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REVIEW ARTICLE

Genetic variability in snake venom and its implications for antivenom development in sub-Saharan Africa

Innocent Ayesiga [a,*], Lenz N. Okoro[b], Chirigo Taremba[c], Michael O. Yeboah[d], Justine T. M. Naab[e], Ruphline M. Anyango[f], John Adekeye[g], and Ivan Kahwa[h]

[a]Department of Research, Ubora Foundation Africa, Kampala 759125, Uganda; [b]Department of Community Medicine, David Umahi Federal University Teaching Hospital, Uburu, Ebonyi State 480101, Nigeria; [c]National University of Science and Technology, Bulawayo 00000, Zimbabwe; [d]School of Public Health, University of Port Harcourt, River State 500001, Nigeria; [e]School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi GA107, Ghana; [f]Department of Veterinary Tropical Medicine, University of Pretoria, Pretoria 0002, South Africa; [g]Virology clinic, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state 111103, Nigeria; [h]Pharm-Biotechnology and Traditional Medicine Centre (PHARMBIOTRAC), Faculty of Medicine, Mbarara University of Science and Technology, Mbarara 40006, Uganda

*Corresponding author: E-mail: ayesiga49@gmail.com

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Snake venom, a complex mixture of proteins, has attracted human attention for centuries due to its associated mortality, morbidity and other therapeutic properties. In sub-Saharan Africa (SSA), where snakebites pose a significant health risk, understanding the genetic variability of snake venoms is crucial for developing effective antivenoms. The wide geographic distribution of venomous snake species in SSA countries demonstrates the need to develop specific and broad antivenoms. However, the development of broad antivenoms has been hindered by different factors, such as antivenom cross-reactivity and polygenic paratopes. While specific antivenoms have been hindered by the numerous snake species across the SSA region, current antivenoms, such as SAIMR polyvalent and Premium Serums & Vaccines, exhibit varying degrees of cross-reactivity. Such ability to cross-react enables the antivenoms to target multiple components from the different snake species. The advent of biotechnological innovations, including recombinant antibodies, small-molecule drugs, monoclonal antibodies and synthetic antivenoms, presents options for eliminating limitations associated with traditional plasma-derived antivenoms. However, challenges still persist, especially in SSA, in addressing genetic variability, as evidenced by inadequate testing capacity and limited genomic research facilities. This comprehensive review explores the genetic variability of snake venoms in SSA, emphasizing the venom composition of various snake species and their interactions. This information is critical in developing multiple strategies during antivenom development. Finally, it offers information concerning the need for extensive collaborative engagements, technological advancements and comprehensive genomic evaluations to produce targeted and effective antivenoms.

Keywords: cross-reactivity, envenomation genetics, genomic studies, snake species, snake venom variability, venom proteins

Introduction

Snake venom genetic variability has hindered envenomation management, especially due to the numerous snake species in sub-Saharan Africa (SSA). Despite the deadly image associated with the snakes, the curative ability of venom has been known about for a long time. Snake venoms exercise their toxicity effect through their complex protein contents.[1,2] Snake venoms reveal high complexity and diversity in composition. Evidence has demonstrated that 90% of total venom proteomes composition consisted of eight, 11 and 10 protein families for *elapidae* (elapids), *viperinae* (true vipers) and *crotalinae* (Pit vipers), respectively.[3] Besides, its compositional diversity plays a significant role in the venom potency.[4,5] The toxin potency and the high variability in snake venom composition predominantly determine clinical manifestations in envenoming. These manifestations can range from local tissue injury to possible severe systemic effects.[2] The administration of precise antivenom has been proven the only efficient treatment for snakebites. However, there is a limitation to the availability and the upscaling of antivenom production due to venom composition variability.[1] Variability of snake venom has a severe effect on the management of snakebite victims.[2]

