylcholinesterases, hyaluronidases, 5′-nucleotidases, phosphodiesterases (PDE), and snake venom metalloprotease inhibitors, and (4) finally, the fourth group includes cobra venom factors (CVF), galactose-binding proteins, aminopeptidases, and waprins.

However, the proteins mentioned above may only be readily available in some venomous snakes. For instance, elapids snakes have Group I PLA_2s and 3FTx, whereas the mambas are known to consist mainly of Kunitz peptides that are also known as dendrotoxins. The viperids consist of PLA_2s and proteases as the most abundant protein groups with varying quantities of serine proteases and metalloproteases [7].

In this study, we present a thorough review based on the existing literature on the bactericidal effect of snake venom PLA_2s, alongside an analysis of the given minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the bacterial strains listed under the study, highlighting the potential of snake venom fraction as promising candidates against several species of antimicrobial resistant bacteria.

2. Methods

2.1. Search Strategy

Relevant studies that reported the bactericidal effects of PLA_2s were searched from PubMed and Embase databases from inception until 30 March 2022. We utilized the search terms ("Bactericidal OR bacterial effect OR Antibacterial") and ("Snake Venom PLA_2s OR Venomous snake PLA_2 OR Snake Venom Peptides OR Snake venom Components OR Fractions OR Enzymes OR Proteins"). The outcome of the searches from the two terms was then combined using the Boolean operator 'AND'. We conducted a manual search of the literature to ensure more comprehensive coverage of the studies in the area of research. Reference lists of included articles that met eligibility criteria were also manually searched to identify any additional articles.

2.2. Eligibility Criteria

We included only original articles published in the English language that reported the bactericidal effect of snake venom PLA_2s in our review. The study excluded reviews, articles on bactericidal effects of snake venoms' fractions other than PLA_2s, those published in a language other than English, and studies that employed the use of commercial venoms

in which the snake specie information was not mentioned, as well as studies involving the effects of venoms on other parasites instead of bacteria.

2.3. Study Selection, Data Extraction and Data Synthesis

PRISMA systematic review procedure was utilized in the process of selecting the most relevant articles to be included in the study [8]. To avoid any potential bias in the search and/or inclusion of studies, two authors (ZUA and SSM) conducted the article screening processes at the title, abstract, and full-text phases. The same authors conducted the extraction of relevant data for the review. A customized excel sheet was used for the data extraction. Any disagreements during the article screening processes and the data extraction were resolved and discussed with all the other authors and then agreed upon by consensus. Data extracted included authors' names, date of publication, snake species, PLA_2s, bacterial isolates, minimum inhibitory concentration, minimum bactericidal concentration, and activities of PLA_2s on the tested bacteria. The synthesis of the extracted data was guided by the "synthesis without meta-analysis" (SWIM) protocol [9].

3. Results

3.1. Search Results

We retrieved 223 reports from searching the databases, 91 and 124 were from PubMed and Embase, respectively. Furthermore, additional eight studies were identified manually through the search of the references of the included studies. After removing all duplicates and the screenings at the titles and abstract phases, the remaining articles were considered for further screening, which led to the exclusion of 163 unrelated articles as well as 17 duplicates. Furthermore, we examined the full texts of the remaining 43 articles, from which 19 studies were excluded (Figure 1). Finally, twenty-four studies met the eligibility criteria and were included. Furthermore, additional eight studies were identified manually through the search of the reference lists, totaling the included reports in this study 26. Table 1 lists the distinguishing features of the included studies, and Figure 1 shows the flowchart of the research search and screening procedures. Moreover, the summary data for MIC and MBC, as well as sequence data for the PLA_2s extracted from the included studies, are provided in Table 2.

Table 1. Features of the analyzed studies.

