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Predicting the Efficacy of Antivenoms against African Vipers and Elapids by Using Immunoblotting and Cytotoxicity Neutralisation Assays

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNNH and NNT managed literature search. Authors MNN Hand MAAH managed for laboratory experiments. Authors MNNH, MAAH and HL wrote the initial draft of the manuscript. Authors NNT and WWM managed literature search and advised for initial draft of the manuscript. Author MNNH wrote final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Snakebite is an important public health problem especially in tropical and subtropical countries. Snake antivenom is the only specific treatment to save the lives. However, antivenoms are relatively expensive, have restricted efficacy to the species of snake whose venom was used to manufacture. Therefore, there is a compelling need to maximize the availability of antivenoms and to know the efficacy of different types of anitvenoms for various species of snakes.
Study Design: *In-vitro* experimental study.
Place and Duration of Study: In the laboratory at the Medical Institution, between February to March 2014.

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Methodology: Venom extracted from the viper and elapid snakes and four different antivenoms, manufactured from Africa, Australia and Asia were used in this study. Sodium dodecyl sulfate (SDS) gel was used to fractionate the venom protein. Immunoblotting allowed the transfer of fractionated proteins from the SDS gel to the nitrocellulose absorbent membrane, and then incubated it in antivenom to observe the binding of all different antivenoms to specific proteins in the venoms of different snakes. Immortalized African green monkey kidney cells (VERO) were used in cell cytotoxicity assay provided a functional measure of antibody efficacy to neutralise the pathological effects of venom in its native state.
Results: In immunoblotting assay, Ipser Afrique polyvalent and SAIMR antivenom exhibited the strong reactivity with elapid and viper venom proteins. *In vitro* cell cytotoxicity assay, Ipser Afrique polyvalent and SAIMR antivenoms were effective in neutralizing the toxicity of *Echi ocellatus* venom, meanwhile, Australian polyvalent and Banded Krait antivenoms were found to be ineffective for the same venom.
Conclusion: Antivenoms from different geographical areas were found to be ineffective against African snakes in this study. Therefore, local pilot trials should be done to ensure the safety and efficacy of antivenoms when introduce to new geographic area.

Keywords: Snake venoms; antivenoms; immunoblotting assay; cytotoxicity neutralisation assays.

1. INTRODUCTION

Snakebite is an important public health problem in tropical and subtropical areas especially in Sub- Saharan Africa, Latin America, South and Southeast Asia [1]. Every year, estimated 421,000 envenoming and 20,000 deaths occurs due to snakebites globally [1]. Snake envenoming mainly occur in rural area, agricultural livelihood, more common in male, peak incidence occur between 15 to 29 years of age and during monsoon period of June to September [2]. Low socioeconomic status in rural community and per capita government expenditure on health are also contributed to the mortality from snakebite [3].

Various snake species produce different types of venoms, which are highly toxic, lethal and composed of several different proteins [4,5]. Snake antivenom is the only specific treatment to save the lives [6]. Antivenoms are mainly produced from the serum of horse and sheep which are inoculated with single venom to produce monospecific antivenom or a mixture of venoms to produce poly specific antivenom. Antivenoms consist of precipitates of whole immunoglobulin G (IgG) or modified to produce antigen-binding fragment (F(ab'))2 or Fab fragments from serum of the horse or sheep which have been inoculated with venom [6].

However, antivenoms are relatively expensive, have restricted efficacy to the species of snake whose venom was used to manufacture. These factors limit the commercial incentive for manufacturers and which leads to the scarcity of antivenoms especially in Africa. This therapeutic necessity drives the influx of non-specific antivenoms manufactured in Asia, which are not effective for African snake venoms, flooding of fake antivenoms in the market [7]. There is therefore a compelling need to maximize the availability of antivenoms and to know the efficacy of different types of anitvenoms for various species of snakes.

In vivo assay is the gold standard to predict the efficacy of antivenom in human patients. However, *in vitro* approaches such as (i) immunoblotting to assess the antivenom's specificity to venom proteins, (ii) enzyme-linked immunosorbent assay (ELISA) to identify the antibody titer and (iii) cell cytotoxicity assay to predict the efficacy of antivenoms are being increasingly used as the alternative of *in vivo* assay in order to reduce the sufferings of the laboratory animals and to save the research funds.

