Investigation of the antibacterial activity of venoms against *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus, a widespread pathogen, is notorious for its ability to become resistant to wide range of antibiotics. There is an urgent need for development of novel antibiotics in order to combat the increased antibacterial resistance. Antimicrobial peptides from venom are attractive candidates for the development of novel therapeutics.
This study identified that venom of Pandinus cavimanus displays antibacterial activity against S. aureus in vitro.

BACKGROUND AND RESEARCH AIM

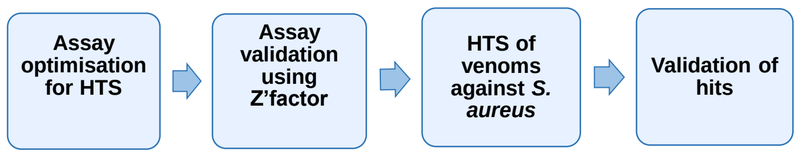
Staphylococcus aureus is not only a leading cause of bacteraemia and skin and soft tissue infections[1], but colonisation of skin with this bacterium also drives skin inflammation contributing to severity of atopic dermatitis[2]. Novel antibiotics that are currently in the clinical pipeline, provide only a short-term solution as these are only modifications of existing drugs[3]. Antimicrobial peptides (AMPs) from venoms are attractive candidates for the development of novel antimicrobials, due to their selectivity for prokaryotic membranes[4], unique membrane-disrupting mechanisms[5] and low resistance rates[4].
The aim of this research was to develop, optimise and validate a robust assay for high-throughput-screening of venoms from *Naja nigricollis*, *Agkistrodon contortix pictigaster*, *Poecilotheria regalis* and *Pandinus cavimanus.* Resazurin was used as the indicator of bacterial cell viability/antimicrobial activity.

MATERIALS AND METHODS

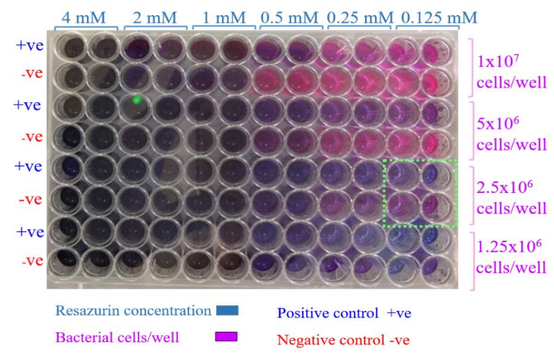
Bacterial strain and growth conditions

Gram-positive bacterium, *S. aureus* (DSM No.: 102262) was used as test organism. An OD_{600} of standardised inoculum (incubated at 37°C, 180 rpm, ~ 18 hours) was measured by spectrophotometer and diluted to obtain $2.5x10^6$ cells/well in an exponential phase.

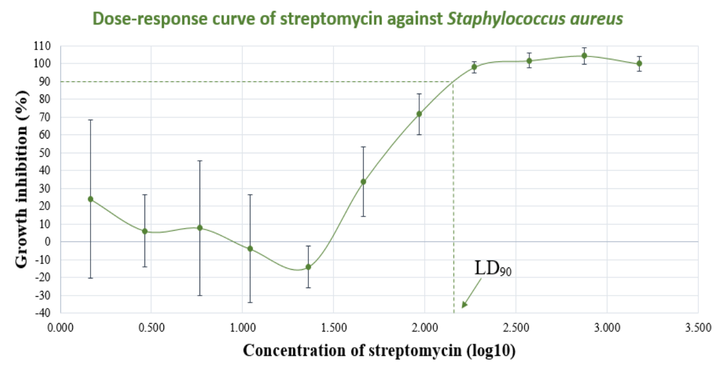
Flow chart illustrating the research process



Optimisation of assay for high-throughput-screening

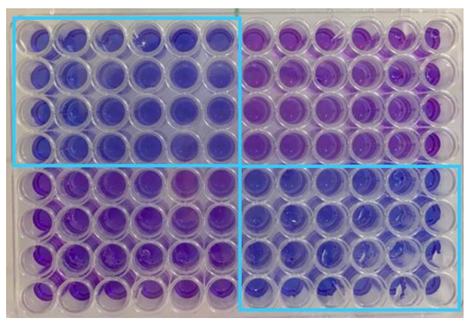


A 96-well plate was used to determine optimal concentration of resazurin and bacterial cells. The wells highlighted in green show the optimal conditions of resazurin (0.125 mM) and bacteria ($2.5x10^6$ cells/well) adapted for this research.



Dose-response curve illustrating the relationship between the dose of streptomycin and growth inhibition of *S. aureus.* Error bars are represented as % standard deviation. The dose that kills 90% of test population (LD_{90}) was determined at 2.15 (log10) = 141 µg/ml.