Study	Snake Specie(s)	PLA_2s	Bacterial Specie(s)	Activity on Bacterial Strains
Nunes et al. [5]	*Bothrops Erythromelas*	BE-I- PLA_2	*Acinetobacter baumanniii*, *Staphylococcus aureus*, *Escherichia coli*	Showed bactericidal activity against *S. aureus* and antibiofilm activity against *A. baumanniii.*
Sudarshan et al. [10]	*Najanaja*	PLA_2 (NN-XIb-PLA_2)	*S. aureus*, *Bacillus subtilis*, *E. coli*, *Vibriocholera*, *Klebshiella pneumonia*, *Salmonella typhi*	Inhibited the growth of all isolates, but more active on *S. aureus* and *B. subtilis.*
Alves et al. [11]	*Crotalusdurissusterrificus*	Crotoxin PLA_2-Crotoxin B	*Ralstonia solanacearum*	PLA_2-CB showed 52% growth inhibition.
Vargas et al. [12]	*Porthidiumnasutum*	$PnPLA_2$	*E. coli* (ATCC 25922), *S. aureus* (ATCC 25923)	Showed bactericidal activity against *S. aureus* in a dose-dependent manner but not on *E. coli.*
Samy et al. [13]	*Echiscarinatus*	PLA_2-EcTX-I	*Bulkholderia pseudomallei* (KHW and TES), *Enterobacter aerogenes*, *E. coli*, *Proteus vulgaris*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*	Strong bactericidal activity was observed in *B. pseudomallei* (KHW) and *E. aerogenes*. It showed only moderate effect on other bacteria.
Sudarshan et al. [14]	*Daboia russelliipulchella*	PLA_2 fraction V (VRV-PL-V)	*S. aureus. B. sub*, *E. coli*, *V. cholerae*, *K. pneumoniae*, *S. paratyphi*	Exhibited bactericidal activity against *S. aureus* and *B. subtilis* more than on *E. coli*, *V. cholerae*, *K.pneumoniae*, *S. paratyphi*
Torres et al. [15]	*B. marajeonsis*	$BmarPLA_2$	*P. aeruginosa*, *S. aureus*	Could not promote any inhibitory activity
Samy et al. [16]	*C. adamanteus*	PLA_2-CaTx-II	*S. aureus*, *B. pseudomallei (KHW)*, *B. pseudomallei (TES)*, *E. coli*, *K. pneumoniae*, *Streptococcus pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *E. aerogenes*	Resulted in bactericidal effect by forming pores and damaging the cell wall membrane of the bacterial isolates.