In this study, we used different antivenoms, which were manufactured with snake venoms from Africa, Europe, Asia and Australia. This study aimed to predict the efficacy of different types of the antivenoms for African viper and elapid snake venoms by using *in vitro* Immunoblotting and Cytotoxicity Neutralisation Assays.

2. METHODS

2.1 Venoms and Antivenoms

Venoms were extracted from the viper and elapid snakes at the research unit in the study

institution (Table 1). The venoms were lyophilized, pooled and re-suspended to 10mg/ml in phosphate-buffered saline (PBS) and stored at -80ºC. They were reduced by adding protein loading buffer, then boiled for 5 minutes and provided as 1 mg/ml concentrations [8].

They were pooled, lyophilised and resuspended to 10mg/ml in phosphate-buffered saline (PBS) and stored at -80°C. The antivenoms used in our study were provided by the manufacturers. They were diluted 1:7,500 with PBS (Table 2).

Table 1. List of venoms

Scientific Name	Common name	Region	Code
Viper venoms			
Echis ocellatus	Saw-scaled viper	Nigeria	EO
Echis leucogaster	Saw-scaled viper	Mali	EL
Echis pyramidum leakeyi	The E African saw-scaled viper	Kenya	EPL
Bitis arietans	The puff adder	Ghana	BA
Bitis gabanica	The gaboon viper	Ghana	BG
Ceraster cerastes	The Saharan horned viper	Egypt	CC
Elapid venoms			
Naja nigricollis	The black-necked spitting cobra	Nigeria	N nig
Naja haje	The Egyptian cobra	Nigeria	N haje
Naja pallida	The red-spitting cobra	East Africa	N pall
Naja nievea	The Cape cobra	South Africa	N niv
Dendroaspis angusticeps	The green mamba	East Africa	D ang
Dendroaspis polylepis	The black mamba	West Africa	D poly

Table 2. List of anti-venoms

Antivenom Name	Formulation	Snake venom used for immunization
Africa		
Ipser Afrique (Institute Pasteur Serum Africa antivenom)	Equine F(ab')2	Puff adder (*Bitis arietans*) Gaboon adder (*Bitis gabonica*) Saw- scaled viper *(Echis leucogaster*) Egyptian cobra (*Naja haje*) Forest cobra (*Naja melanoleuca*) Black-necked Spitting cobra (*Naja nigricollis*) Black mamba (*Dendroaspis polylepis*) Green mamba (*Dendroaspis viridis*) Jameson's mamba (*Dendroaspis jamesoni*)
SAIMR Polyvalent (Polyvalent antivenom produced by South African Institute of Medical Research (SAIMR))	Equine, F(ab')2	Puff adder (*Bitis arietans*) Gaboon adder (*Bitis gabonica*) Black mamba (*Dendroaspis polylepis*) Green mamba (*Dendroaspis angusticeps*) Jameson's mamba (*Dendroaspis jamesoni*) Banded Egyptian cobra (*Naja annulifera*) Forest cobra (*Naja melanoleuca*) Cape cobra (*Naja nivea*) Mozambique spitting cobra (*Naja mossambica*) Rinkhals (*Hemachatus haemachatus*)
South East Asia		
Banded Krait antivenom	Equine IgG	*Bungurus fasciatus*
Australasia		
Australian polyvalent antivenom	Equine F(ab')2	Death adder (*Acanthophis antarcticus)* Eastern brown snake *(Pseudonaja textilis)* Tiger snake *(Notechis scutatus)* King brown snake *(Pseudechis australis)* Taipan *(Oxyuranus scutellatus)*

2.2 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting

The venom proteins were fractionated based on their molecular weight by using SDS gel. Immunoblotting allowed the transfer of fractionated proteins from the SDS gel to the nitrocellulose absorbent membrane, and then incubated it in antivenom overnight at 4°C with gentle agitation. Which allowed us to observe the binding of all different antivenoms to specific proteins in the venoms of different snakes [9].

The venoms were reduced by adding of protein loading buffer and boiling for five minutes. The amount of 12 µl of each of the venoms and molecular weight markers were added to 15% SDS-PAGE gel, fractionated under 200 volts and the fractionate proteins were visualized by staining with Coomassie Blue R-250.