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Significantly, regional variation is directly linked to ecological variance and neutral evolution, which is evidence of the dynamism of venom evolution.[6] In toxin isoforms, a significant amount of snake venom is encoded by a multilocus gene family and varies across snake species. Notably, there are limited studies on genetic variability in low- and middle-income countries.[6] Understanding the significance of the genetic variability of snake venom, its composition, associated pharmacokinetics and its long-term harmful effects, is among the requirements for developing an effective management plan.[7] Besides, some antivenoms have associated adverse reactions, which, according to the WHO, are classified into early reactions (which occur within 24 h of its administration and are characterized by fever, chills, headache, nausea, sweating and an increase in heart rate) and late reactions (which occur between 5 and 24 d of treatment with clinical manifestation like rash, fever, malaise, arthralgia and arthritis).[8,9] In addition, antivenoms can sometimes be ineffective against several snakebite-related complications.[9] Therefore, this review intends to offer comprehensive insights concerning the genetic variability of snake venom and its potential implications for antivenom development in SSA.

Snake species and their venom variability in SSA

SSA has abundant snake species, especially among tropical and subtropical countries.[10] Some snake species are non-venomous, while others are venomous, significantly contributing to most human deaths in rural areas.[11] The many medically important and venomous snake species in SSA include various cobras, mambas and vipers.[12] Significantly, most venomous snakes exist in pastoral and agricultural communities, and specific antivenoms to these snake species are scarce, impacting envenomation management.[13] Several venomous snake species have been identified in Ethiopia, and the most common ones include *Bitis arietans*, *Bitis arietanus somalica*, *Echis pyramidum* (known as the Northeast African Carpet viper) and *Naja pallida* (red spitting cobra).[10] In Tanzania, the following venomous species were identified: *Dendroaspis polylepis* (black mamba), *Ramphiophis rostratus* (rufous-beaked snake), *Bitis arietans* (the puff adder) and *Naja nigricollis* (black-necked spitting cobras). Other non-venomous species identified include the *Lamprophis fuliginosus* (the brown house snake), *Eryx colubrinus* (Kenyan Sand boa) and *Dasypeltis scabra* (the egg eater).[13] However, there is still limited knowledge about the distribution of predominant venomous and non-venomous snakes in the different countries.[10] SSA countries must prioritize safe and protective snake-catching activities, such as utilizing the expertise of experienced herpetologists and ecologists, to ensure that the geographical distribution of the different species is well documented.

An understanding of the variation in snake venom composition is necessary to select toxin immunogens that may be used to generate quality, safe and efficacious antivenom used to treat envenomation.[14] For example, mamba snakes have highly potent venoms of pharmacologically active peptides, including extremely rapid-acting neurotoxins. The venoms vary in composition in the genera, and the difference can be attributed to dietary variations.[14] Moreover, due to the divergent terrestrial ecology, black mamba venom has abundant dendrotoxin I and K. Among green mambas, the predominant venom is three-finger toxin (3FTx) due to their arboreal habitat.[15] Genomic variations of the venom proteins can also result from alteration of the amino acids encoding for these proteins. Conventionally, some predisposing factors to genetic variation among human proteins, for example, include radiation, random mutations and cross-fertilization.[16] Among snake species, some predisposing mechanisms to variation include evolutionary tinkering of expression, trans-splicing, domain loss, alternative splicing and duplication.[17] However, most of these mechanisms are still postulated, creating a significant implementation barrier in evaluating venom variability. Thus, empirical and experimental studies with diverse snake species are necessary in the SSA setting to understand particular variability. Other factors affecting venom variability include the availability of prey in different habitats, the age of the snake, sex and captivity effects, including stress and temperature variations. Thus, leveraging these variant determinants is necessary to understand the location-specific venom composition from the different snake species. Through this understanding and utilization of modern genetic analyses, specific antivenom to SSA snake species can be produced, thus combating envenomation.