Table 1. *Cont.*

Study	Snake Specie(s)	PLA_2s	Bacterial Specie(s)	Activity on Bacterial Strains
Jia et al. [17]	*Agkistrodon piscivorusleucostoma*,	PLA_2	*B. subtilis*, *S. aureus*, *E. coli*, *V. cholera*	*A. pleucostoma* PLA_2 proteins namely AplAsp49 and AplLys49 did not show any bactericidal activity against any of the bacterial isolates.
Samy et al. [18]	*A. halys*	PLA_2- AgkTx-II	*S. aureus*, *P. vulgaris*, *P. mirabilis*, *B. pseudomallei*, *E. coli*, *E. aerogenes*, *B. pseudomallei* (TES and KHW), *P. aeruginosa*	Caused potent bactericidal activity against *S. aureus*, *P. vulgaris*, *P. mirabilis*, and *B. pseudomallei* with rapid killing effect on *S.aureus*, *P. vulgaris*, *B. pseudomallei* in a dose dependent pattern. It was suggested that the activity was through membrane permeability and damage.
Samy et al. [19]	*C. durissuterrificus*, *Vipera ammodytes ammodytes*, *C. scutulatusscutulatus*, *Bungarusmulticinctus*, *Oxyuranus scutellatusscutellatus*, *Pseudechis australis*, *D. russelli*	C.d.t- CA C.d.t-CB V.a.a-a C.s.s-m B.m- β-b O.s.s-t P.a-m D.r-d	*B. pseudomallei* (KHW), *B. pseudomallei* (TES)	Presented bactericidal activity which was incriminated to be due to activity of cytotoxin and the PLA_2.
Samel et al. [20]	*N. oxiana*, *Viperalebetina*, *V. berusberus*	$NNOPLA_2$ $VLPLA_2$ $VBBPLA_2$	*B. subtilis*, *E. coli*, *Vibrio fishera*, *S. aureus*	Only $VBBPLA_2$ from *V. berusberus* completely inhibited the growth of *B. subtilis*. Moreover, the effect of $VBBPLA_2$ was reported to be due to other properties of the protein rather than catalytic activity. To *S. aureus*, $NNOPLA_2$ (from *Najanaja*) inhibited its growth and resulted in just a slight inhibition of the growth of *B. subtilis*. However, none of the three svPLA$_2$s showed inhibitory effect on *E. coli* even at the highest concentration tried.
Roberto et al. [21]	*B. jararacussu*	BthA-I-PLA_2	*E. coli* (ATCC 29648) *S. aureus* (ATCC 25923)	Presented bactericidal activity against both bacteria.
Xu et al. [22]	*Bungarusfasciatus*	BPFA-PLA_2	*E. coli*, *S. aureus*	Showed activity against both bacteria.
Corrêa et al. [23]	*B. neuwiediurutu*	BnuTX-I PLA_2	*E. coli* (ATCC 25922), *S. aureus* (ATCC 29213), *K. pneumoniae* (ATCC 13883) *P. aeruginosa* (ATCC 27853)	Showed bactericidal activity against both Gram-positive and Gram-negative isolates, with greatest inhibitory effect on *P. aeruginosa*.
Denegri et al. [24]	*B. alternatus*	Ba SpII RP4- PLA_2	*S. aureus* (ATCC 25923), *E. coli* (ATCC 25922)	Showed no bactericidal activity against the two bacteria.
Abid et al. [25]	*Walterinnesia aegyptia*	$WaPLA_2$-I $WaPLA_2$-II	*B. subtilis* (ATCC 6633), *B. cereus* (ATCC 14579), *E. faecalis* (ATCC 29122), *S. aureus* (ATCC 25923), *S. epidermis* (ATCC 14990), *E. coli* (ATCC 25966), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 43972)	$WaPLA_2$-I presented bactericidal activity against all the gram positive and negative bacteria with the highest activity recorded from *E. coli*, *S. enteric* and *S. aureus*. Lower $WaPLA_2$-II bactericidal effect was recorded from *P. aeruginosa*. Notably, *E. faecalis*, *S. epidermis*, *S. aureus*, *E. coli* and *S. enteric* showed more sensitivity to $WaPLA_2$-II than to $WaPLA_2$-I. Using agar dilution method to determine IC_{50}, $WaPLA_2$-II presented an IC_{50} of 9 ± 0.2 to 20 ± 1 μg/mL against the human pathogenic strains whereas $WaPLA_2$-I presented 10 ± 0.3 and 17 ± 1.4 μg/mL. Noteworthy is that, $WaPLA_2$-II was more effective than $WaPLA_2$-I against *S. epidermis*, *E. coli*, *P. aeruginosa*, and *S. enteric*.
Barbosa et al. [26]	*B. jararacussu*	BthTX-I BthTX-II	*Xanthomonas axonopodispv.passiflorae*	Both PLA_2 presented bactericidal activity against Gram-negative bacteria.
Shebl et al. [27]	*B. arietans*, *N. naja*, *C. cerastes*, *P. australis*, *N. nigricolis*, *V. lebetina*, *E. carinatus*	PLA_2s	*S. aureus* *E. coli* *S. typhimurium* *P. aeruginosa*	The highest PLA_2 activity was identified in *B. arietans*, *P. australis*, *N. naja* and *N. nigricolis*. Moderate effect was recorded from *V. lebetina* and *C. cerastes*. *N. haje* presented the least activity.
Almeida et al. [28]	*C. oreganusabyssus*	CoaTX-II	*P. aeruginosa* (31NM) *E. coli* (ATCC 25922) *S. aureus* (BEC 9393) *S. aureus* (Rib1)	Presented bactericidal activity against *P. aeruginosa*, *E. coli*, MRSA *S. aureus* (Rib1 and BEC9393).

Table 1. *Cont.*

Study	Snake Specie(s)	PLA_2s	Bacterial Specie(s)	Activity on Bacterial Strains
Toyama et al. [29]	*C. durissusterrificus*	F15	*X. axonopodis.pv.passiflorae*, *Claribacter michiganensis michiganensis*	Reduced the bactericidal activity of *X. axonopodis.pv.passiflorae* by up to 58.2% and that of C. *michiganensis michiganensis* by up to 98%.
Bacha et al. [30]	*Walterinnesiaaegyptia*	$WaPLA_2$	*B. cereus*, *B. subtilis*, *E. faecalis*, *S. epidermis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. enteric*	Presented highly significant bactericidal activity against all Gram-positive and Gram-negative strains *B. cereus*, *B. subtilis*, *E. faecalis*, *S. epidermis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. enteric.*
Santamaría et al. [31]	*B. asper*, *Bothriechis schlegelii*, *Cerrophidion godmani*, *Atropoides nummifer*	*B.a*- myt- I, II and III B. s-myt I, C.g-myt- I, II, A.n- myt- I B.a-spo-myt- II	*S. typhimurium*, *S. aureus*, *Brucella abortus*	The eight PLA_2myotoxinsincludingL ys49 and Asp49-type isoforms presented bactericidal effect, with an indication that the activity could be due to group IIA PLA_2 protein family. In vitro assay for bacterial, cytolytic, and anti-endotoxic effects of the peptides implies a correlation between the number of tryptophan substitutions presented and microbicidal potency, against *S. typhimurium* and *S. aureus*.
Costa et al. [32]	*B. brazili*	MTX-I MTX-II	*E. coli* (ATCC 29648)	Showed bactericidal effect.