Immunoblotting was carried out the same method as above. After separation, the fractionate proteins were transferred to the nitrocellulose paper, blocked with 5% non-fat milk and incubated for one hour at room temperature. The filter was washed (3 changes of mixture of Tris-buffered saline and Tween 20 (TBST) over 30 minutes), added diluted antivenom IgG (1:7,500 with TBST) and incubated overnight at 4°C with gentle agitation. After then, the filter was washed (6 changes of TBST over 60 minutes) and placed in the diluted Horse Radish Peroxidase-conjugated second antibody (1:1000 with TBST) for 2 hours at room temperature. The filter was finally washed (6 changes of TBST over 60 minutes) and visualised the results by adding 50 ml of DAB substrate (200 ml PBS, 100 mg DAB, mix, add 50 µl H_2O_2) [8].

2.3 Cell Cytotoxicity Assay

Cell cytotoxicity assay provides a functional measure of antibody efficacy to neutralise the pathological effects of venom in its native state. Immortalized African green monkey kidney cells (VERO) were used in this study. Venoms (10 µl) and antivenoms (200 µl) were pre-incubated before adding to the cell culture plate, thus the residual cytopathic effect of venom, which had not been bound to the antibodies, was examined. The neutral red indicator was a dye, which could be taken up by the living cells and incorporated into lysosomes. So that the results of neutral red cytotoxicity assay were mainly dependent on (i) the number of variable cells in the culture and (ii) the precise lysosomal function of these cells [10]. Pre-immune sheep serum, the normal sheep serum, was used as negative control and venom alone was used to confirm its cytopathic effect. Antibody was added to the wells to validate the toxic effect on the cell. Media control wells were used to indicate growth of cells without venom or antivenom.

In brief, serial dilution of antivenom or pre-immune serum (PIS) were prepared in assay medium (45 ml of Dulbecco's Modified Eagle Medium (DMEM) and 5 ml of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)) and placed in fridge (4°C) overnight. The amount of 10 µl of *Echis ocellatus* venom was diluted with DMEM/HEPES buffer (1:100 dilution) and transferred the venom solution, 100 µl/well, into rows B-F columns 2-11 and G5-G7. The plate was incubated for 30 minutes at 37°C to allow the venom and antivenom to bind to each other.

The 96 well cell culture plate was provided, which had been seeded with 100 µl/ well of a suspension containing 0.75 x 10^5 VERO cells/ml and grown in a 37°C incubator overnight in pink culture media (DMEM/10% Foetal bovine serum). The diluted antivenom and pre-immune serum (100 µl/ well) was transferred to the corresponding well of the cell culture plate and incubated for 4 hours at 37°C. After that, Neutral red dye (2 ml neutral red to 10 ml pre-warmed PBS/plate) was added 50µl/well to the culture plate. After 2 hours of incubation at 37°C, the plate was washed twice with PBS, added 150 µl/ well of destrin (50% ethanol, 1% acetic acid in water) to the plate and rocked for 10 minutes. Absorbance was detected in a microtitre plate reader at 540nm. The analysis result of mean absorbance of antivenom and pre-immune serum against the log dilutions were plotted on y-axis and x-axis respectively. The mean absorbance of antivenom, venom and medium wells were calculated. The EC_{50} absorbance was calculated by using the following formula and read off the corresponding antivenom dilution from the graph [9].

$$EC_{50} \text{ absorbance} = [(A_{med} - A_V)/ 2] + A_V$$

3. RESULTS

3.1 SDS-PAGE Profiles of Viper and Elapid Venoms

The SDS-PAGE profiles revealed the variation in molecular mass and quantitative representation

of venom proteins in different types of viper and elapid venoms. In elapid venoms, lower molecular weight toxins were predominant, whereas in viperic venoms, both higher and lower- mass toxins were identified (Fig. 1).