Notably, venom production genes have evolved using different genetic processes such as gene duplication, although some duplicates are retained by natural selection, and some are lost through deletion. These often result in functional divergence and variability. Further, the efficacy of these venoms is altered following neo-functionalization, whereby a new function is acquired, or through sub-functionalization, whereby the original function is partitioned through mutation. Over time, snakes have devised chemical means of capturing their prey by producing, storing and delivering venoms.[18] Further, biotic and abiotic factors will also influence the genetic variability of the venom. These include prey availability as a determinant of venom composition and variation.[16,17] Other factors determining this variation include gene flow, environment and population structure. Importantly, the genes involved in venom production include those that encode for the protein families that make up the venoms. For instance, venom serine proteases are encoded by multilocus gene families that are rapidly duplicating.[19] Other genes include metalloproteases (MP) and Vascular Endothelial Growth Factor.[15] However, there is little information on the genes involved in antivenom production from a SSA context, creating significant challenges in the same. Thus, enhancing the capacity of SSA countries to identify the mechanisms of variation among African snake species within their geographical distribution is essential for producing antivenoms to reduce envenomation in the region.

Venom proteins from different snake species

Predominantly, snake venoms consist of primary proteins, such as MP, phospholipase A_2s (PLA_2; the most common across all front-fanged snakes), 3FTxs and serine proteases.[17] Secondary proteins comprise cysteine-rich secretory proteins, natriuretic peptides, C-type lectins/snacks, disintegrins, L-amino acid oxidases and Kunitz peptides.[16] The Eastern Green Mamba (*Dendroaspis angusticeps*) predominantly consists of 3FTx at an estimated

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composition of 87% for the orphan and short-chain aminergic toxin subgroups.[20] Conversely, the Western Green Mamba (*Dendroaspis viridis*) predominantly has long neurotoxin (lNTx) estimated at 32.98%, followed by 3FTx at 29.36%. Black Mamba (*Dendroaspis polylepis*) venom has 74.53% composition of the Kunitz-type proteins followed by lNTx at 12.91%.[20] An evaluation by Kazandjian et al. and Lauridsen et al. found >57.1% mean composition of 3FTx among Naja species, such as *Naja melanoleuca*.[21,22] Nguyen et al. also found a 64% approximate 3FTx venom protein composition from the forest spitting cobra (*Naja melanoleuca*).[20] Other venom proteins identified in the *Naja melanoleuca* included the Kunitz type, sNTx and cytokines at 3.1%, 6.06% and 27.36%, respectively. However, Kazandjian et al. further revealed a distinction in PLA_2 phylogenetic analyses across three lineages of the Naja species.[23] The distinction characterized by duplication in these lineages is a marker of evolution and genetic variability among this species. By contrast, the viper snakes produce venoms containing serine proteases, MP and PLA_2.[17] For example, analysis of the West African Gabon Viper (*Bitis gabonica*) demonstrated a variable composition of the venom proteins with snake venom metalloproteases (SVMPs) contributing the highest percentage at 26.97%.[20] Other venom proteins present included Kunitz-type at 5.11%, PLA_2 at 3.1% and disintegrins at 14.93%.[20] Other *Bitis* species, such as *Bitis arietans*, also had a variable composition, with SVMPs contributing the highest percentage at 61.60%. However, *Bitis rhinoceros* had a higher composition of PLA_2 at 39.36%, while other species such as *Bitis nasicornis* and *Bitis arietans* had 0.73% and 6.71%, respectively.[20] The distinction in this composition of venom proteins further suggests variability occurrence in the venom composition of the same species.

Antivenom cross-reactivity and snake venom genetic variation in relation to antivenom efficacy