Abbreviations: s. p. o = short peptide of *D. ruselli*, B. a-myt- = *B. asper* myotoxin, B.s-myt = *B. schlegelii* myotoxin, C. g-myt = *C. godmani* myotoxin, a. m-myt = *A. nummifer* myotoxin.

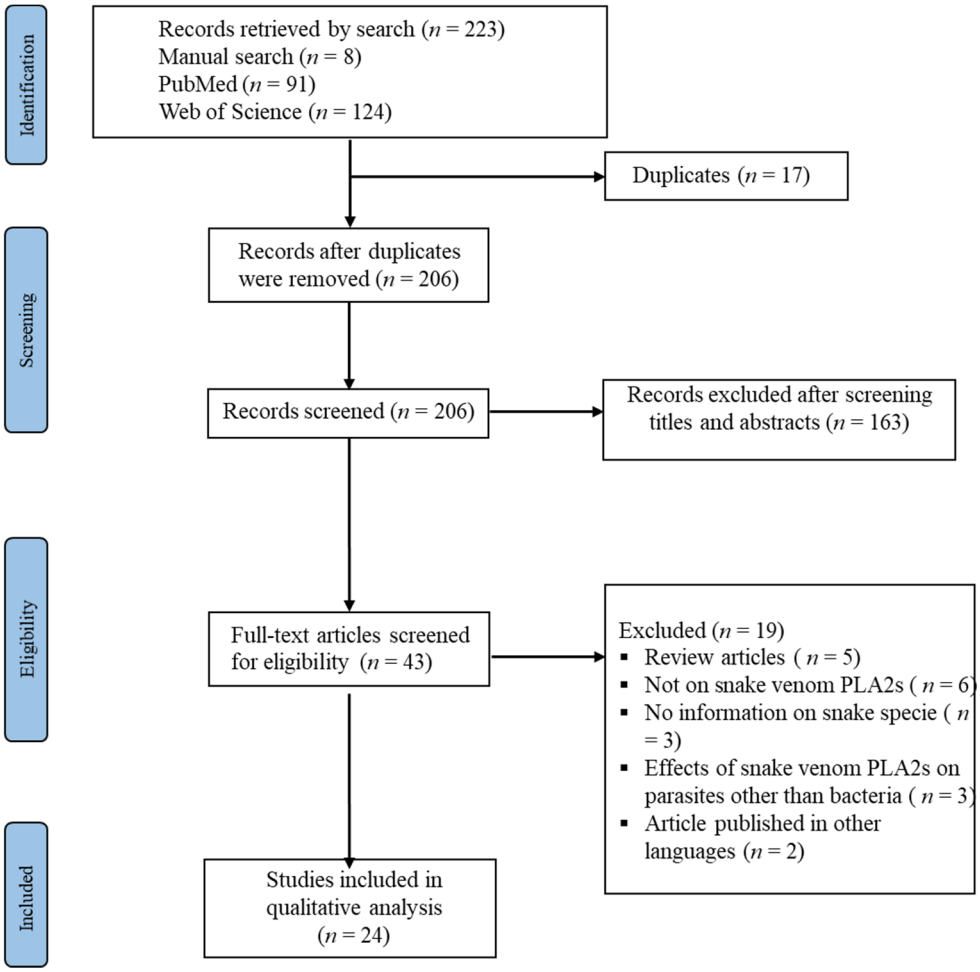

Figure 1. PRISMA diagram for the study search and selection processes.

Table 2. Summary table for MIC, MBC, and sequence data extracted from the included studies.