The SDS-PAGE separated the proteins largely on the basis of molecular mass so that the researchers could not identify particular enzymes exactly. However, by visualising the molecular weight of the toxin proteins, i.e., by comparing the bands, we could estimate the likelihood enzymes in the venoms. The most prominent bands in viperic venoms were at ~ 55 kDa, ~ 24 kDa and ~15 kDa. By correlating them with reference molecular weight, viperic venoms might mainly compose of Metalloprotease P III (43-85 kDa), Metalloprotease P I (20-24 kDa) and Phospholipase A_2 enzyme (13-15 kDa). The two most prominent protein bands in elapid venoms appeared at ~ 15 kDa and ~ 9 kDa. These bands might correlate with the Phospholipase A_2 (13-15 kDa) and Three-finger toxins (6-9 kDa) [11].

3.2 Immunoblotting for Antivenom's Venom Protein-specificity

The researchers used four different antivenoms manufactured from Africa, Australia and Asia to predict their efficacies against African Vipers and Elapids. The researchers assessed the SAIMR poly antivenom's specificity on different venom proteins. It is a polyvalent, equine F(ab')2 antivenom manufactured from Africa. Both of the African viper and elapid venoms were used to manufacture SAIMR poly antivenom's so that it had clinically efficacy for most of these species. The immunoblot revealed that this antivenom had strong cross-reactivity with viper venom proteins and elapid venom proteins (Fig. 2A).

Ipser Afrique polyvalent is equine F(ab')2 antivenom, produced by using both viper and elapid venoms and it has clinical efficacy against wide variety of these species. The immunoblot of Ipser polyvalent antivenom exhibited the strong reactivity with all elapid venom protein especially to lower molecular weight protein toxins. It also showed reactivity with all viper venom proteins (Fig. 2B).

Commonwealth Serum Laboratories (CSL) poly antivenom was manufactured in Australia by using the venoms of Australian snakes. Mostly Australian snakes belonged to the elapid family and their venoms mainly caused coagulopathy. Only a few are neurotoxic in humans. Despite the CSL poly antivenom was produced by using Australian elapid snakes' venoms, it revealed cross reactivity to both African elapid and viper venom proteins, especially with *Naja haje and Naja pallida*, in the immunoblotting assay (Fig. 2C).

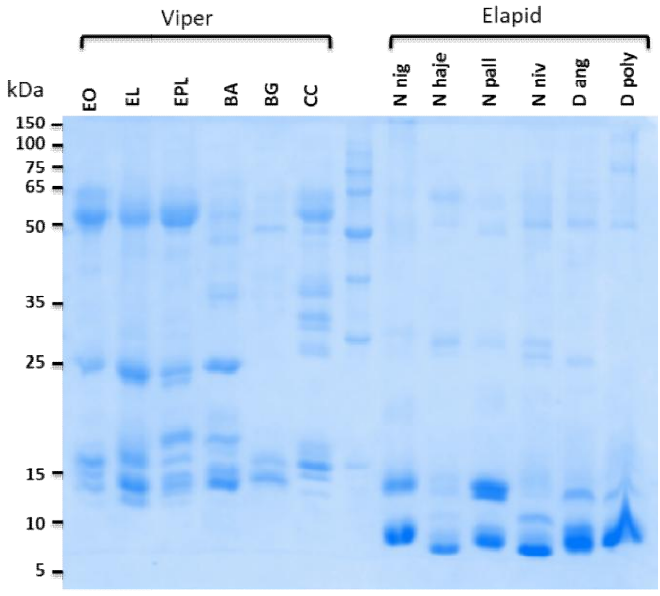

Fig. 1. Venom proteins of vipers and elapids visualised using SDS-PAGE

Banded Krait monovalent antivenom is equine, IgG antivenom manufactured by using the venom of *Bungurus fasciatus*. It is produced in Asia intended to use in Asia snake envenoming patients. In immunoblotting, fairly weak cross-reactivity with African viper venom protein had been observed. There was some cross-reactivity especially with low molecular mass proteins of African elapid viper venoms (Fig. 2D).

3.3 *In vitro* Cell Cytotoxicity Assay

In this study, the ability of different antivenoms to neutralise the cytotoxic effect of *the Echi ocellatus* venom were tested by *in vitro* cell assay. Because of the limitation of study period, only one venom, i.e, *Echi ocellatus* venom, was selected for cytotoxicity assay. The *Echi ocellatus* venom was selected because it is the commonest cause of morbidity and mortality due to snakebite in West Africa [12].