Evidence about antivenom cross-reactivity among snake species in SSA remains sparse and deficient. With substantial ability to adapt across various ecological niches, snakes, alongside their venoms, continue to pose a significant barrier to specific antivenom production. Evidence from an in vivo study found no difference in the concentration of antibodies following administration of three different antivenoms: Bothrops-Crotalus-Lachesis (BCL), Anti-Lachesis (AL) and Anti-Bothrops (AB), manufactured from Instituto Clodomiro Picado, Costa Rica.[24] Evaluation of the interactive patterns of antivenoms—such as Bioclon: Antivipmyn; Premium Serums & Vaccines: Pan Africa; Inosan: Inoserp; VINS: African; South African Vaccine Producers: SAIMR; and Sanofir Pasteur: FAV—demonstrated significant cross-reactivity, evidenced by poor k-*mer* interactions and poor binding profiles to the selected venoms.[25] The study further demonstrated poor toxin recognition among the selected antivenoms. Thus, the absence of this antivenom specificity to the multiple snake venom species continues to pose a significant threat to developing a common antivenom. In Thailand, the available antivenoms, such as Hemato Polyvalent Snake antivenom, *C. rhodostoma* antivenom, *T. albolabris* antivenom and *D. siamensis* antivenom, also demonstrated significant cross-reactivity with limited binding affinity to the different venoms.[26] Furthermore, these antivenoms demonstrated low venom identification, creating more challenges for envenomation management. Noteworthy, most of these antivenoms, especially those obtained from immunized serum, contain monovalent antibodies or whole IgG.[27] Often, the specificity and structural configuration of the variable segments contribute to the cross-reactivity among antivenoms, even though the exact mechanisms are not yet fully understood. In the SSA perspective, characterized by limited technological mechanisms that accurately identify the different epitopes and structural integrations, evaluating the antivenom cross-reactivity is still far from achievable. Thus, exploring the different avenues for establishing laboratory facilities to identify the antivenom cross-reactivity can significantly contribute to manufacturing targeted antivenoms. These can be distributed across various SSA countries, leading to quality-of-life improvement from snakebite envenomation.

Currently, SAIMR polyvalent and Premium Serums & Vaccines antivenom have proven some efficacy in neutralizing the venom from different snake venoms across SSA, such as from *B. arientans*, *D. polylepis*, *N. haje*, *N. pallida* and *N. nigricollis*.[28] Analysis of other antivenoms available on the SSA market, such as INOSAN and Sanofi Pasteur, demonstrated the least cross-specificity binding to venoms from multiple species. Notably, the antivenom effectiveness on the diverse snake species depends on the venom protein composition of the different snake species. Also, this effectiveness is influenced by the amount of immunoglobulins, such as IgG, present in the specific antivenom.[25] SSA still lacks sufficient testing capacity to identify the specific immunogenic titers responding to the specific protein titers in snake venom.[28] Current assays have evaluated the venom protein binding intensity to the antivenom compared with the protein specificity, yet exploring the venom protein specificity binding would offer more conclusive insights about antivenom diversity to multiple snake species. Moreover, analyses of the characteristic venom proteins from the diverse snake species abundant in the SSA region are still deficient, posing other significant threats to establishing universal antivenoms.

Approaches to studying snake venom variation

Technological advancement has continuously advanced study approaches toward snakebite venom variation. Various approaches, such as plasma clotting assays, western immunoblotting, chaotropic, end-point titration and avidity ELISA and fractionation methodologies, have been critical in studying the structure, patterns and behavior of snake venoms and their interaction with antivenoms.[24–26,28] Other approaches to evaluation of the venom proteins and their interactions with different antivenoms include neutralization assays, antivenomics, immunochemical studies and high-density microarray analysis that evaluates antivenom and toxin interactions.[25] However, most of these analyses are present in developed countries, leaving underdeveloped countries, such as those in SSA, with a greater burden concerning snake venom genomic studies.

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In turn, the scarcity of these genomic approaches limits the development of specific and general antivenoms to counteract the envenomation from multiple snake species. Kenya is the only country in SSA equipped with a facility housing an inventory of snake venoms detailing their murine toxicity and venom protein composition.[28] The absence of these analytical approaches among other African countries affects antivenom costs and affordability, thus affecting the mitigation of the envenomation. Therefore, exploring effective approaches towards establishing genomic testing facilities is paramount to promote comprehensive evaluations of the snake venom proteins from the different snake species in SSA. Multiple collaborations, such as south-south and north-south engagements between the different existing institutes conducting genomic studies, must be encouraged through multilateral ties and strategic partnerships to ensure the establishment of these centers of excellence in different SSA countries. We believe that increased coverage of genomic studies will offer substantial information concerning the snake venom proteins among the multiple snake species in SSA, offering an opportunity for antivenom development.