Author	Minimum Inhibitory Concentration(s) (MIC)	Minimum Bactericidal Concentration (MBC)	PLA_2 Sequence Data
Nunes et al. [5]	NA	NA	SLVQFETLIMKIAGRSGVWYYGSYGCYCGSG
Sudarshan et al. [10]	26.1 ± 3 µg/mL	NA	NA
	21.3 ± 2 µg/mL		
	23.3 ± 3 µg/mL		
	25.1 ± 1 µg/mL		
	19.3 ± 3 µg/mL		
	21.4 ± 2 µg/mL		
Alves et al. [11]	NA	NA	NA
Vargas et al. [12]	32 µg/mL	32 µg/mL	DLLQF-DMMKCC
	DPIA	DPBA	
Samy et al. [13]	DPIA	DPBA	SVVELGKMIIQETGKSPFPSYTSYGCFCGG (N) SLLELGKMILQETGKMPSKSYGAYGCNCGVLGR
	120 µg/mL	18 µg/mL	
	60 µg/mL	26 µg/mL	
	60 µg/mL	25 µg/mL	
	30 µg/mL	2 µg/mL	
	60 µg/mL	9 µg/mL	
	15 µg/mL	1 µg/mL	
	60 µg/mL	22 µg/mL	
Sudarshan et al. [14]	13 ± 2 µg/mL	NA	NA
	12 ± 3 µg/mL		
	15. ± 1 µg/mL		
	12. ± 2 µg/mL		
	14. ± 3 µg/mL		
	12 ± 3 µg/mL		
	13 ± 1 µg/mL		
Torres et al. [15]	DPIA	DPBA	SLLELGKMILQETGKMPSKSYGAYGCNCGVLGR
Samy et al. [16]	7.8 µg/mL	7.8–15.6 µg/mL	NA
	7.8 µg/mL	7.8–15.6 µg/mL	
	15.6 µg/mL	7.8–15.6 µg/mL	
	62.5 µg/mL	NA	
	250 µg/mL	NA	
	31.25 µg/mL	NA	
	62.5 µg/mL	NA	
	31.25 µg/mL	NA	
	125 µg/mL	NA	
Jia et al. [17]	DPIA	DPBA	*Apl Lys49* = KK-YKA YFKLKCKK *Apl Asp49* = SKTYWK YPKKNCKE
Samy et al. [18]	10.63 µM	NA	HLLQFRKMIKKMTGKEPVVSYAFYGCYCGSGGRGKPKD
	21.25 µM		
	21.25 µM		
	85 µM		
	42.5–85 µM		
	21.25 µM		
	(KHW)—10.63 µM		
	85 µM		

Table 2. *Cont.*

Author	Minimum Inhibitory Concentration(s) (MIC)	Minimum Bactericidal Concentration (MBC)	PLA_2 Sequence Data
Samy et al. [19]	Cdt-Cb and CDt-Ca 0.5–0.03125 mg/mL	NA	NA
	Cb and Dt- 0.5–0.03125 mg/mL		
	V.a-aa, Cs- m, Bm-β-b, P.a-m, O. sst, D.r-d = DPIA		
Samel et al. [20]	NA	NA	NA
Roberto et al. [21]	NA	NA	MRTLWIMAVLLVGVEGSLWQ
Xu et al. [22]	NA	NA	MNPAHLLVLLAVCVSLLGAA
Corrêa et al. [23]	NA	NA	SLFELGKMILQETGKNPPAKSYGAYGCNCGVLGRGKPKD ATDRCC
Denegri et al. [24]	NA	NA	DLLQFEGMLKIAGKSGFWYYGAYGCYCGAGGQCTPVDA TDRCCQVHDCCYKKTNCN
Abid et al. [25]	NA	NA	NLQFGMIKLTTGKPEALSYNAYCGWGGQGKPQDATDHC CFVHDCC
Barbosa et al. [26]	NA	NA	NA
Shebl et al. [27]	B.a—NA P.a—625 µg/mL N.g—NA N.n.n—625 µg/mL V.l—NA E.c—NA C.c—1250 µg/mL		NA
	B.a—312.5 µg/mL P.a—156 µg/mL N.g—312.5 µg/mL N.n.n—156 µg/mL V.l—625 µg/mL E.c—625 µg/mL C.c—625 µg/mL		
	B.a—NA P.a—312.5 µg/mL N.g—625 µg/mL N.n.n—312.5 µg/mL V.l—625 µg/mL E.c—1250 µg/mL C.c—1250 µg/mL		
	B.a—NA P.a—NA N.g—1250 µg/mL N.n.n—1250 µg/mL V.l—NA E.c—NA C.c—NA		
Almeida et al. [28]	NA	NA	SLVELGKMILQETGKNAIPSYGFYGCNCGWGGRGKPKDA TDRCCFVHKCC
Toyama et al. [29]	NA	NA	HLLQFNKMIKFETRKNAVPFYAFYGCYCGWGGQRRPKD ATDRCCFVHDCCYGKLTKCNTKWDIYRYSLKSGYITCGK GTWCKEQICECDRVAAECLRRSLSTYKNEYMFYPKSR CRRPSETC