Pre-immune serum was used as the negative control and calculated the EC_{50}, medium effective concentration of antivenom to neutralise venom-induced cytotoxicity in 50% of the cells.

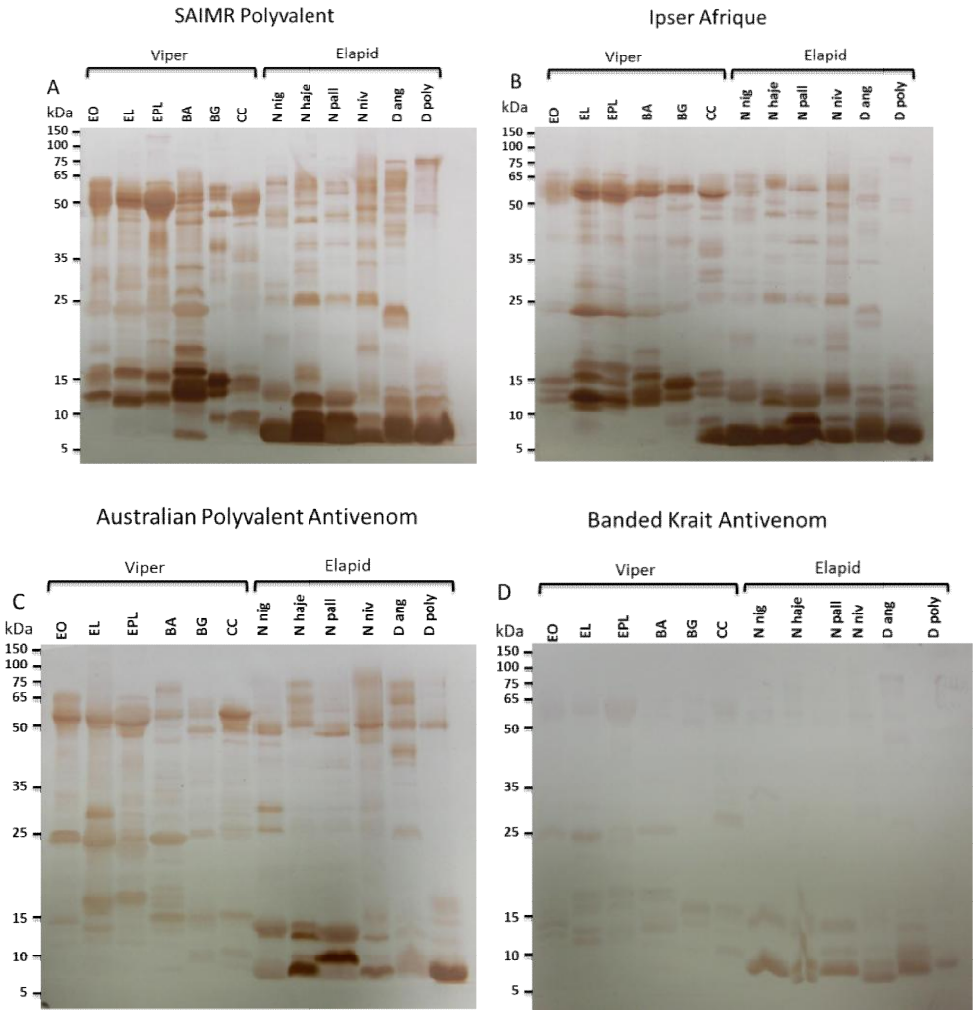

Fig. 2. Different types of antivenoms exhibit cross-specifity venom protein reactivity

Immunoblotting showed extensive cross-specific reactivity with SAIMR polyvalent antivenom to African viper and elapid venoms (A), extensive cross-specific reactivity with Ipser Afrique polyvalent antivenom to African viper and elapid venoms (B), cross-specific reactivity with Australian polyvalent antivenom to African viper and elapid venoms (C) and weak cross-reactivity with Banded Krait antivenom to African viper and elapid venoms.