Biotechnological innovations in antivenom development

Biotechnological innovative approaches to antivenom development can overcome some limitations, especially in SSA.[29] Recombinant human antibodies, known as next-generation treatments, have demonstrated greater efficacy, limited cross-reactivity and enhanced safety.[30] One of the biotechnological and innovative approaches that was invented during the study of human antibodies against snake venom toxins is phage display technology. Using recombinant antibodies has the benefits of lower adverse reactions and higher active antibody content. As such, recombinant antivenoms promise to reduce morbidity and mortality among snakebite victims. Another benefit of recombinant antivenom has been the low production cost compared with plasma-derived antibodies.[29] Because snakebite envenomation, a neglected tropical disease, has been associated with poor communities, the cost implications of recombinant antivenoms are critical. For example, the costs of manufacture for recombinant monovalent and polyvalent antivenoms have been estimated at US$20–225 and US$48–1354 per treatment, respectively, unlike the cost of US$56–640 for plasma-derived antibodies.[30,31] The higher costs of production, coupled with minimal budget allocations for health and innovation in SSA countries, creates a significant barrier to antivenom development, hindering envenomation management.

Different initiatives are being undertaken to address the limitations associated with conventional plasma-derived antivenoms. Synthetic antivenoms have proven to eliminate such challenges and several approaches are being employed to produce these synthetic antivenoms. These synthetic approaches focus on toxins of high toxicity and abundance.[32] Antivenom components are synthesized, either as polyvalent or monovalent antivenoms. They are also produced either as peptide inhibitors, such as metalloproteinase and phospholipase inhibitors, or as small molecule inhibitors.[9,31] Notably, monoclonal antibodies against African venoms is a promising area with the potential of broadly neutralizing specific venom toxins. The current antivenoms are predominantly obtained from horse serum with the potential of predisposing to serum sickness; however, monoclonal antibodies present safer ways against envenomation.[33] Researchers at the Technical University of Denmark developed Antibody 2554_01_D11, which demonstrated potential against the α-cobratoxin from the forest and monocled cobra.[34] Another monoclonal antibody that demonstrated higher binding affinity, especially to α-elapitoxin from the elapids, was the antibody 2551_01_A12. Besides, both antibodies still demonstrated higher cross-reactivity to α-elapitoxin and α-cobratoxin, further indicating cross-neutralization. These developments present a significant opportunity to develop a remedy against envenomation.[35] Another monoclonal antibody, Anti-African *Bitis arietans* Snake Toxin Phospholipase A2, developed by Brazilian researchers targeting the toxins from *Bitis arietans* and tested in Uppsala, Sweden, demonstrated the potential of neutralizing the snake venom.[36] Significantly, most of these advances have taken place in developed countries and not in SSA, because of certain obstacles, such as limited infrastructure including facilities and a lack of trained personnel and equipment, as well as different regulatory frameworks to guide the development of these monoclonal antibodies. Although synthetic antibodies developed using phage and yeast display technologies show promise, addressing the limitations and challenges associated with these studies, particularly in the context of SSA, is critical.

Additionally, small molecule drugs have shown promise in neutralizing African venom toxicity, offering a potential solution for the treatment of snakebite envenoming in SSA. These drugs, such as batimastat, a metalloproteinase inhibitor, marimastat, an SVMP inhibitor, and varespladib, have demonstrated greater efficacy against the neurotoxic effects of snake venom.[37] Importantly, evidence from in vivo studies exploring the effect of combined small molecule drugs, such as varespladib and marimastat, has demonstrated a combined and stronger efficacy against various effects of snake venom, such as hemorrhage and coagulopathies.[38] However, while these experiments exploring the efficacy of these drugs have demonstrated significant efficacy against snake venom, very few of these experiments were performed in SSA. Besides, even the materials used, like citrated plasma and lyophilized venoms, were not obtained from SSA, even although they are from African snake species.[39] SSA still has various challenges affecting the implementation and utilization of small molecule drug development. These include venom variability, the absence of scalability opportunities and ineffective regulatory frameworks to guide development and deployment. However, they also provide a potential opportunity for SSA to further address venom toxicity.