Table 2. *Cont.*

Author	Minimum Inhibitory Concentration(s) (MIC)	Minimum Bactericidal Concentration (MBC)	PLA_2 Sequence Data
Bacha et al. [30]	>9 µg/mL	NA	NLYQFKNMVQCVGTQLCVAYVKYGCYCGPG
	>12 µg/mL		
	>7 µg/mL		
	>12 µg/mL		
	>5 µg/mL		
	>7 µg/mL		
	>8 µg/mL		
	>10 µg/mL		
	>9 µg/mL		
Santamaría et al. [31]	NA	NA	KKWRWWLKALAKK
Costa et al. [32]	NA	NA	MTX-I = SLWEFGQMIIKETGKLPFPYYGAYGCYCGWGGRRGPK-DATDRCCYVHDC MTX-II = SLFQLGKMILQETGKNPAASYGAYGCNCGVLGRGKPK-DATDRCCYVHKC

Abbreviations: NA = not available, DPIA = did not promote inhibitory activity, DPBA = did not promote bactericidal activity, C.d.t CB = *C. dirussus terrificus* CrotoxinB, C.d.t CA = *C. dirussus terrificus* Crotoxin A, V. a-aa = *V. ammodytes* ammodytoxin, Cs-m = *C. scutulatus* mojave toxin, Bm-β-b = *B. multicinctus* β-bungarotoxin, P.a-m = *P. australis* mulga toxin, O. sst = *O. scutellatus* taipoxin, D. r-d = *D. ruselli* daboia toxin.

3.2. Bactericidal Effects of Snake Venom PLA_2s

Snake venom PLA_2s (svPLA_2s) are enzymatic proteins with low molecular weights. They catalyze the hydrolysis of the 3-sn-phosphoglyceride-dependent calcium 2-acyl ester bond, yielding lysophospholipids and fatty acid products [6]. Snake venom PLA_2s have similar toxicological profiles, including cytotoxicity, myotoxicity, oedema, inflammation, neurotoxicity, hypotension, anticoagulant activity, hemolysis, hyperalgesia, and microbicidal activity [5]. Some of the evaluated PLA_2s (either acidic or basic) could present an IC_{50} against at least Gram-positive or Gram-negative bacteria, whereas others could not show any activity against any strain. Nunes et al. [1] reported that the acidic PLA_2 from *B. erythromela* presented IC_{50} against Gram-positive bacteria but not negative bacteria. Torres et al. [15] reported that the basic PLA_2 isolated from *B. marajeonsis* showed no inhibitory effect on neither *P. aeruginosa* or *S. aureus*.

Moreover, Jia et al. [17] reported that the PLA_2 from *Agkistrodon piscivorus leucostoma*, AplAsp49, and AplLys49, presented no bactericidal effect against any of the bacterial strains. Moreover, Sudarshan et al. [10] reported that the basic PLA_2 of *D. russelliipulchella* showed higher bactericidal activity against Gram-positive bacteria when compared with Gram-negative bacteria. It has been reported that the bactericidal activity of PLA_2, especially the basic is related to the disturbances of bacteria membrane integrity [23]. Gram-negative bacteria have a cell wall that is made up of an inner membrane formed of phospholipids, an outer membrane made of asymmetric lipids, and a layer of peptidoglycans. This structure acts as a barrier to medications that have been established. A similar scenario exists for the PLA_2, as the outer membrane naturally resists its activity [33]. On the other hand, Gram-positive bacteria possess just one layer of peptidoglycans and then an internal cell membrane, showing their prompt susceptibility to the action of PLA_2. As such, the low bactericidal activity of some PLA_2s in Gram-negative bacteria compared with Gram-positive bacteria could be due to the difference in the structure of their respective cell walls. Sudarshan et al. [10] reported that there was a strong, established relationship between the hemolytic and bactericidal activity of *D. russelliipulchella* PLA_2.