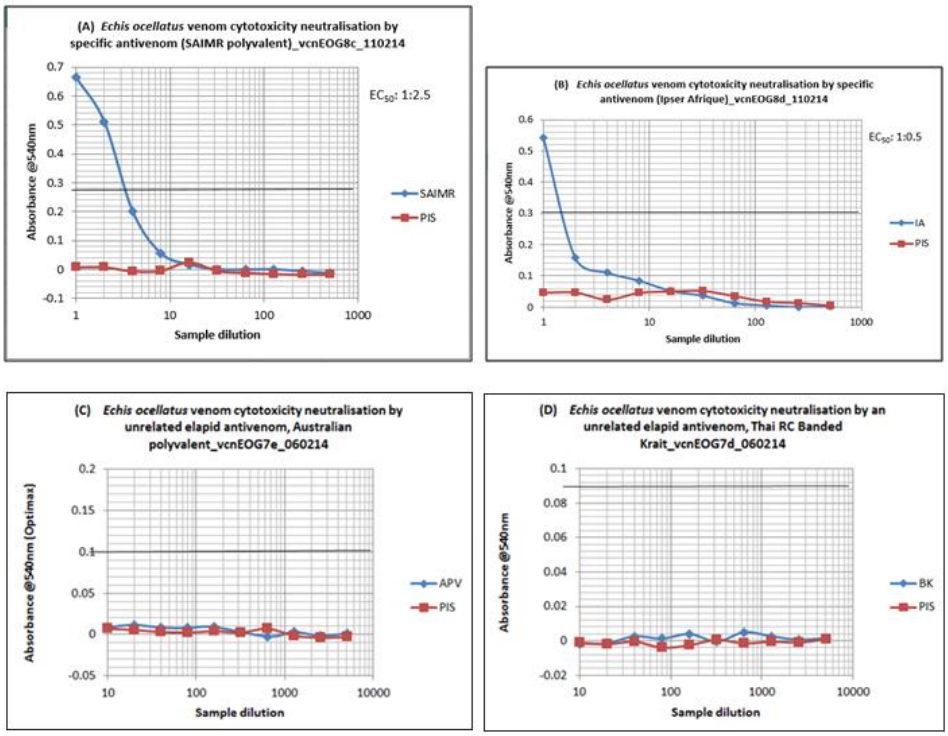

Fig. 3. Neutralisation of antivenoms against the cytotoxic effect of the *Echi ocellatus* venom

The absorbance of the sample containing 10µl of the venom and serially diluted anti-venoms (blue line) and PIS (red line) were measured by microplate reader and plotted on the graph. The SAIMR polyvalent antivenom was effective and EC_{50} was 1:2.5 dilution (A). Ipser Afrique polyvalent antivenom was also effective and its EC_{50} was 1:0.5 dilution (B). Australian polyvalent antivenom and Banded Krait antivenoms were ineffective against the Echi ocellatus venom toxin since their absorbance values were similar with PIS (C and D).

The SAIMR polyvalent antivenom was effective in neutralising the toxicity of *Echi ocellatus* venom in cell cytotoxicity assay. The graph obtained with the neutral red uptake essay was sigmoidal in shape and EC_{50} was 1:2.5 dilution (Fig. 3A). Ipser Afrique polyvalent antivenom was also found to be effective in neutralising the toxicity of the same venom. The graph obtained was exponential in shape and EC_{50} was 1:0.5 dilution (Fig. 3B).

However, Australian polyvalent and Banded Krait antivenoms were found to be ineffective for the *Echi ocellatus* venom toxin. Both of them did not reach to the medium effective concentration (EC_{50}) and the graph lines of antivenoms were the similar with those of the pre-immune serum, i.e., used as the negative control in cell cytotoxicity assay (Fig. 3C and 3D).

4. DISCUSSION

Snakebite is one of the neglected tropical diseases and the vast majority of mortality occurs in Africa and Asia. Different species of snakes produce a variety of venoms, which can cause the life threatening manifestations such as cytotoxic, haemorrhagic, neurotoxic and myotoxic effects [13]. Effective and appropriate antivenoms could save the lives and reduce the morbidity due to snake envenoming. However, identifying the snake species is often a challenge to choose the appropriate monovalent antivenoms. In order to overcome this challenge, the polyvalent antivenoms are manufactured to cover a number of species of snakes.