Prospects concerning genetic variability in antivenom development

Over time, snake venoms have undergone significant enhancements of >100-fold in all major animal groups, resulting in the development of highly efficient biochemical weapons.[40] Several inquiries regarding the development of toxin arsenals remain

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unresolved, such as the origins of venom genes, the role of venom in the survival of venomous animals and the specific genomic, transcriptomic and protein alterations that propel their evolution.[41] OMIC technologies have facilitated the comprehensive characterization of snake venom compositions, the identification of molecular mechanisms that drive venom variation and the clarification of consequent functional implications for toxicologists.[6] The primary goal of OMIC technologies is to attain a thorough identification of proteomes, metabolites, mRNA (transcriptomics) and genomes inside a particular biological sample.[42–44] Scientists have extensively researched the growth and development of snake species and their venom using standard approaches that focus on morphological features, core genetic analysis and the study of venom proteins. Although there have been significant advancements in genomics in the past two decades, mostly due to the implementation of next-generation sequencing technologies, the examination of snake genomes is still in its nascent phase.[45] Biobank research and genetic/genomic research possess particular characteristics that give rise to special ethical and regulatory concerns. These concerns have been vigorously debated to formulate suitable solutions that may be applied globally and locally. The primary ethical and regulatory concerns revolve around the principles of autonomy, privacy and maintaining the secrecy of information, especially in animal studies. Therefore, ethical and regulatory frameworks must be followed and facilitate the establishment of biobanks and the implementation of genetic/genomic research.[46] Genetic studies involve conducting interviews to gather information and insights on the genetic causes of diseases, obtaining informed consent, establishing guidelines for data usage and sharing, discussing the advantages and disadvantages of participating and setting expectations for after the study.[47] Globally, there is a production deficit to fulfill the demands of medical requirements, especially for antivenoms in regions with limited manufacturing facilities. Insufficient funding and inadequate management of antivenom markets at the national level may lead to the production of low-quality antivenom.[48] This is most evident accurate when firms fail to register their products in the countries where they are intended to be used, or when insufficient assessment criteria allow for registration without conducting evaluation.

Conclusion

Snake venom has evolved over decades with a direct pathway of transcription of toxin genes for onward translation into toxin proteins. Its genetic composition and phenotypic expressions depict variations among various snake populations. Genetic variations in populations of snake species have been expressed due to factors such as genetic duplication, deletions and sub- and neofunctionalization. The production of various genetic compositions in snake venom in different geographic populations is connected with the concept of natural selection as a universal phenomenon and a key driver of interspecific variation. Snake venom toxins have been classified into 63 families, with most of them found in negligible amounts in a small fraction of snake species. The four predominant families of highest relevance are PLA_2, 3FTxs, SVMPs and Snake Venome Serine Proteases. Venom derived from different geographic locations with variations in genetic composition demonstrates the potential for antivenom production, particularly in SSA. Genetic variability in the potency of antivenoms has, among other factors, caused shortfalls in the number of effective antivenom products available in SSA. The WHO earlier estimated a pending antivenom supply failure in Africa due to a compromise in supply and value chain protocol complicated by the production of non-specific antivenom candidates that are unable to universally neutralize the constantly mutating genetic composition of snake venoms predominant in SSA. Thus, there is a need for several future research efforts targeted at studying the geographical and intraspecific variation of venom, particularly in SSA, as well as enhanced improvement in antivenom production with far-reaching neutralizing abilities. Further exploration of the ontogeny and phylogenetic evolutionary changes in snake venoms is necessary, with an emphasis on adapting experimental designs to the peculiar research needs in SSA.

Authors' contributions: IA conceptualized the manuscript. MOY & JA developed the challenges in addressing genomic variability. CT drafted the biotechnological innovations concerning snake venom. JTMN developed the genomic approaches to snake venom variability. RMA presented the snake species and their venom variability. LNO offered conclusive remarks on the manuscript. IA developed the first manuscript draft. IK reviewed the first and final manuscript draft. All authors approved the final manuscript draft and accepted it for publication.

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