Furthermore, it was reported that p-bromophenacyl bromide (p-BPP) presents a significant decrease in enzymatic activity and associated antibacterial activities, thereby

destabilizing the membrane bilayer. Regarding PLA_2 crotoxin A or B (PLA_2- CA and PLA_2- CB), Alves et al. [11] reported that both from *C. durissus terrificus* showed high bactericidal activity against *R. solanacearum*. Similarly, Samy et al. [19] reported that crotoxin B of *C. durissus terrificus* and daboiatoxin of *Daboia russelli* presented the most robust bactericidal activity against the two strains of *B. pseudomallei* (TES and KHW). Some PLA_2s were reported to have shown bactericidal activity against both Gram-positive and Gram-negative bacteria [16,31]. Others presented a bactericidal effect on Gram-positive and not Gram-negative bacteria [12]. Of interest to note is that some PLA_2 showed more vigorous bactericidal activity on some Gram-negative bacteria and moderate on other Gram-negative and Gram-positive bacteria [13].

3.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for Bacterial Strains

Minimum inhibitory concentrations (MICs) are the lowest concentration of an antimicrobial that could inhibit the visible growth of a microorganism after overnight incubation. MICs are utilized by diagnostic laboratories mainly to confirm resistance but are most often used as a research tool to determine the in vitro activity of new antimicrobials [34]. In the works of Samy et al. [13] and Samy et al. [16], Vargas et al. [12], and Jia et al. [17], broth micro-dilution assay was employed. The procedure entails suspending fresh overnight bacterial cultures to a turbidity of 0.5 McFarland units and further subjecting them to a dilution in Mueller Hinton (MH) and tryptic soy (TS) broth, followed by a dilution to approximately 1.5×10^5–3.2×10^6 CFU/mL (CFU = colony forming unit). On the other hand, minimum bactericidal concentration (MBC) is referred to as the minimum bactericidal density required to kill bacteria; as such, it is opposed to mere bacteriostatic densities [35]. According to Samy et al. [13] and Samy et al. [16], the plating technique was carried out whereby the broth from the MICs well and those from above the wells were plated for each bacterial strain 3.2×10^6 CFU/mL onto approximate drug-free growth media MH and TS agar plates used for determining antibacterial activity. Surviving bacteria were then quantified using the dilution plate technique. On the starting inoculums, the quantitative colony counts were assessed. The lowest concentration that killed up to $\geq$99.9% of the starting inoculums was defined as the MBC. In the work of Jia et al. [17], the plating technique involved adding two hundred microliters of 1/1000 diluted overnight bacterial cultures to 1 mg/mL, 0.75 mg/mL, 0.1 mg/mL 50 μg/mL 25 μg/mL, 10 μg/mL PLA_2 in the 96-well plates in parallel 50 μg/mL of the control (antibiotic), and PLA_2 buffer as a negative control. Bacterial cultures that presented no growth were then plated on agar and incubated overnight to achieve colony-forming unit (CFU) enumeration.

4. Discussion

Overall, our systematic search identified 24 articles on the bactericidal effect of svPLA_2s. The majority of the studies (80%) found through the search were on the PLA_2s of snakes from the Viperidae family, whereas 20% were on the PLA_2s from snakes of the Elapidae family. There are considerable variations in the composition of snake venoms recorded even from closely related species and within species [6]. Evidence of intra-genus or intra-specific variation in venom compositions has been documented in pit vipers and adders [36]. This variation was attributed to diet [37] or topography [38]. Repeated toxin-encoding genes, production processes, as well as functional and structural diversifications are other attributable factors [39]. For instance, the sea snake *Laticauda Semifasciata S. venom* has a simple composition, with only two prominent protein families, the 3FTxs and the PLA_2s. However, about 50–100 peptides or proteins representing about 10–20 protein families are present in the venoms of rattlesnakes and mambas [7]. In general, cobra, kraits, and the hydrophids particularly have more negligible toxins such as 3FTxs and PLA_2, whereas the venoms of vipers are composed of the more significant fractions with enzymatic roles such as snake venom metalloproteinase and snake venom serine protease [7]. For instance, amino acids, small peptides, carbohydrates, lipids, biogenic amines, and enzymes are

contained in the venom of *C. durissus terrificus*, whereas that of *B. jararaca* is composed of peptides serine and metalloproteases [40]. As such, the activity of snake venoms varies due to differences in concentrations and compositions.