The SAIMR polyvalent antivenom is produced in South Africa by using the venoms of various species of snakes found in Africa (Table 2). Immunoblotting assay in this study had been shown the extensive cross-specific reactivity to African viper and elapid venoms and cell analysis revealed that it was effective to neutralise *E. ocellatus* toxin. This finding did not support the study of *in vitro* cell cytotoxicity assay conducted in Sudan, where the SAIMR polyvalent antivenom failed to neutralise the cytotoxic effect

of *E. ocellatus* [14]. Even though the same *E. ocellatus* venoms were used, the geographic locations of these studies were different. Geographical variation has effect on the venom variability of the same species as the diet, climate, habitant are diverse around the world [15]. The potential variation in the component of the venoms of *E. ocellatus* from Africa and Sudan might be the reason of the discordant findings. Therefore, it is important for the clinicians to understand the variability of venoms and to choose the appropriate antivenoms in treating the snakebite patients.

Murine *in vivo* study of SAIMR polyvalent antivenom had been identified that it was effective to neutralise the *Bitis arietans* venom and treatment of choice for the *B. arietans* envenoming patients [16,17]. However, another consideration is that the initial dose of SAIMR polyvalent antivenom is higher than the monovalent antivenoms as it is produced by using venoms of various snake species in Africa. Four to ten ampules of initial dose is needed and which can increase the cost and risk of early anaphylactic reactions up to 76% of the patients treated [18]. Therefore, it is crucial to give antivenom under closely medical supervisions.

Ipser Afrique polyvalent antivenom is produced from both of the African viper and elapid venoms. Not surprisingly, extensive cross-specific reactivity to African viper and elapid venoms had been observed in Immunoblotting assay. The EC_{50} in cell cytotoxicity analysis was 1:0.5 dilution. However, the murine *in vivo* efficacy test is yet to be done for this antivenom. The initial dose of this antivenom is similar with SAIMR antivenom, i.e., 4-10 ampoules depending on species. A large-scale clinical trial with 223 envenomed patients was conducted in Cameroon to determine the safety of Ipser Afrique antivenom. The intermittent infusion schedule, started with low dose of two (10 ml) ampules and repeated depending on the clinical and biological markers of envenomation, was used and it was found to be safety. Among the patients, only less than 1% occurred anaphylactic shock and serum sickness [19]. Both SAIMR and Ipsar Afrique polyvalent antivenoms are effective against the African vipers and elapids envenoming and initial dosages are the same. However, SAIMR antivenom has higher risk of early anaphylactic reactions than Ipsar Afrique antivenom. The clinicians need to be aware of this adverse effect and should give SAIMR antivenom with full resuscitation facilities.

Commonwealth Serum Laboratories (CSL) Australian polyvalent antivenom is Equine, F(ab')2 antivenom, produced from a variety of Australian elapid species. It is effective not only for the snakes which venoms were used to manufacture but also for the other snake species such as New Guinea small-eyed snake (*Mircopechis ikaheka*) , Inland Taipan (*Oxyuranus microlepidotus*), etc [20,21]. In the immunoblotting assay, CSL antivenom revealed cross reactivity to African viper and elapid venom proteins, especially to low molecular weight elapid venom proteins. Since CSL antivenom is produced from Australian elapid species, the cross reactivity in immunoblotting assay can occur with elapid venoms. This finding was in line with the study of Minton, in which the researcher found out that CSL antivenom was effective in neutrallising the venoms of exotic elapids in mice [22].

Although CSL polyvalent antivenom had cross-reaction with African snake venoms, it was found to be ineffective to neutralise *E. ocellatus* venom in cell cytotoxicity assay in our study. The possible explanation for this discrepancy of the findings is that antivenom might bind to the non-toxigenic proteins in the venoms and appeared the bands in immunoblotting assay. However, we did not used African elapid venoms for cell cytotoxicity assay. Further research should be continued to identify the efficacy of this antivenom against African elapid venoms especially for *Naja haje* and *Naja pallida*, which had intense cross-reaction in immunoblotting. Generally, this study finding predicts that it is not effective against African viper venoms. A study conducted in Sudan found out that this antivenom had only partial neutralising efficacy against the cytotoxicity of *E. ocellatus* venom [15]. Therefore, CSL was predicted to be not fully effective against *E. ocellatus* venom.