Validation of assay for high-throughput-screening



Z' assay showing positive (streptomycin,158 µg/ml), highlighted in blue and negative controls. Z' factor was calculated using formula below, where σ is the standard deviation and µ is the mean of positive (*p)* and negative (*n*) controls.
The Z'factor value identified the assay as an excellent assay (Z'factor = 0.7).

$$Z'factor = 1 - \frac{3x(\sigma p + \sigma n)}{|\mu p - \mu n|}$$

High-throughput-screening of venoms

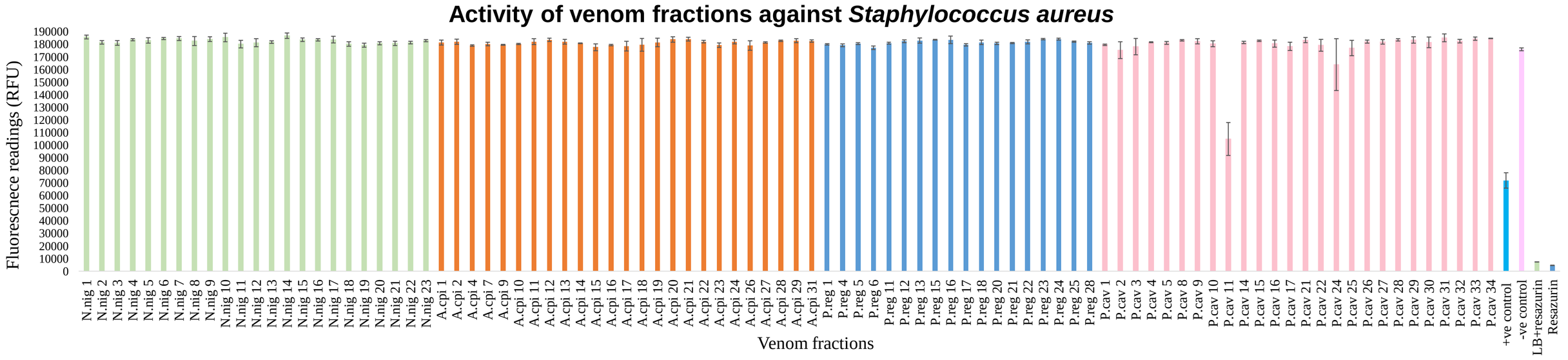
Venom fractions (Table 1) in T-VDA^{microbe} array were rehydrated with 90 µl of dH_2O. Each fraction was tested in triplicates, therefore 3x30 µl of each venom fraction (2µg per treatment) was added to wells containing $2.5x10^6$ bacterial cells. Plates were incubated for 2 hours at 37°C. Resazurin was added and fluorescence readings were taken every 30 minutes using FLUOstar® Omega plate reader.

Code	Species (Latin name)	Common name
N.Nig	*Naja nigricollis*	Black-necked spitting cobra
A.cpi	*Agkistrodon contortrix pictigaster*	Western copperhead
P. reg	*Poecilotheria regalis*	Indian ornamental tree spider
P. cav	*Pandinus cavimanus*	Tanzanian red clawed scorpion

Table 1: Illustration of the venoms used and the origin of venoms

RESULTS

Activity of venom fractions against *Staphylococcus aureus*

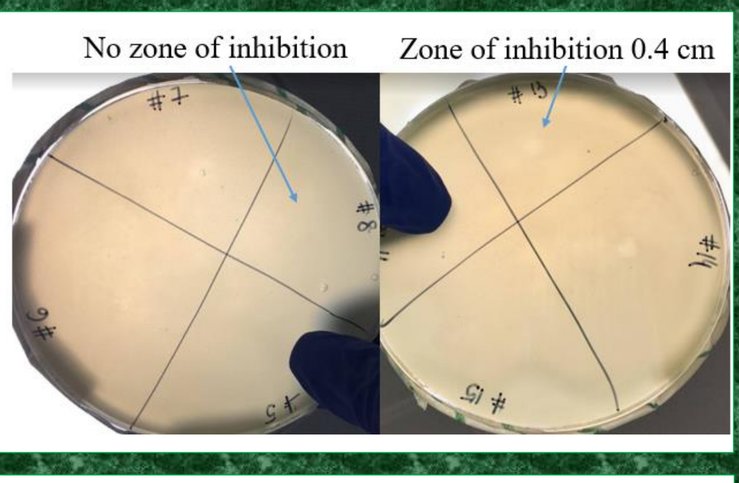


Graph illustrating the antimicrobial activity *of Naja nigricollis, Agkistrodon contortix pictigaster*, *Poecilotheria regalis* and *Pandinus cavimanus* first-dimension venom fractions against *S. aureus*. The controls were as follow: positive (streptomycin, 158 µg/ml), negative (no ATB), resazurin only and LB broth+resazurin only. The fluorescence readings are plotted as means, whereas the error bars represent % standard deviation. Both fractions No. 11 and 24 in *P. cavimanus* venom displayed bactericidal activity, No. 11 indicated stronger inhibition of bacteria.

VALIDATION OF HITS

Agar-diffusion test

An agar-diffusion test was used to validate the hits. Venom fractions were rehydrated with 0.9% NaCl and diluted to a concentration of 0.5 µg/µl. 2 µl of each sample was pipetted into a 1% LB agar plate seeded with *S. aureus* (OD_{600} =0.361). Plates were incubated at 37°C, ~ 24 hours. The results confirmed antibacterial activity of fraction No. 13 (24 in the venom array). Fraction No. 8 (11 in the venom array) showed no activity against *S. aureus*.



Acknowledgement

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CONCLUSION AND FURTHER DIRECTIONS

The optimised assay for high-throughput-screening of venoms was validated using Z'factor identifying the assay as excellent. The high-throughput-screening of first-dimension venom fractions identified two hits in *Pandinus cavimanus* venom, fraction No. 11 and 24. An agar-diffusion test confirmed that one fraction, No. 13 (24 in the venom array) inhibits the growth of *S. aureus*. Further experiments would include dose response of second-dimension fractions to determine minimal inhibitory concentration (MIC). Mass spectrometry analysis would identify the active compound and determine whether it is a novel antimicrobial peptide.

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