It has been reported that svPLA$_2$s makes a considerable component of the venoms of the vipers and elapids [41] due to their biomedical importance over the other compositions of snake venoms [42]. They are proteins that belong to groups I and II. The group I PLA$_2$s are those of snakes from the Elapidae family (Elapidae and Hydrophiinae) [7]. In contrast, those belonging to group II are from snakes of the Viperidae family (Viperinae and Catalina) [7]. The latter comprises two subgroups: the catalytically active Asp49-PLA$_2$ and the catalytically inactive PLA$_2$ homologs containing Lys49 residue [43]. The GIIA PLA$_2$s were reported to have an essential role in the defense against bacteria. The ASP49- PLA$_2$ and LYS49- PLA$_2$ homologs were reported to have acted synergistically, increasing Ca2+ ions in the plasma membrane, resulting in the rapid death of myotubes [43].

Crotoxin is a non-covalent heterodimeric neurotoxin of two subunits; an active PLA$_2$ and a chaperone peptide called crotaperone. The molecule has three peptide chains connected by seven disulfide bridges [44]. It is the main neurotoxin in the venom of the South American rattlesnake (*C. durissus terrificus*) and accounts for about 50% of its dry weight and it acts at the presynaptic membrane level. Pharmacologically, crotoxin promotes pre- and post-synaptic effects, indicating several interactions with excitable cells [29]. Crotoxin B, a basic neurotoxic phospholipase A, has three chain proteins that promote the lethal potency of crotoxins [19]. In contrast, crotoxin A (CA) is the acidic subunit of the crotoxin (Crtx) on which the essential subunit crotoxin B (CB) depend for the ability to bind specifically to the cell membrane [45].

There are variations in the mechanism of actions of svPLA$_2$s. For instance, CaTx-II from *C. adamanteus* was reported to have inhibited the growth of *E. aerogenes* through the disintegration of its cell wall by generating pores in the membrane. Furthermore, the protein has been reported to promote the healing of wounds [16]. It is noteworthy that peptides produced by the breakdown of svPLA$_2$ can interact with lipopolysaccharide (LPS), specifically, the lipid A component of S. aureus, causing membrane permeabilization and acting as a bactericide [16]. Various cationic peptides from *B. asper's* svPLA$_2$s exhibit bactericidal activity against *K. pneumoniae*, protect mice from *S. enterica*-induced peritonitis, and cause membrane permeabilization in *S. aureus* when they are derived from cationic peptides [31,46,47]. The derivatives of the carboxy terminus of svPLA$_2$s found in these peptides, which range from ten to twenty-two amino acids, are crucial. Compared to the parent compounds, they are less toxic to eukaryotic cells and have greater bactericidal activity. Similarly, it has been documented that the C-terminal cationic/hydrophobic segment (residues 115–129) of svPLA$_2$s has bactericidal potential. As such, identifying bactericidal positions in svPLA$_2$s because of developing new therapeutics is promising [48–50]. Furthermore, there have been reports of varying MICs and MBCs against various bacterial strains, as well as sequence data of each PLA$_2$.

4.1. Strength

Our systematic review has the following strengths; firstly, we conducted a thorough and extensive search of literature via PubMed and Embase databases, as well as a manual search of the literature, which enriched our search coverage. Secondly, we adopted appropriate quality rating tools for assessing the qualities of the included studies.

4.2. Limitations

The study is associated with some limitations that should be considered when interpreting the reported findings. Firstly, we excluded studies based on language (only those published in English), thereby limiting the ability to incorporate relevant data from studies in languages other than English. Moreover, most of the included studies did not include information on accession numbers. However, we have included the sequence information found from each study in our work to have representative information for at least each

included study. Nevertheless, in our future research, we will deeply investigate the DNA sequence of PLA_2 with nucleotide accession numbers when the data become available.

5. Conclusions

This systematic review provides a comprehensive overview of the bactericidal effect of snake venom PLA_2s and analyses the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)of PLA_2s for bacterial strains. Varying bactericidal effects of various PLA_2s were reported, presenting compelling concepts to the alternative search for therapies against bacterial resistance. However, more data are needed to investigate the bactericidal effects of other snake venoms PLA_2s using purified snake toxins. Thus, it is imperative to study other bacteria of public health importance using snake venoms and their associated purified snake toxins.

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