An Asian product, Banded Krait monovalent antivenom, is equine, IgG antivenom and manufactured by using *Bungurus fasciatus* venom. It had very weak cross reactivity with African elapid venoms and much weaker with African viper venoms in immunoblotting. It also showed no efficacy to neutralise the *E. ocellatus* venom in cell cytotoxicity assay.

Table 3. Summary of efficacy of antivenoms against African viper and elapid snakes

Antivenom	EC_{50}	ED_{50} (µl/mouse)	Clinical efficacy
SAIMR polyvalent	1:2.5 dilution	++ (BA)	ND
Ipser Afrique polyvalent	1:0.5 dilution	ND	ND
Australian polyvalent	NE	ND	ND
Banded Krait antivenom	NE	ND	ND

The antivenom's neutralising efficacy is expressed as medium effective concentration (EC_{50}) in vitro, ED_{50} in vivo and clinical efficacy trials.
BA = Bitis arietans
ND = Not done
NE = Not effective

The clinical and geographic effectiveness of antivenom is restricted to the snake species whose venom was used to produce. In Africa, antivenom market is inconsistent, low demand and suboptimal utilisation, which could lead to increase manufacturing cost and reduce output of antivenoms [3]. This therapeutic vacuum causes the influx of antivenoms from other regions of the world, which are not appropriate to the local needs [7]. Some antivenoms used in Africa are lack of efficacy against some snake species that they are targeted [23]. Some Indian antivenoms intended for use in South Asia are labelled with English snake names which can lead to confusion for clinicians, increase mortality of patients [24]. It is virtually important to know the effectiveness of antivenoms against species of snakes and appropriate geographic regions.

This study results were based on the immunoblotting and cell cytotoxicity assays. There were some limitation of immunoblotting, i.e., the bands obtained were not be able to quantified and identify the clinical efficacy. In order to quantify the results, we need to conduct ELISA assay. *In vitro* assays should be followed by *in vivo* assays. However, preclinical *in vivo* tests have physiological limitations such as the venom, antivenom inject protocols do not represent the natural situation, the physiological response and pathological process of rodent envenoming may different from human physiology. Nevertheless, murine *in vivo* ED_{50} test is the currently useful test to assess antivenom potency [25]. Although the World Health Organization (WHO) recommended performing the preclinical tests especially LD_{50} and ED_{50} in animal models, about half of the antivenoms, which we used in this study, have not done *in vivo* assay (Table 3 above).

Clinical trials in human patients are useful despite there are challenges such as difficulty to recruit snakebite patients at one area. Although all these antivenoms' pre-clinical results need to be verified by clinical trials, most of the antivenoms in the market did not complete that stage. Prospective observational studies are crucial to ensure the safety and efficacy of antivenoms when used in the new geographic area. Post-marketing surveillance studies also help to evaluate the safety and efficacy of antivenoms [26].

New research approaches are progressing to treat the snake envenoming. Camelic unique IgG is less immunogenic and less likely to induce complement activation than equine and ovine IgG. It has the potential advantages for safety and treating local effect [27]. Recombinant multiepitope DNA immunization of *Echis ocellatus* venom is a new approach for toxin specific antivenom production. This approach is the beginning of the future novel antivenom production and other cases where targets have hypermutation or antigenic variation [28].

5. CONCLUSION

In conclusion, this study demonstrated that African polyvalent antivenoms, SAIMR and Ipsar Afrique are effective against African viper and elapid envenoming. However, antivenoms from different geographical areas were found to be ineffective against African snakes. The result of this study revealed that CSL Australian polyvalent antivenom is not effective for African viper venom but further research need to be continued to identify it's efficacy against African elapid venoms. Meanwhile, Asian antivenom, Banded Krait antivenom, might not be suitable to be used in Africa. There is therefore, local pilot trials should be done to ensure the safety and efficacy of antivenoms when introduce to new geographic area. Moreover, efforts should also be devoted to produce more effective, safe and affordable life-saving antivenoms in future.

CONSENT AND ETHICAL APPROVAL

In this study, the live animals such as snakes were not included. The researchers used the extracted, pooled and lyophilized venom provided from the research unit. Therefore, consent and ethical approval were not applicable for our study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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